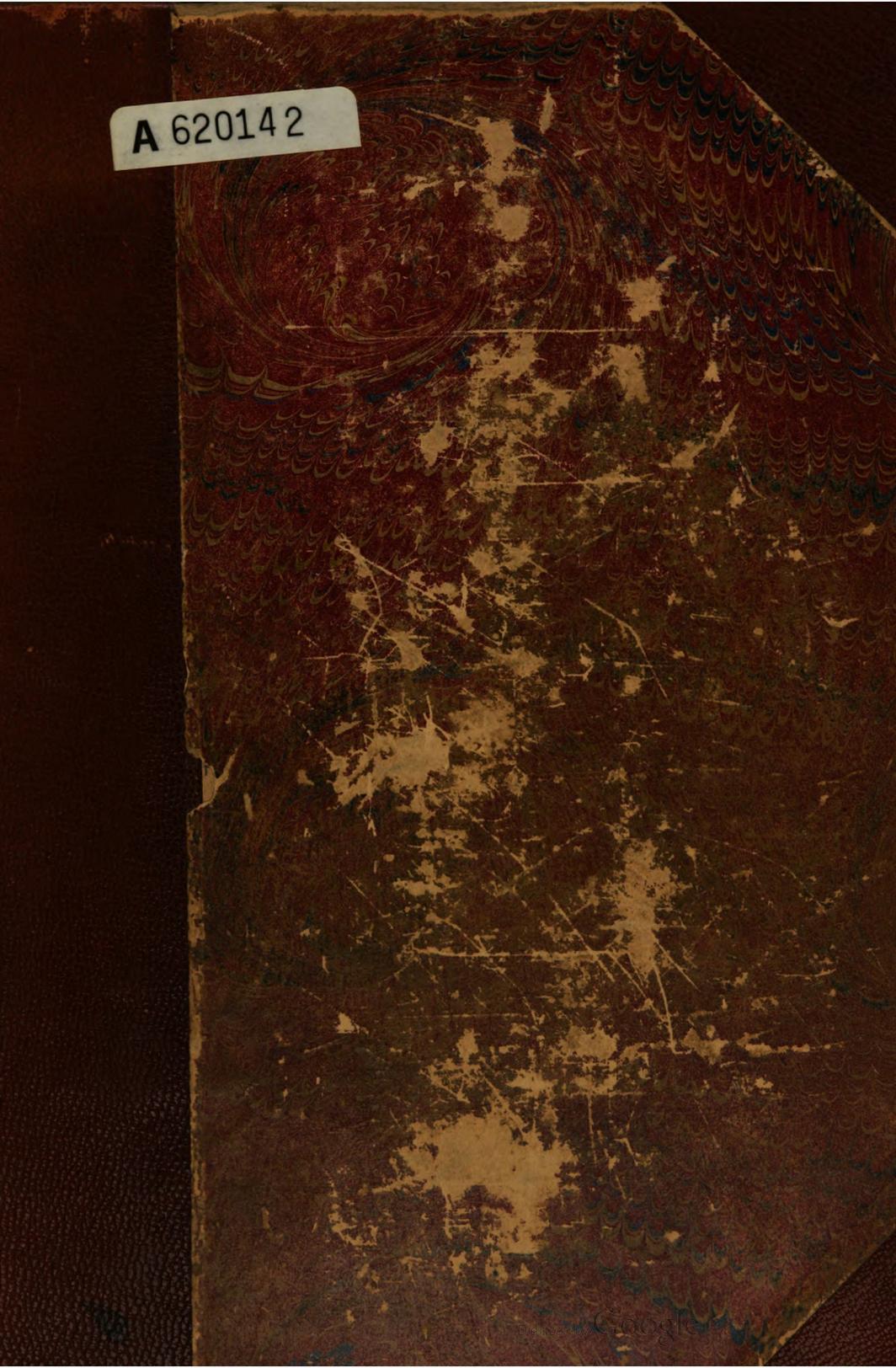
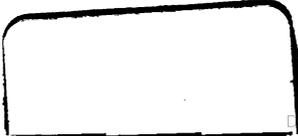
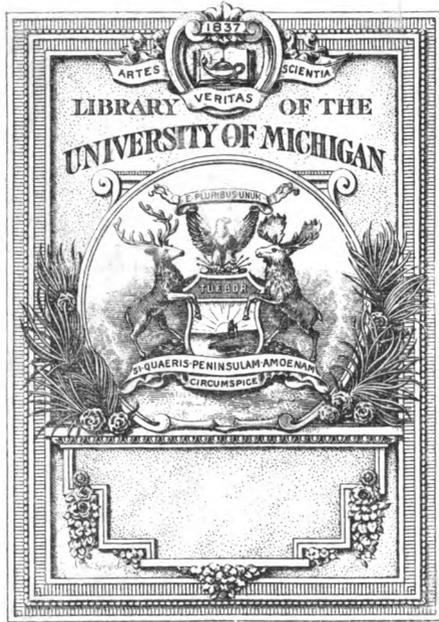


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LECTURE NOTES

ON

CHEMICAL



PHYSIOLOGY AND PATHOLOGY.

BY

VICTOR C. VAUGHAN, M. D., Ph. D.,

Lecturer on Medical Chemistry in the University of Michigan; Author of "Osteology and Myology of the Domestic Fowl," "Charts for the Analysis of Abnormal Urine," "A New Method of Detecting and Separating Arsenic, Antimony and other Poisons," etc.

SECOND EDITION, REVISED AND ENLARGED.

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PREFACE.

The speedy exhaustion of the first edition of these notes has led the author to issue another and enlarged edition. The nature of the work is expressed in its title. It has no claim to completeness, but is given as a guide to the student, who may desire to pursue this branch of study. In the preparation of these notes, various writers have been consulted; but especially is the author indebted to the following: M. Foster, T. Lauder Brunton, J. Burdon-Sanderson, F. W. Pavy, K. B. Hofmann, Hoppe-Seyler, and Gorup-Besanez. The various writings of these distinguished physiologists have been the basis of the author's study for years, and whatever of merit these pages may contain is in great part due to the above mentioned names.

The plates representing the crystals of the most important substances discussed in these notes will soon be issued on charts with references to the pages of this book under each cut. This has been decided to be better than placing the cuts in the text, for the following reasons: (1) the chart will be more convenient for constant reference as it can be framed, and preserved indefinitely; (2) it is intended to issue a series of these charts illustrating the majority of the crystals met with in the study of chemical physiology and pathology.

These notes have been especially prepared for the use of the students of the author, and embrace the work done by them.

ANN ARBOR, MICHIGAN,

January, 1879.

CHEMICAL PHYSIOLOGY AND PATHOLOGY.

DIGESTION.

All living things absorb and excrete matter. Thus, the plant takes up carbonic acid and gives off oxygen. That microscopic speck of albuminous matter, known as the moneron, thrusts out any part of its body and takes in its food, digests it, utilizes a part and casts off the remainder. Many of the lower forms of living beings absorb their food directly from the surrounding world and without first subjecting it to any particular changes. The plant absorbs gases from the atmosphere and thus builds up its tissues. The gases, previous to absorption, are not subjected to the action of any digestive fluids secreted by the plant. However, there is a digestive process going on in many plants whereby substances otherwise insoluble are dissolved by the juices of the roots and thus fitted for absorption. Man resembles the plant inasmuch as important articles of nutrition are received directly from the inorganic world. Oxygen, inhaled by animals, enters the circulation, and takes part in the various changes which support life, produce muscular activity and call into existence the intellectual and moral faculties of man. Oxygen is a food, but it is absorbed without the action of digestive fluids.

The process of digestion consists in certain physical and chemical changes which food undergoes while in the alimentary canal and whereby the nutritive parts of the food are fitted for absorption. The foods of man are inorganic, starchy, fatty and albuminous. Of these, we will not take into consideration the inorganic foods since some of these are absorbed unchanged and

the changes which take place in others are simple and in no way to be distinguished from those produced by various physical and chemical agents outside of the animal world. The digestive fluids, to which these foods are subjected, are the saliva, the gastric juice, the pancreatic juice, the bile and the intestinal juice. The different foods are effected variously by the several juices.

In the mouth, food is masticated and mixed with the saliva. The mixed saliva is furnished from four sources, the parotid, submaxillary and sublingual glands, and the mucous membrane of the mouth. The saliva from these sources varies in its composition and the intensity of its action upon food. The parotid saliva of man is a clear fluid, of feebly alkaline reaction and of specific gravity from 1004 to 1007. It contains no morphological elements, but upon standing deposits carbonate of lime, which in the recently obtained secretion is held in solution by carbonic acid gas. Parotid saliva contains from one to one and a-half per cent of solids. Of these, about one-half are inorganic constituents, the most interesting of which is the sulphocyanide of potash; besides this, there are traces of alkaline chlorides, phosphates and sulphates and calcic bicarbonate. The most important of the organic constituents is ptyaline: while an albuminous substance coagulable by heat is present.

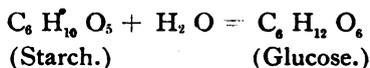
The secretion of the submaxillary gland varies with the means by which the gland is excited; thus, by excitation of the chorda tympani or by irritating the tongue with a drop of acid, a peculiar secretion known as *chordal* saliva is obtained. On the other hand, by irritation of that branch of the cervical sympathetic which supplies this gland or by irritation of the tongue with pepper or an alkali (Hofmann), a different secretion, known as *sympathetic* saliva, appears. Again, if all the nerves supplying this gland be severed, or if their function be destroyed by curare, a saliva differing in composition from either of the others and known as *paralytic* saliva is secreted.

Chordal saliva is a clear, strongly alkaline fluid, with a specific gravity varying from 1003 to 1005. It contains globuline and traces of alkaline chlorides and phosphates and calcic bicarbonate. Sympathetic saliva is cloudy with morphological elements and its

specific gravity is about 1008. Paralytic saliva is poorer in solids than either of the other two. It has an alkaline reaction and specific gravity from 1001 to 1002.

Saliva from the sublingual gland is tenacious and ropy, alkaline in reaction and contains ten per cent of solids. Mucus is present in considerable quantity and to this constituent the viscosity of this secretion is due. Bicarbonate of lime is present in small quantity, but is not deposited in a crystalline form as it is in parotid saliva. The secretion of the mucous membrane of the mouth resembles sublingual saliva, since both are rich in mucine. The former contains epithelial scales, salivary corpuscles and at times traces of cholesterine. Fat may also be present either from the food or from a diseased condition of the mucous membrane.

Upon all solid foods, saliva exerts a physical influence, rendering the formation of a bolus possible and deglutition more easy. Upon the starchy food only, does saliva exert any chemical action. Under the influence of the peculiar ferment, ptyaline, starch takes up water and is converted into glucose or grape sugar.



Starch consists of cellulose and granulose. The former is not colored blue by the action of iodine alone; but it is so colored by iodine after being subjected to the action of sulphuric acid. Granulose is colored blue immediately by the application of iodine. In the starch grain, cellulose and granulose are arranged in alternating layers. The saliva acts upon the granulose, but is without action on the cellulose. Consequently, raw starch is acted upon very slowly by saliva, since the coats of cellulose must be penetrated; but if the grains be ruptured by boiling, the granulose is exposed and is rapidly converted by the saliva into glucose. Thus, during mastication a part of our food is converted into grape sugar, or is fitted for absorption. It must be understood that the short sojourn of the food in the mouth is not sufficient for the conversion of *all* the starch.

During its passage through the œsophagus, no part of the food is materially changed. The stomach furnishes two secre-

tions which differ essentially in their composition and action upon foods. These are known as the *succus gastricus* and the *succus pyloricus*. As its name implies, the latter is secreted from the pyloric extremity of the stomach; while the *succus gastricus* is poured from the walls of the fundus of the same organ. The flow of the pyloric secretion is constant; while that of the true gastric juice is intermittent.

The *succus pyloricus* is a viscid, yellowish fluid, of alkaline reaction, specific gravity about 1010, and contains from fifteen to twenty per cent of solids. When pure, it changes starch into sugar, but is without action upon albuminous food. However, after it has been rendered acid with dilute hydrochloric acid, this juice digests albumen with readiness. It has no action upon fats. (Hofmann.)

† The gastric juice is colorless, has a specific gravity which varies from 1001 to 1002, and does not contain more than one per cent of solids. It is poured out during digestion, and has an acid reaction which is soon imparted to the entire contents of the stomach. Besides the free acid, which normally is hydrochloric, a ferment, pepsine, is present. By the combined action of the acid and pepsine, assisted by the movements of the stomach, the albuminous parts of the food are changed. The principal products of stomachic digestion are *peptones* and *para-peptones*. Various kinds of peptones have been described by authors; but for our purpose, it is, at present, necessary to note only the broad distinctions. The great physiological difference between peptones and para-peptones, is that the former is ready for absorption, while the latter must be farther changed before it can enter the circulatory system. Moreover, para-peptones can not be changed by the action of gastric juice into peptones; or, in other words, cannot be fitted for absorption by this digestive fluid.

Upon starch the gastric juice has no effect and often the acidity is so great as to arrest the action of the saliva upon this part of our food. Fat itself is not chemically changed by the gastric juice. When fatty food is taken, the albuminous envelopes of the globules of fat are digested in the stomach, and the fat, thus freed from its proteid covering, is the more readily acted upon by the

juices with which it meets in the intestines. From this fact, a practical lesson in physiology may be learned. It is very necessary to healthy digestion that the proteid envelopes of the fat should be digested in the stomach. In order that this may be fully accomplished, the fat of the food should be well distributed. If lumps of fat be swallowed, the gastric juice does not gain access to all the proteid matter and consequently, the fat still enveloped with albuminous matter passes into the intestines. It is true that the pancreatic juice acts upon proteids, but this action is slow unless the proteid has been previously converted into a parapeptone by the action of the gastric juice. If a lump of butter be swallowed, a disagreeable sensation and probably nausea will be produced; while if the same amount of butter be spread upon bread, the whole may be eaten and relished. Children, who refuse fat meat will frequently consume a quantity of butter, containing several times as much fat as the meat refused. Fats constitute a very important and necessary part of our food, and if prepared properly never interfere with healthy digestion.

Some albuminous articles of food are digested more readily than others. Generally the rapidity with which proteids are digested in the stomach is in direct proportion to the comparative extent of surface exposed directly to the action of the juice. Muscular fibre is dissolved much more rapidly than an equal weight of hard-boiled egg; because, the first readily separates into parts and is permeated by the juice, while the second is acted upon only from the outside. Again, unboiled albumen forms a clot, when taken into the stomach, and is dissolved with more difficulty than albumen which has been coagulated by heat. But if the raw white of the egg be shaken well with air, the bubbles of gas prevent the formation of a dense clot and thus render the albumen more susceptible to the action of the digestive fluid. (Foster.)

The question is frequently asked, if the gastric juice dissolves albuminous food, why are not the walls of the stomach digested by their own secretion? This does occur, sometimes, after death. It has already been stated that the acidity of the gastric juice is normally due to hydrochloric acid. This acid is supposed to be

obtained from the sodic chloride of our food. Under the influence of the peptic glands a chemical reaction between sodic chloride and water takes place, whereby free hydrochloric acid and sodic hydrate are formed. The sodic hydrate permeates the walls of the stomach, prevents their digestion by imparting an alkaline reaction, is taken into the blood and unites with carbonic acid forming a carbonate. This carbonate is supposed to be carried to the liver and there to enter into new combinations, whereby the base for glycocholate and taurocholate of soda is furnished. This theory, which is especially insisted upon by Thudicum, may account for *one* of the causes which prevent the digestion of the walls of the stomach; but there are other and equally important conditions which must be considered. In the first place, the fact that the walls of the stomach are permeated by blood-vessels containing an alkaline fluid must be recognized as a prevention of digestion of the organ itself. In the second place, it has already been stated that the secretion of the pyloric extremity of the stomach is alkaline. Moreover, this secretion is constantly being poured out, while the production of gastric juice is not continuous. Consequently, for the greater part of the twenty-four hours, the reaction of the mucous membrane of the stomach is neutral or alkaline. It is true that several observers have reported that the mucus of the dog's stomach examined through a fistula is constantly acid, even after a fast of many days duration. Others have found the mucus neutral or alkaline in healthy dogs during the intervals of digestion. My experience belongs to the latter class and I have never obtained an acid reaction in the empty stomach, after the wound made for the establishment of the fistula had completely healed. However, in the few cases, where an accidental fistula in man has afforded an opportunity of investigation, the mucus of the stomach has invariably been found neutral or alkaline when digestion was not going on. Again, the greater specific gravity of the pyloric secretion, together with its viscosity may aid in protecting the walls. Indeed it has been ascertained by testing the reaction at different depths in the long glands of birds, that the acidity is confined to the surface.

The food which has been fitted for absorption by the action of

the saliva and the gastric juice, is, in part at least, absorbed directly from the stomach. The chyme, as it passes through the pylorus, is rich in parapeptones, but contains little or no peptones. The latter, together with glucose, has been taken up by the capillaries, and thence into the gastric veins.

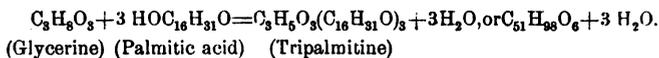
Almost immediately after leaving the stomach, the food is acted upon by the pancreatic juice. This digestive fluid produces changes in the starchy, albuminous and fatty food. Starch is quickly and completely changed into glucose by the action of the pancreatic juice. Thus, the process, began in the mouth, is completed in the intestines and in a healthy condition, all the starchy food is now fitted for absorption. The parapeptones prepared in the stomach, are farther changed in the intestines into leucine, tyrosine, asparagic acid, and glutamic acid. Leucine is amido-caproic acid, has the formula, $\text{NH}_2, \text{C}_6\text{H}_{10}, \text{CO}_2\text{H}$, and is related to a true fat. Thus, we see that before the food has been taken into the system, the complex albumen yields leucine which is known to be a link in the chain of retrograde metamorphosis. Already, the chemical changes of the body have brought a part of the food from the condition of the highly complex albuminous molecule to that of the fatty: from its high position in the organic world, one step nearer the confines of inorganic nature. Tyrosine belongs to the group of aromatic bodies, bears a close relation to benzoic acid and has the formula, $\text{C}_9\text{H}_{11}\text{NO}_3$. Glutamic acid has the composition represented by the formula, $\text{N H}_2, \text{C}_3\text{H}_5 (\text{C O}_2\text{H})_2$; while asparagic acid is known as $\text{N H}_2, \text{C}_2\text{H}_3 (\text{C O}_2\text{H})_2$. The latter is easily obtained by boiling asparagus with alkalis.

Both, glutamic acid and asparagic acid may be prepared by digesting plant-fibrine with dilute acids. All of the albumen of the food is now fitted for absorption: the first change in the proteids, taking place in the stomach, where peptones and parapeptones were formed. The peptones were absorbed from the walls of the stomach: the parapeptones as such could not be absorbed and consequently passed into the intestines. Here by the action of the pancreatic juice, the parapeptones are farther changed as has been stated above.

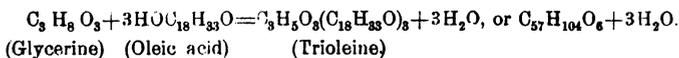
If albumen be directly digested with the pancreatic juice, pep-

tones, leucine, tyrosine, glutamic acid and asparagic acid are produced. The albumen is first broken up into peptones and parapeptones, and the latter is instantly decomposed, a change which could not have been produced by the action of gastric juice. However, there is a difference between the peptones formed by the gastric juice and those produced by the action of the pancreatic secretion. In the former instance, the peptones are acid-albumins; while in the latter they are alkali-albumins. They are, however, physiologically identical, since both are absorbable. Consequently, if a small amount of albuminous food escapes digestion in the stomach, it is acted upon by the pancreatic juice.

The pancreatic juice emulsifies fats and splits up neutral fats into glycerine and fatty acids. Thus, palmitine, or more properly tripalmitine, which exists as a fat in our food, consists of palmitic acid combined with glycerine. Glycerine is represented by the formula, $C_3 H_8 O_3$. When an acid combines with glycerine, the former replaces one or more of the atoms of hydrogen in the latter. There are three of these atoms replacable and consequently the formation of a neutral compound requires the substitution of an acid for the three atoms of hydrogen. Palmitic acid is monobasic; therefore, three parts of this acid are required in order to combine with the glycerine, and thus form the neutral fat, tripalmitine:



Oleine or trioleine, another fat of our food and a constituent of olive and other oils, consists of oleic acid combined with glycerine:



Tristearine has a composition similar to the two fats mentioned above. Now, the pancreatic juice has the power of decomposing these neutral fats into their constituents, glycerine and fatty acids. The fatty acids, thus freed, combine in part with alkalis forming soaps and in this condition are absorbed. Other portions of the fatty acids are emulsified; the formation of the emulsion being hastened by the presence of phosphate of sodium, which is con-

tained in the food, or is furnished by the bile. If palmitic or stearic acid be boiled with phosphate of sodium, a fine emulsion is formed; while if neutral fats be substituted for the fatty acids, the salt of sodium fails to produce any effect. Thudicum thinks that the chief effect that the bile has upon the absorption of fats, is due to the presence of phosphate of sodium in the biliary secretion.

In some instances, the pancreas becomes diseased and fails to perform its function. When this is the case, much of the fat of the food is excreted unchanged with the faeces; nutrition is necessarily imperfect; the patient becomes very anaemic, and often, especially in the latter stage of the disease, is unable to retain any food. In one case of this kind, I found that the pancreas had undergone fatty and calcareous degeneration. In this case, but little food could be retained for six weeks previous to death. Even a drink of ice water caused nausea and vomiting and food was administered per rectum. The vomited matter had a peculiar coffee ground appearance, which caused some physicians to diagnose cancer of the stomach, an error revealed by the post mortem examination.

+ Bile, by virtue of its alkalinity, aids the pancreatic juice in the action of the latter upon food. As the food passes through the pylorus, the gall-bladder contracts and the bile rushes into the intestine. This is probably due to reflex action and the flow of bile can be produced by irritating the pyloric orifice with any acid solution, but not with alkalis. The bile assists in destroying the acidity of the food; precipitates the parapeptones and carries down mechanically with this precipitate any pepsine that may have passed from the stomach with the food. Bile furnishes bases which unite with the fatty acids forming soaps. Evidently, bile aids the absorption of fats; thus fats pass more readily through animal membranes, or filter papers which have been moistened with bile, than through membranes or papers moistened with water. Assisted by one of my former students, Mr. Worden, I made quite a number of experiments upon the effects of bile upon the absorption of fats from the intestines. Animals, cats and dogs, were put under the influence of chloro-

form, the walls of the abdomen were opened and loops of the intestines ligated, care being taken not to separate the intestine from its attachments. The first object was to find from what part of the small intestines, fats were absorbed with the most readiness. For this purpose, a section of two inches of intestines near the pyloric extremity of the stomach was ligated at each end, a second and similar section in the lower part of the duodenum, and still a third, near the termination of the small intestines, were ligated in the same manner. In all cases, the section was freed from its contents by gentle pressure. Into each of these knuckles of intestine, were injected equal volumes of cod liver oil. After from four to six hours, each section was removed and the oil remaining in it was estimated. It invariably occurred that the section from the lower part of the duodenum contained less oil after removal, than either of the other sections.

In the second place, contiguous sections at the lower part of the duodenum were ligated as above. Into one of these, oil containing bile, and into the other oil freed from bile were injected. Upon removal of the sections, and estimation of the remaining oil as above, it was always found that the presence of bile had favored absorption of the oil. However, the proportion of bile to the oil, most suitable for absorption seemed to be limited; thus the addition of an undue amount of bile to oil did not hasten the passage of the latter through the intestinal walls.

In some animals, at least, bile has the power of converting starch into sugar; but this action is so slight and the same property is possessed in a similar degree by so many animal fluids which are in no way concerned in digestion, that it may be considered as of little importance.

Of the action of the intestinal juice upon food, but little is positively known. From the best evidence which we have upon the subject, it seems that this fluid acts upon proteids in the same manner as the pancreatic juice does. That there are certain fermentative changes going on in the intestines and due to the presence of bacteria is probable.

ANALYSIS OF SALIVA.

MICROSCOPICAL EXAMINATION.

Examine a drop of saliva under the microscope. Observe mucous corpuscles and pavement epithelium, and, if the mouth has not been kept clean, particles of food, cryptogamic sporules, and sometimes vibriones.

METHODS OF OBTAINING MIXED SALIVA.

A quantity of saliva, sufficient for analysis, may be obtained by artificially stimulating the glands. This may be done in either of the following ways :

1. By attempting to chew a glass stopper.
2. By depressing the lower jaw and tickling the fauces with a feather.
3. Fill the mouth with vapor of ether, carry it back into the pharynx and retain it for some time.
4. Touch the end of the tongue with a crystal of citric or tartaric acid, or with one of carbonate of soda.
5. Exert a strong pressure under the chin, and at the same time tickle the palate with a feather.

General Properties.—The mixed saliva is turbid, bluish-white, and devoid of taste and odor. The normal reaction is alkaline, and during mastication, the alkalinity is increased ; but while fasting, it again gradually decreases until just before the next meal, when it may be neutral, or even faintly acid. In some cases of diseased saliva, especially when the flow is scanty, or when the person suffers from dyspepsia (Foster) the reaction is constantly acid. The specific gravity varies from 1002 to 1009, the usual variation being between 1004 and 1006. Upon standing for some time, saliva forms a grayish-white deposit, which by examination with the microscope, will be found to consist of leucocytes and pavement epithelium. Owing to the mucine which it contains as a normal ingredient, saliva is somewhat viscid, and can be drawn out into threads after having been stirred briskly for a few moments with a glass rod. No other animal fluid decomposes more readily than this, consequently it is necessary that all specimens for examination in physiological research, or

for diagnostic purposes, should be perfectly fresh. A disregard of this fact caused Wright (Lancet, 1842,) to ascribe to saliva a sharp, saline, and slightly astringent taste, and the property of poisoning vegetable and animal organisms. (Lehmann.)

QUALITATIVE ANALYSIS OF INORGANIC CONSTITUENTS.

In order to obtain tests for the inorganic constituents the saliva must be filtered; but as it decomposes so rapidly and filters so slowly, some caution is necessary. Everything used must be perfectly clean, for the precipitates will be so slight that they may not be seen; or, what is more likely to occur, one may think that he has a sufficient test for some base or acid, when the turbidity is solely due to the test tube not being clean, or the filter paper containing some impurity. Before passing the saliva through the filter, it would be well to pass several ounces of water through and then test the water for each of the constituents soon to be given. Of course, in this case, all the results should be negative. Having tested the filter paper in this way, the saliva is mixed with about three times its bulk of boiling distilled water, and filtered. The filtrate may now be tested as follows:

For Chlorides.—Acidify a part of the filtrate strongly with nitric acid, and add a few drops of nitrate of silver: the appearance of a white precipitate insoluble in acids but soluble in ammonia indicates the presence of hydrochloric acid.

For Sulphates.—Acidify a part of the filtrate with hydrochloric acid, and add a few drops of chloride of barium: the insoluble sulphate of barium will appear.

For Phosphates.—To some of the filtrate add a few drops of acetate of soda, and then some uranic acetate; a yellowish white precipitate, insoluble in acetic, but soluble in hydrochloric acid, shows that phosphoric acid is present, and has been precipitated as phosphate of uranium.

For Calcium.—Calcium will be precipitated as an oxalate upon the addition of oxalate of ammonia.

For Magnesium.—This will appear as an ammonio-magnesian phosphate, upon adding to the clear filtrate some ammonia, chloride of ammonia and disodic hydric phosphate.

For Sulphocyanic Acid.—This acid is not always present. It is derived from the parotid gland, and is not always found in the secretions of the other glands. It should be tested for by distilling 300 c. c. of saliva, rendered acid with dilute sulphuric acid; neutralizing the concentrated distillate with sodic hydrate and adding a drop of dilute ferric chloride, when if sulphocyanic acid be present, a blood-red color will be produced.

This acid may also be obtained by the following method:

Evaporate the saliva to dryness on the water bath, treat the residue with alcohol and filter, evaporate this filtrate, dissolve the residue thus obtained in a little water, and test this solution with ferric chloride, as in the preceding method.

In some cases the blood-red color may be obtained upon the addition of ferric chloride directly to the saliva. If the reaction fails when the test is applied in this way, it is no proof that sulphocyanogen is wholly absent; while on the other hand, if the red color is produced by the direct application of the ferric salt, it must be remembered that this alone is not positive proof of the presence of sulphocyanide; for the perchloride of iron produces the same color with meconic acid, which may be present in the patient's mouth from opium. Consequently a farther test is necessary, and any doubt may be removed by the addition to the colored solution of a little mercuric chloride, when, if the color had been produced by a sulphocyanide, the solution will become colorless; while, if meconic acid be present, the mercuric chloride will cause no visible change. Ferric salts also strike a red color with strong acetic acid, with a decoction of mustard, and with an infusion of Iceland moss; but these are never present in quantities sufficient to give the reaction, and even if this were possible, they would be recognized by their other properties.

For Sodium and Potassium.—Evaporate a small dish full of saliva to dryness on the water-bath. Place some of the residue thus obtained on a platinum wire and heat it in the colorless flame of a Bunsen burner. The flame as seen through a blue glass, presents the violet color characteristic of potassium; while without the glass the flame is seen to be of a yellow color, due to the presence of sodium. These bases are, in part, com-

bined with the acids, already referred to, and partly with organic substances. The latter combination is feeble, and the organic substances are freed directly upon the addition of any inorganic acid; as for instance, it will be seen under ptyaline that on the addition of phosphoric acid, this substance is set free, and falls with other precipitated matter.

Determination of the amount of Water and Solids.—

Place a small crucible with its cover in an air-bath or box water-bath and keep at 100° C. for half an hour. Remove the crucible to a dessicator, which contains a dish of sulphuric acid, and after the crucible has cooled, weigh it. Again put the crucible in the bath and keep at 100° for another half hour, cool in the dessicator and weigh as before. This must be repeated if necessary, until the weight is constant. Then fill the crucible two-thirds full of saliva and weigh again. The difference between the weight of the crucible containing the saliva and that of the empty crucible, will be the weight of the saliva. Place the crucible containing the saliva, after being weighed, in the bath, and keep at 100° until all the water has been driven off. Cool in the dessicator and weigh. Repeat the heating, cooling, and weighing, until the weight remains constant. The difference between the weight of the crucible containing the saliva and that of the crucible with the residue will be the weight of water in the saliva taken: From this the per cent. of water must be calculated. The difference between the weight of the crucible with the residue and that of the empty crucible, will be the amount of solids in the saliva taken, and from this the per cent. of solids may be obtained.

Place the crucible with the residue over a Bunsen burner and keep at a red heat for half an hour, cool in the dessicator, weigh and repeat this operation until the crucible ceases to lose any weight. By the continued application of heat the organic constituents of the total residue have been driven off, and only the inorganic matter is left. The difference between the weight of the crucible with the total residue and that of the crucible with the inorganic residue is the weight of organic matter in the saliva taken; while by subtracting the weight of the empty crucible

from that of the crucible with the inorganic residue, the weight of the latter is obtained. From these results the per cent. of organic and inorganic solids should be calculated.

ORGANIC CONSTITUENTS. 

The principle organic constituents are albumen, mucine, and ptyaline.

Albumen.—If saliva be strongly acidified with nitric acid, it becomes more turbid. If it then be boiled, the coagulum takes a yellow color and is not dissipated, thus showing the presence of albumen. A confirmatory test may be obtained by adding to a second portion of saliva a mixture of acetic acid and ferrocyanide of potassium when a white precipitate is produced.

Mucine.—The tenacity of saliva is due to mucine. To some saliva in a small beaker add gradually acetic acid, stirring with a glass rod; the mucine separates in white stringy flakes.

Ptyaline.—Collect 600 c. c. of saliva, acidify it strongly with phosphoric acid, then add milk of lime till the mixture is faintly alkaline, and filter. The ptyaline is now on the filter paper, but contains many impurities. Remove the filter paper with its contents to a clean beaker, and add distilled water not exceeding in quantity the saliva originally employed; stir well and filter again. The ptyaline is now in the filtrate, and may be precipitated by the addition of absolute alcohol, and dried over sulphuric acid.

Ptyaline from the Salivary Glands.—As ptyaline exists already prepared in the salivary glands, it may be obtained from these more easily and in greater quantity than from the saliva. Cut the salivary glands of any animal into very small pieces, place these in a flask, and cover with absolute alcohol. Cork the mouth of the flask and let it stand for twenty-four hours. Pour off the alcohol and press the remainder in a cloth, in order to remove as much of the alcoholic extract as possible. The cake thus obtained is placed in a beaker, covered with glycerine, and allowed to remain for several days, being thoroughly stirred occasionally. It is then strained through a cloth and afterwards

through paper. From this filtrate, ptyaline is precipitated by absolute alcohol.

Amyolytic Action of Saliva.—To some filtered saliva, add a few drops of Fehling's solution (or some dilute solution of sulphate of copper and then an excess of potassic or sodic hydrate.) A blue precipitate is thrown down and on boiling the solution takes a pale rose color from the action of the copper solution on the albumen, but the copper is not reduced. This shows that sugar and other substances which reduce copper are not present in normal saliva. Now boil one gramme of starch in one litre of distilled water, and filter. To some of the filtrate in a test tube add some Fehling solution. A blue precipitate falls, and on boiling, the solution becomes black. Again the copper is not reduced. Now to some filtered saliva add twice as much of the starch solution and place the mixture on the water-bath and keep at about 40° C. for some minutes; then to some of this mixture add Fehling's solution and boil. A yellow or yellowish-red precipitate of the suboxide of copper appears. The starch has been converted by the action of the saliva to sugar, which reduces the copper. If the saliva, before being mixed with the starch, is heated to 60° or higher, it loses permanently its power of converting starch into sugar. The amyolytic action of saliva is arrested by freezing or by the addition of strong acid, but is regained by raising the temperature and by neutralizing the acid. Caustic potash and soda destroy the action of saliva on starch, and in this case it is not renewed by neutralization. The carbonates of these alkalis arrest the diastatic power which is restored upon carefully adding an acid until the neutral point is reached. At ordinary temperature starch is converted into sugar by saliva, but the change goes on most rapidly at about 40° C. Potato starch is converted more quickly than corn starch. Instead of saliva, a solution of ptyaline, prepared according to either of the methods already given, may be used in all the cases referred to.

The student should test experimentally all of the above assertions with regard to the action of saliva on starch.

ABNORMAL SALIVA.

Iodine and Bromine.—The saliva may be filtered as directed

and the filtrate tested for iodine and bromine, with chlorine and bisulphide of carbon. I have detected iodine in the saliva within five minutes after the administration of a ten grain dose of the iodide of potassium. Besides iodides and bromides many other medicinal substances appear in the saliva.

Mercury.—Slightly acidify the filtrate, and place in it a strip of clean copper; metallic mercury will be deposited upon the copper. During salivation from mercury, sulphocyanogen disappears from the saliva.

Urea.—Evaporate the filtrate almost to dryness, then add nitric acid equal in bulk to the part left. Set aside in a cool place for five minutes and at the expiration of this time examine under the microscope. Flat rhomboidal crystals of nitrate of urea will appear. Urea appears in the saliva only after the kidneys have ceased to perform their duties.

Urates.—Evaporate some of the filtrate to one-half its bulk, then acidify with nitric acid, only using enough to make strongly acid, and after five minutes examine under the microscope for crystals of uric acid; also apply murexide test. (See under Urates in Urine.)

Pus.—May be detected under the microscope, the corpuscles are identical with the white corpuscles of blood, and, upon the addition of acetic acid, present from one to three, generally three, nuclei. The albumen will also be increased in saliva containing pus.

Excess of Chlorides.—An excess of chlorides will be indicated by the bulk of the precipitate obtained with nitrate of silver.

Excess of Phosphates.—Will be shown by bulk of precipitate with uranic acetate. The phosphates are often deposited upon the teeth.

Excess of Carbonates.—Shown by brisk effervescence with acetic acid. The carbonates, when the saliva is being poured into the mouth, are dissolved in an excess of carbonic acid, which escapes, and the carbonates are then deposited upon the teeth, forming tartar.

Bile.—Saliva containing much bile, takes a dull, yellowish color, gradually deepening into a faint olive color, upon the addi-

tion of nitric or hydrochloric acid. The tests for bile-acids and bile-pigments should also be made. (See under Bile.) Dr. Fenwick (London Lancet, September 1, 1877,) calls attention to the examination of the saliva for bile in "billiousness." The patient complains of his liver being out of order, and of a bitter taste in his mouth on rising in the morning. His skin and conjunctivæ are not yellow, and the examination of the urine fails to reveal the presence of bile. But if an ounce of the saliva be evaporated to dryness on the water-bath, a yellow or reddish-brown residue is left, which is soluble in chloroform and gives the reaction of bile-pigment. Upon examination, the back part of the patient's tongue on rising in the morning will also be found colored yellow or reddish-brown. During the night the heat of the mouth evaporates the saliva (which in waking hours is swallowed) and affords the same indication of bile as is obtained by evaporation on the bath. A smaller quantity of bile can be detected in the saliva than in the urine, because the normal coloring matter of the latter interferes with the test. The physician who fails to make use of this means of diagnosis will often fail to recognize the hepatic derangement. In some cases of "billiousness," bile acid can be detected by evaporating two ounces of the saliva to dryness on the water-bath, treating the residue with boiling absolute alcohol, filtering, evaporating the filtrate to dryness, redissolving in water and applying to this solution Pettenkoffer's test. But in the majority of instances, the quantity of bile-acids is too small to afford the test, and the physician must rely upon the colored residue and the bitter taste, which are sufficient proof of the presence of the hepatic secretion in the blood.

Blood.—The presence of blood in the saliva is shown by the color, which varies from red to black; also by the appearance of corpuscles under the microscope. It must be observed whether the blood comes from the cavity of the mouth or whether it is poured out with the saliva. This can be ascertained by carefully inspecting the parts.

Tyrosine and Leucine.—Concentrate the saliva without filtering, and examine under the microscope. Tyrosine will be found in

needle-shaped crystals, which are not soluble in acetic acid, and in this way are to be distinguished from phosphate of lime. Leucine appears in globules resembling oil in appearance, but insoluble in ether. Tyrosine and leucine have been found in the saliva of hysterical persons.

Milky Saliva.—Opaque, curdy, and acetic acid increases the coagula.

Sugar.—Ferments with yeast, alcohol and carbonic acid being formed. Also reduces the copper solution as given under urine.

Excess of Fat—Evaporate some saliva to dryness on the water-bath, extract with ether and judge of the quantity of fat under the microscope.

Acid Saliva.—Indicated by action on litmus. Acid saliva contains lactic acid. (Thudicum.) Wright holds that acidity of the saliva may accompany any of four classes of diseases: (1) idiopathic affections of the salivary glands; (2) those diseases in which there is an excess of acid in the system, as rheumatism, scrofula, phthisis, rachitis and amenorrhœa; (3) inflammatory affections of the mucous membrane of the stomach and intestines; (4) in dyspepsia. Lehmann found the saliva always acid in cancer of the stomach and in diabetes mellitus, and frequently but not invariably, acid in catarrh of the gastric and intestinal mucous membranes and in ulceration of the stomach. Frerichs states that the acid reaction is always due to the secretion of the buccal mucous membrane.

Salivary calculi are usually composed of phosphate and carbonate of lime and organic matter. I once analysed one which was two inches long and about one-quarter of an inch in diameter and weighed forty-eight grains. Upon boiling some of it with acetic acid, carbonic acid gas was given off and the disagreeable odor of putrid saliva was observed. On making a cross section of the calculus its interior was seen to be composed of layers of a chalky white substance, principally carbonate of lime. The surface was rough, and covered with a greenish deposit of organic matter. Dental calculi form in decayed teeth of men and animals. They resemble salivary calculi in composition; but con-

tain more organic matter and phosphate of lime. Dental calculi are often covered with, and form nests for vibrios.

GASTRIC JUICE.

For physiological purposes gastric juice is obtained artificially or by the establishment of fistulæ in the lower animals. For diagnostic purposes the physician must examine this secretion as contained in vomited matters. The gastric juice of man is a clear, watery fluid and contains but a small amount of solids. The secretion of the stomach of the dog has a specific gravity of about 1008, and contains from 3 to 4 per cent of solids. In some herbivorous animals gastric juice has a brownish color and contains less acid and less pepsine than that of the carnivora. Normally the acidity of this secretion is due to hydrochloric acid and acid phosphates, but in certain diseased states these may be entirely replaced by lactic, butyric and acetic acids, one or all.

The action of gastric juice upon albuminous food is due to the combined effects of hydrochloric acid and pepsine. The latter has never been obtained in a perfectly pure state, consequently its chemical formula is not known.

Nature of the Acids.—Certain cases of acute anemia are due to the abnormal condition of the gastric secretion. The same is true of gastric catarrh and certain febrile affections. The vomit of persons affected with these diseases is intensely acid, but contains neither hydrochloric acid nor pepsine. The nature of the acidity can be ascertained as follows: The vomited matter, if not sufficiently liquid, should be mixed with a little water and filtered. If the filtrate is not clear, filter again either through cloth or paper or through both. Put the filtrate into a large retort, connected with a Liebig's condenser, and distil at about 130°. If during the process of distillation a thick scum should form over the contents of the retort, the liquid should be removed, freed from the pellicle by filtration, then replaced in the retort. Continue the distillation until the retort fills with a dense white cloud. Neutralize the distillate with carbonate of sodium, evaporate it to dryness on the water-bath, extract the residue with absolute alcohol, filter the alcoholic solution, again evaporate to dryness on the

water-bath, and dissolve this residue in a little distilled water. To a small portion of this aqueous extract, in a test tube, add a few drops of a neutral solution of ferric chloride; a blood-red color which is destroyed by the subsequent addition of hydrochloric acid appears if acetic acid be present. To a second portion of the watery extract, add nitrate of silver; a white precipitate insoluble in nitric acid, soluble in ammoniac hydrate, shows that free hydrochloric acid was present in the substance under examination. To the remaining portion of the aqueous extract, add a few drops of dilute sulphuric acid, allow to stand for an hour, observing the odor from time to time. If butyric acid be present, the peculiar odor of rancid butter will be recognized.

Any lactic acid that may have been present in the matter under examination, remains in the retort. In order to ascertain the presence of this acid, shake the residue in the retort with much ether, remove the ethereal layer with a pipette and evaporate this to dryness on the water-bath. Dissolve the residue in water and boil this solution with the oxide or carbonate of zinc. Remove the excess of zinc by filtration, concentrate the filtrate on the water-bath and allow to stand, when, if lactic acid were originally present, crystals of lactate of zinc form in square prisms with one or two oblique surfaces at the ends. (Brunton.)

Lactic and butyric acids are sometimes found in large quantities, as much as five grammes of the two having been obtained. The latter is supposed to originate from the former by the liberation of CO_2 and H . It very seldom or never happens that acetic, lactic and butyric acids are all present. The process given above for detecting these acids in vomited matter, will also apply to the examination of gastric juice obtained through a fistula or the contents of the stomach after death.

Estimation of the Amount of Free Hydrochloric Acid.—The amount of free hydrochloric acid present in gastric juice, the contents of the stomach, or vomited matters, is best ascertained by the method proposed by Schmidt, and which is as follows: To a measured amount of the clear filtrate (from which insoluble substances and albumen, if present, have been removed by heat and filtration) add nitric acid and nitrate of silver. Collect the pre-

precipitated chloride of silver upon a filter and wash well with distilled water. (Reserve the united filtrate and wash-water for further examination.) Dry the precipitate on the filter in an air or steam oven. Shake the dry chloride of silver from the filter upon a piece of glazed paper. Burn the filter paper, allowing the ashes to fall into a small crucible, the weight of which has been previously ascertained. The chloride of silver which adhered to the filter now exists with the ash and as metallic silver. To the ash add a few drops of nitric acid; this dissolves the silver forming the nitrate. To this add a few drops of hydrochloric acid which again forms the chloride. Evaporate the contents of the crucible to dryness at the temperature of the water bath. To this residue add the chloride of silver which has been placed on the glazed paper. Again dry the crucible with its contents and weigh. From the weight of the whole subtract the weight of the crucible and the remainder will represent the weight of chloride of silver. Every part of chloride of silver will represent .247 parts of chlorine; from this the total amount of chlorine in the gastric juice or vomited matters taken, is calculated.

T. The filtrate from which the chlorine has been removed with nitrate of silver, is placed in a large crucible, evaporated to dryness, and the residue heated until all the organic matter is driven off. The ash is dissolved in water slightly acidified with acetic acid, and this solution, which may be diluted to any desired extent, is carefully measured and divided into five parts. It is not necessary that these five be equal parts, but the exact amount of each, and its relation to the whole must be noted.

In one of the parts the phosphoric acid is estimated volumetrically with uranic acetate as given under the quantitative examination of the urine.

In a second portion the sulphuric acid is estimated as follows: Render the solution strongly acid with hydrochloric acid and then add chloride of barium as long as a precipitate is formed. Collect the precipitated baric sulphate on a filter, wash with hot water, dry, transfer to a weighed crucible, burn the filter paper, adding the ash to the contents of the crucible, heat to a dull red

heat, cool over sulphuric acid and weigh ; one part of BaSO_4 represents .412 parts of SO_4 .

In a third portion estimate the amount of calcium and magnesium, as follows : Render the solution strongly alkaline with ammoniac hydrate, then add ammoniac oxalate. Heat the mixture gently and collect the precipitate upon a small filter. (Reserving the filtrate for the estimation of magnesium.)

Dissolve the oxalate of lime on the filter in dilute hydrochloric acid. To this solution, concentrated if necessary to a small volume, add an excess of alcohol and then dilute sulphuric acid as long as a precipitate is formed. Collect the precipitated sulphate of lime, wash with dilute alcohol, dry, transfer to a weighed crucible, burn the filter paper, heat the whole to redness, cool over sulphuric acid and weigh. Each part of CaSO_4 represents .294 parts of calcium.

Concentrate the reserved filtrate, from which the oxalate of calcium has been removed, to a small volume. Add chloride of ammonia, ammoniac hydrate and phosphate of sodium. Cover the beaker containing the mixture with a piece of glass and allow to stand for twenty-four hours. Collect the precipitate upon a small filter, wash with a mixture of one volume of ammoniac hydrate and three volumes of water. Dry the filter with its contents and transfer to a weighed crucible, burning the filter paper and adding the ash to the contents of the crucible. Heat the crucible to an intense redness. Dry over sulphuric acid and weigh. The magnesium was precipitated as a phosphate of ammonia and magnesium, which by the high heat has been converted into the pyrophosphate of magnesium, and in this form it is weighed. Each part of the pyrophosphate, $\text{Mg}_2\text{P}_2\text{O}_7$ contains .216 parts of magnesium.

In a fourth portion of the aqueous solution of the ash, estimate the amount of potassium and sodium as follows ; Treat the solution with chloride of lime as long as a precipitate is produced, then add baric hydrate until the mixture has a feebly alkaline reaction. Remove the precipitated matters by filtration ; to the filtrate add ammoniac hydrate and carbonate as long as a precipitate forms. Remove the excess of calcium and barium now

precipitated as carbonates, by filtration; wash the precipitate; evaporate the united filtrate and wash-water to dryness. Heat the residue to bright redness, and maintain this temperature for some time in order to drive off the excess of carbonate of ammonia; cool; dissolve the residue in water; filter; wash any residue, that may rest on the filter, well with water. Unite the filtrate and wash-water; concentrate, if necessary; pour into a small weighed crucible; evaporate to dryness; heat the residue; cool and weigh. This gives the combined weight of the chlorides of sodium and potassium. In order to separate these bases dissolve the weighed residue in a little water, add some dilute alcohol and then chloride of platinum as long as a precipitate forms. Cover and allow to stand for 24 hours. Then collect the precipitate on a small filter, dry at the temperature of the water-bath, and weigh. Each part of the double chloride of potassium and platinum, K_2PtCl_6 , contains .306 parts of potassic chloride, KCl . The weight of the latter subtracted from the weight of the combined chlorides already found, gives the amount of chloride of sodium. From the weights of their respective chlorides the amount of each base is calculated.

The fifth portion of the solution of the ash which has been held as a reserve in case any accident should happen during the examination of one or more of the other portions, is now, if the above estimations have been satisfactory, discarded.

The amount of each base and acid contained in certain measured portions of gastric juice, or extract of the contents of the stomach or vomited matters, is now known. From these figures the amount of each base and acid in the *same* quantity (100 c. c.) of the fluid is calculated. From the equivalence of each acid and base, the amount of the various salts are calculated, observing the following rules: 1. The sulphuric acid is to be considered as combined with potassium, forming K_2SO_4 ; and any excess of this acid over the base is supposed to combine with sodium forming Na_2SO_4 . 2. The phosphoric acid is to be regarded as forming acid phosphates, RH_2PO_4 . In this formula, R represents calcium, magnesium, or sodium, one or all, in the order given. 3. Any remaining bases which may not have been taken up by the sul-

phuric and phosphoric acids are supposed to have existed as chlorides. 4. Any excess of hydrochloric acid remaining, existed originally as free acid.

The above method obviates the possibility of hydrochloric acid being set free by the action of lactic acid upon the chlorides of lime and magnesium, this having been urged by some as the source of the free acid; and which may possibly happen if the old method of obtaining the acid by distillation be employed.

Artificial Gastric Juice.—Take the stomach of a recently killed animal, dog, pig, or fourth stomach of a calf; open and spread upon a board with the mucous side upward. Wash this with a gentle stream of water; then scrape off all the mucus; rub this up in a mortar with powdered glass and water; allow to stand for two hours; filter and dilute the filtrate with an equal bulk of a 0.2 per cent. solution of HCl. This will digest fibrine rapidly and may be kept in closed bottles for a long time.

Remove the mucous membrane from the stomach of a pig, wash with water and cut into fine pieces. Cover these with dilute hydrochloric acid (made by adding 4 c. c. of hydrochloric acid to one litre of water); allow to stand for four hours, stirring the mixture frequently; filter off the fluid and extract the residue with another portion of the dilute acid. Repeat this process as long as the filtrate acts upon fibrine, as given below. In this way, from one to six litres of an active extract may be obtained from the stomach of one pig. The juice thus obtained acts very energetically but contains such quantities of peptones that it decomposes in a few days.

Action of Gastric Juice.—This is best shown upon fibrine from blood. Stir some fresh blood with a rough stick, a bundle of glass rods, or a piece of whale bone; collect the fibrine which has been coagulated, and wash it until it is perfectly white. Put a small piece of this fibrine into a test tube with some gastric juice, made as above, or obtained through a fistula, or by means of a stomach pump or gastric syphon, and keep on the water-bath at 35° C. The fibrine will swell and soon dissolve.

Extraction of Pepsine.—Cut open a stomach and wash as above, remove the pyloric part, and dissect off the remainder of

the mucous membrane. Cut this into small pieces, put into a beaker and cover with glycerine, allow to stand for two days; then strain off the glycerine. This will be found to have taken up the pepsine and gastric juice may be formed by adding to a little of the glycerine a 0.2 per cent. solution of HCl. That pepsine alone will not digest fibrine may be proven by diluting some of the glycerine extract with water and adding a piece of fibrine and keeping on the water-bath at 35° C.; the fibrine will not be dissolved. That the dilute HCl will not by itself digest the fibrine should also be proven. But as soon as the pepsine and dilute acid are mixed and the experiment tried, it is at once successful. These three experiments should be made at the same time, in as many test tubes.

Precipitation of Pepsine.—Von Wittich first introduced the following method of removing pepsine from the mucus of the stomach: Wash the mucous membrane gently with water, cut into fine pieces and cover with alcohol. Allow the pieces to remain in the alcohol until they partially harden; then pour off the alcohol; dry the pieces of membrane by pressure between folds of blotting paper; pulverize them; cover with glycerine and allow to stand from one to two weeks. Filter the glycerine and add to the filtrate a large excess of absolute alcohol, when a flocculent precipitate containing impure pepsine falls. Filter and dissolve the residue on the filter with dilute hydrochloric acid (made by adding 20 c. c. of hydrochloric acid to 980 c. c. of distilled water). This solution contains no albuminous substances; and digests fibrine rapidly.

Pepsine, as prepared above, is a dirty white powder, soluble in water, glycerine and dilute acids, insoluble in alcohol and ether. The aqueous solution of pepsine does not pass through animal membranes, is not precipitated by nitric acid, or by acetic acid and potassic ferro-cyanide, but is precipitated by the acetate of lead. The aqueous solution of pepsine is, when it contains no free acid, without action upon fibrine, but when fibrine is added to such a solution, a part of the pepsine is taken up and held mechanically by the fibrine. From this combination pepsine cannot be removed by water nor glycerine, but it is readily set

free by a two per cent. solution of hydrochloric acid. Pepsine, in dilute hydrochloric, nitric or lactic acid, digests fibrine. This reaction goes on most rapidly at a temperature of about 40° . Dry pepsine can be heated to 100° without decomposition, and after having been subjected to that temperature, may be dissolved, and be found to digest albumen; but if a solution of pepsine be heated to 100° , the ferment is decomposed and does not regain its original properties on cooling. At the freezing point, the gastric juice of mammals is inert. If bile be added to the gastric secretion, the pepsine of the latter is carried down mechanically with the precipitate formed by the action of glycolic acid upon the products of digestion. Moreover the alkalinity of the bile is sufficient to arrest gastric digestion.

Digestion a Chemical Process.—Digest some coagulated albumen in gastric juice and test the solution for albumen with nitric acid, or with acetic acid and ferrocyanide of potassium; no precipitate occurs, proving that the process has not been one of simple solution, but one of chemical change. The products of stomachic digestion are in part at least albuminous, and consequently their molecular composition is very complex. The albuminous nature of the contents of the solution may be shown by boiling them with sodic hydrate and a few drops of a dilute solution of sulphate of copper, when a violet color, changing to blue on the addition of more of the copper solution, appears; this reaction is characteristic of albuminous substances. The division proposed by Meissner of the products of gastric digestion into peptones, parapeptones, metapeptones and dyspeptones is not founded upon facts; since it is now well-known that the substances which Meissner analyzed, and to which he gave these names, were not chemically pure. However, there are certain chemical and physiological differences which enable us to divide these products into two classes. The substances of one of these classes are capable of being absorbed, while those of the second class will not pass through animal membranes. Moreover, the latter cannot be fitted for absorption by the action of the gastric juice. To represent these two classes, I have, in speaking of digestion, used the words *peptones* and *parapeptones*;

Kühne has proposed the terms *antipeptones* and *hemipeptones*.

For diagnostic purposes, the physician often desires to know whether the gastric juice, as obtained from vomited matter is capable of performing its physiological duties; this may be done as follows: The substances under examination, if not sufficiently liquid, are stirred with water and filtered. To a portion of the clear filtrate, a piece of fibrine prepared from blood and well washed, is added, and the whole is kept at about 40° in an air-bath for twelve hours. If at the expiration of this time, the fibrine has not been perceptibly dissolved, or if putrefaction, as manifested by the odor, has begun, the gastric juice contained in the vomited matters is inert. This want of activity may be due to the absence or paucity of either the pepsine or free normal acid.

To another portion of the clear filtrate add an equal volume of a one-tenth per cent. solution of hydrochloric acid. To this add a small piece of fibrine and treat as above. If now the digestive action proceeds normally, the physician recognizes the fact that the indigestion of his patient is due to an insufficient supply of the normal acid; while if the fibrine remains insoluble the pepsine is deficient.

If the piece of fibrine be dried and weighed before being added to the solution and the fibrine remaining at the expiration of the twelve hours be also dried and weighed, the exact degree of action may be ascertained.

Grünhagen has introduced the following method of approximately estimating the amount of pepsine: Some washed fibrine is covered with a two per cent. solution of hydrochloric acid and allowed to stand at the ordinary temperature for an hour or two. The jelly-like mass of fibrine is then freed from the dilute acid by pressure and the solid cake is placed on a filter. The funnel supporting the filter is set in a beaker which is placed in an air-bath with the temperature at 40°. The fluid to be tested is now poured upon the fibrine and the amount of pepsine is estimated from the rapidity with which the fibrine is dissolved and the solution passes through the filter.

In uremia, vomited matters often contain urea, carbonate of

ammonia and bile. The urea is detected by concentrating the filtrate on the water-bath to a small volume and then adding nitric acid, when on standing crystals of nitrate of urea form. Bile-acids and bile-pigment are detected respectively by Pettenkoffer's and Gmelin's tests. (See under Bile.)

The vomit of persons suffering with cholera is alkaline in reaction, is turbid from the presence of mucus and epithelium, contains albumen, and frequently urea.

Vomit produced mechanically from an empty stomach is neutral or alkaline, and consists principally of mucus. The same condition has been observed in the vomit of hysterical persons and of those addicted to the excessive use of alcoholic drinks.

In hæmatemesis, the blood, unless excessive in quantity, is disintegrated by the action of the acid of the stomach, the hæmoglobin being converted into hæmatin. This may be dissolved in an alkali and examined with the spectroscope.

7 ANALYSIS OF BILE.

Bile may be obtained for analysis after the death of the animal, or during life by means of a biliary fistula. Fresh bile can be procured also by putting an animal under the influence of chloroform, laying open the abdomen and drawing the bile from the gall-bladder with a trochar, aspirator, or hypodermic syringe. It is very seldom that an opportunity is presented of making an analysis of the bile of a living man. Consequently an examination of this secretion is not undertaken as an aid to treatment in the individual case. But we are prompted in this work by the following considerations: (1) We hope to understand more fully its chemical composition and physiological action; (2) an obscure case which has terminated fatally may present an abnormal condition of this secretion. Thus, by studying the dead, we may be better prepared to protect the living.

In man, and most other omnivorous and carnivorous animals, bile is of a yellow, reddish-yellow, or brownish-yellow color; the principal coloring ingredient being bilirubin. In the herbivora, the bile has a green or brown color, which is due to the presence of biliverdin. As taken from the gall-bladder, this secretion contains more or less mucus, and is neutral or feebly alkaline in

reaction. I once found that the bile taken from a patient who died of peritonitis from cancer of the rectum and colon, was strongly acid. In order to ascertain the reaction, the bile should be diluted with distilled water, filtered and tested with litmus paper. The inorganic constituents of bile are sodic chloride, calcic phosphate, sodic phosphate, oxide of iron, and traces of copper. The organic are mucine, cholesterine, lecithine, oil, bile-pigment, and bile-acids. In the bile of man, glycocholic is the principal acid; while in the cat, taurocholic acid predominates; and in birds and hogs hyoglycocholic acid is present. In all cases these acids are combined with sodium or potassium.

Crystallized Bile-Acids.—Place the bile in an evaporating dish on the water-bath, and concentrate to one-sixth its volume. To the residuum, add absolute alcohol, stir and filter through animal charcoal. Repeat this operation with several successive portions of alcohol. The alcoholic filtrate (which, if not perfectly colorless, should be again filtered through animal charcoal) is concentrated on the water-bath to one-quarter its volume and poured into a clean flask. To this add ether in great excess. The ether precipitates the taurocholate and glycocholate of soda. The former of these only is present, if the bile used was that of the cat or dog. Place the flask containing the suspended precipitate in a freezing mixture and allow to stand for twenty-four hours. At the expiration of this time, (it will frequently require a longer time than twenty-four hours for crystallization) the precipitate will have formed in needle-shaped crystals. Decant the mixture of alcohol and ether. Wash the crystals by decantation with pure ether; dissolve them in water and use as a solution of purified bile.

Pettenkoffer's Test for Bile-Acids.—To a dilute solution of purified bile in a test tube add a drop of a solution of cane sugar and then sulphuric acid drop by drop; keeping the temperature as near 70° C. as possible by placing the tube in cold water if too warm, or by gently heating it if too cool. If bile-acids be present in the proportion of one part of the acids to five hundred of water, or in greater quantity, a white precipitate will appear upon the addition of the first drop of sulphuric acid.

The addition of the acid must be continued until this precipitate is dissolved. The solution now takes a cherry-red color, becoming purple on standing. If only minute quantities of the bile-acids be present, the white precipitate does not appear, but the solution becomes colored as before. This solution generally has a characteristic purple foam, but if no foam exists, as is sometimes the case, it can be obtained by the addition of a few drops of carbonate of soda to the cherry-red solution. When this is done the froth produced by the liberated gas has a purple hue, which instantly disappears. The student should ascertain the limit of this test and the various shades of color according to the amount of bile present, by diluting his solution of purified bile with definite proportions of water. This test fails to reveal bile acids in the presence of alcohol, and oxidizing substances as nitrates and chlorates.

Dilute some crude bile with an equal volume of water, filter and apply Pettenkoffer's test. To some urine in one test tube and to some saliva in another, add bile, filter and test each as above.

To some urine add bile, filter and apply the following modification of Pettenkoffer's test. Render the urine alkaline, with ammonia, then add acetate of lead as long as a precipitate is formed; filter; dry the precipitate at 100° C.; remove it to a dish, add absolute alcohol and boil. Again filter and evaporate the alcoholic filtrate to dryness on the water-bath. Dissolve the residue in water, rendered slightly alkaline with sodic hydrate. To this solution apply the sugar and sulphuric acid as given above.

GLYCOCHOLIC ACID— $C_{26}H_{43}NO_6$.

This substance exists in the bile of man and that of the ox. It is not present in the bile of the dog whether that animal be fed upon animal, vegetable, or mixed food. It is found in small quantities in the urine of jaundiced persons. Traces of this acid can generally be found in the fæces of oxen.

Preparation. (1) To a solution of purified bile, add dilute sulphuric acid until a cloudiness appears and remains on being stirred or shaken. Care must be taken not to add enough sulphuric acid to redissolve this precipitate. The dilute acid combines with

the base of the glycocholate of sodium and frees glycocholic acid. The latter, after standing some hours, crystallizes in needles. Collect the crystals on a filter, wash them with a little cold water, dissolve in absolute alcohol, reprecipitate by the addition of ether, collect and dry by pressing between folds of blotting paper.

(2) Gorup-Besanez employs the following method: Evaporate fresh ox-gall almost to dryness on the water-bath. Extract the residue with ninety per cent. alcohol. Distil or evaporate the alcohol, dissolve the residue in water, add milk of lime, warm and filter. To the filtrate, after cooling, add dilute sulphuric acid until a cloudiness appears and remains (avoiding an excess of the acid). Allow to stand for some hours, when the glycocholic acid will have crystallized. Collect on the filter, wash with cold water, redissolve in much lime water, reprecipitate with dilute sulphuric acid as above, collect, and dry with blotting paper.

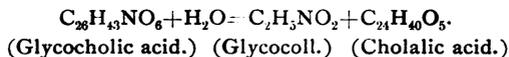
(3) Concentrate fresh ox-gall to half its volume. Place it in a tall graduated measure, add one-twentieth as much hydrochloric acid, then add ether. The glycocholic acid is precipitated and after a few days crystallizes. Collect the crystals on the filter and wash with cold water until the filtrate is colorless. Dissolve the crystals as they remain on the filter, in hot water. As the filtrate cools, glycocholic acid recrystallizes.

General Properties.—Glycocholic acid is soluble in 303 parts of cold, or 120 parts of hot water. From its solution in the latter it crystallizes, on cooling. It is freely soluble in absolute alcohol, insoluble in ether. If the alcoholic solution be evaporated, glycocholic acid remains as a hard, amorphous mass: while if water be first added to the alcoholic solution, the acid separates in fine crystals. Its solution colors blue litmus red. Glycocholic acid dissolves without decomposition in strong sulphuric, hydrochloric and acetic acid. It is freely soluble in the hydrated alkalis, forming glycocholates. When heated with an alkaline carbonate, carbonic acid is liberated. Its alkaline salts give the same reactions that the free acid does. Glycocholates of the alkalis, alkaline-earths and silver are soluble in water; while the combinations with other bases are insoluble or soluble with difficulty in water. All glycocholates are soluble in alcohol. Sodic.

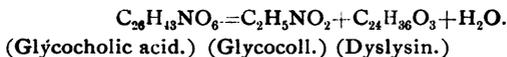
potassic and argentic glycocholates form in needle-shaped crystals on the addition of ether to their alcoholic solutions, while the corresponding salts of barium and lead are amorphous. Neutral acetate of lead precipitates glycocholic acid from aqueous solution; this precipitate being soluble in hot alcohol, and forming a flocculent deposit on cooling.

If glycocholic acid be dissolved in concentrated sulphuric, or hydrochloric acid and the solution warmed, *cholonic acid*, $C_{26}H_{41}NO_5$, separates on cooling. It will be seen, by comparison of the formulae, that cholonic acid is formed from glycocholic acid by the abstraction from the latter of one molecule of water. Cholonic acid is insoluble in water, soluble in alcohol and is never crystalline. Its barium salt is insoluble in water and by this means is easily distinguished from glycocholic, also from cholalic acid. (Hoppe-Seyler).

If a solution of glycocholic acid be boiled with a caustic alkali, a saturated solution of baric hydrate, or dilute acids, it takes up a molecule of water and forms *glycocoll* and *cholalic acid*:



By further action of dilute acids on glycocholic acid, *dyslysin* is produced:



(Hofmann.)

TAUROCHOLIC ACID, $-C_{26}H_{45}NSO_7$.

Taurocholic, also known as choleinic acid, is found together with glycocholic acid in human and ox bile. It will be seen from its formula, that this acid contains sulphur, which exists also in *hyotaurocholic* and *chenotaurocholic* acids.

Taurocholic acid is best obtained from the bile of the dog, in which it is the only acid. Evaporate dog-bile to dryness on the water-bath. Extract the residue with hot alcohol and filter through animal charcoal. Evaporate the filtrate on the water-bath, dissolve the residue in a small quantity of absolute alcohol. Precipitate the taurocholate of sodium from this alcoholic solution by the addition of ether. Dissolve the precipitate in water,

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render the solution alkaline with ammonia, and reprecipitate with the basic acetate of lead. Wash the precipitated taurocholate of lead with water, dissolve in hot alcohol and filter before allowing to cool. Treat the filtrate with a stream of hydrosulphuric acid gas, and remove the sulphide of lead by filtration. Concentrate the filtrate and precipitate by the addition of ether. Taurocholic acid forms first in an amorphous mass, then in fine crystals, which on exposure to the air dissolve into a syrup.

Taurocholic acid gives a decidedly acid reaction, is soluble in water and alcohol, insoluble in ether. In both the free and combined state, it is easily decomposed. By the evaporation of the aqueous solution of the free acid, its molecules are broken up, forming *taurine* and *cholalic acid*, in the same manner that glycocholic acid is with much greater difficulty changed into glycocholl and cholalic acid. The same decomposition is produced by the action of alkalis and dilute acids, and also occurs normally in the intestines. This acid, as well as glycocholic, forms salts with vegetable alkaloids.

Taurocholic is separated from glycocholic and cholalic acids by means of the different reactions of these substances with neutral acetate of lead. This reagent completely precipitates glycocholic and cholalic acids from aqueous solutions of their salts; but throws down only a trace of taurocholic acid unless the solution be strongly alkaline. Thus, if to a solution of purified ox-bile, prepared as directed under crystallized bile, neutral acetate of lead be added, the glycocholate of lead is precipitated and may be removed by filtration. If now to the filtrate, basic acetate of lead or the neutral acetate with ammonia be added, the taurocholate of lead is precipitated. From this precipitate the taurocholic acid may be separated by the following method: Place the lead-precipitate in an evaporating dish, add a solution of carbonate of soda and evaporate to dryness on the water-bath. Extract the residue with hot absolute alcohol and filter. Concentrate the filtrate and precipitate the taurocholate of soda by the addition of ether. If the taurocholate of soda, thus obtained, be transferred to a platinum dish, nitrate of potash be added, the whole burnt until all the organic matter is destroyed, the

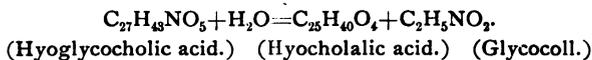
sulphuric acid of the ash estimated in the usual way with baric chloride, the amount of taurocholic acid may be ascertained. From the amount of sulphuric acid, calculate the sulphur, one part of which represents 16.28 parts of taurocholic acid.

HYOGLYCOCHOLIC ACID, $-C_{27}H_{43}NO_5$.

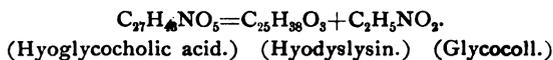
This acid, known also as hyocholic, has been found as yet only in the bile of the pig. The bile of this animal is decolorized by agitation with animal charcoal and filtration. To the filtrate, crystallized sulphate of soda is added to saturation. This precipitates the bile-acid which is now collected upon the filter, washed with a saturated solution of sulphate of soda, and then dissolved in water. From the aqueous solution of this compound the free acid is precipitated by the addition of hydrochloric acid and collected upon the filter paper.

As thus prepared, hyoglycocholic acid is a colorless, amorphous mass, insoluble in water, slightly soluble in ether, and freely soluble in absolute alcohol. Its solution has an acid reaction and a bitter taste. It combines with bases forming salts. Its alkaline salts are freely soluble in water; while the corresponding salts of the alkaline-earths and metals are insoluble in the same menstruum. All its salts are soluble in alcohol. From aqueous solutions of its salts, the acid is precipitated by the addition of alkaline sulphates or chlorides.

On being boiled with hydrated alkalis or with dilute acids, the atoms of the hyoglycocholic acid molecule are rearranged forming *glycocoll* and *hyocholalic acid*. This change, as will be seen from the equation, corresponds to that by which glycocoll and cholalic acid are formed from glycocholic acid.



On further boiling with dilute hydrochloric acid, *hyodyslysin* is produced.



HYOTAUROCHOLIC ACID, $-C_{27}H_{45}NSO_6$.

This acid, which is identical with Strecker's hyocholeinic,

exists in minute quantities with hyoglycocholic acid in the bile of the pig. It has never been obtained in the pure state. It will be seen from comparison of the formulae that the molecule of hyotaurocholic acid contains one atom of carbon more and one of oxygen less than the taurocholic acid molecule. The same relation exists between hyoglycocholic and glycocholic acids. By long boiling with alkalis or dilute acids, hyotaurocholic acid is converted into *taurine* and *hyocholalic acid*.

λ CHENOTAUROCHOLIC ACID, $-C_{29}H_{49}NS_6$.

This acid is found in the bile of the goose. The gall of this bird is evaporated, the residue extracted with absolute alcohol, the solution decolorized with animal charcoal and filtered, and the filtrate treated with ether. The pasty mass precipitated by the ether is collected on the filter, washed with a saturated solution of sodic sulphate and dissolved in absolute alcohol. After the addition, to the solution, of ether and after standing, chenotaurocholate of sodium is deposited in a crystalline mass. This is dissolved in water and treated with basic acetate of lead. The lead precipitate is suspended in alcohol and treated with hydrosulphuric acid gas. The sulphide of lead is removed by filtration and the filtrate evaporated, when an amorphous mass of chenotaurocholic acid remains.

This acid, prepared as above, is soluble in water and in alcohol. It combines with the alkalis forming salts. From aqueous solution of its alkaline salts, the acid is liberated by the addition of hydrochloric and other mineral acids. The chenotaurocholates of lead, silver and barium are insoluble in water. On being heated for a long while with a saturated solution of baric hydrate, chenotaurocholic acid is decomposed with the formation of *taurine* and *chenocholalic acid*, $C_{27}H_{44}O_4$.

CHOLALIC ACID, $-C_{24}H_{40}O_5$.

This acid is by some authors known as *cholic acid*. (See Burdon-Sanderson's Handbook for the Physiological Laboratory, p. 504.) But the name, cholic acid, has been used to designate different substances and consequently is now quite worthless. For instance, the cholic acid of Gmelin and Strecker is identical

with the glycocholic acid of other chemists, while the cholic acid of Demarcay and Berzelius is identical with the cholalic acid of Strecker.

Cholalic acid combined with glycocholl forms glycocholic acid ; combined with taurine, forms taurocholic acid. Cholalic acid exists free in the intestines and in the excrement of man and the domestic animals. It is also often found in the urine. By spontaneous decomposition of bile, this acid is set free from its combinations with taurine and glycocholl. Cholalic acid is prepared as follows: Boil ox-, or dog-bile for twenty-four hours, or until no ammonia is given off, with a solution of baric hydrate saturated at the boiling point. (Adding water whenever necessary to prevent evaporation to dryness.) Add to the mixture hydrochloric acid and collect the precipitate, which forms, upon the filter. Wash with water, dissolve in sodic hydrate, avoiding an excess of the solvent, add ether and hydrochloric acid, and set aside for a few days. During this time impure cholalic acid is deposited in crystals. Collect the crystals, press them between folds of blotting paper, redissolve in a little hot alcohol and add water to the alcoholic solution until a cloudiness appears. Allow to stand for some days when cholalic acid forms in tetrahedrons.

Hofmann gives the following method for preparing cholalic acid: Dissolve crystallized bile in a concentrated solution of potassic hydrate. Heat this solution in hermetically sealed tubes at 100°C ., for twenty-four hours, and then at 120° for one hour. Treat the contents of the tubes with water to which a little ether has been added, then add hydrochloric acid. Pure cholalic acid is deposited on standing.

Cholalic acid appears both in an amorphous and a crystalline condition. If a solution of the amorphous variety in ether be concentrated, the acid forms in four-sided prisms, the ends of which terminate in two oblique faces. From its saturated solution in hot alcohol, cholalic acid forms on cooling in modified octohedrons or tetrahedrons. The crystals are permanent in the air, slightly soluble in ether, freely soluble in hot alcohol, and insoluble in water. They are brittle, opaque and colorless. The amorphous acid has a waxy appearance and a doughy consis-

tency. Solutions of free cholalic acid have an acid reaction and a bitter taste. The acid is freely soluble in alkalis and on being heated with an alkaline carbonate liberates carbonic acid. The alkaline salts are sparingly soluble in hot alcohol and freely soluble in water. The baric salt crystallizes in fine needles, is freely soluble in alcohol, soluble in 30 parts of cold, or 23 parts of hot water. From aqueous solutions of its baric and alkaline salts, the acid is precipitated by the addition of silver nitrate, or lead acetate. The cholalates of lead and silver are soluble in hot alcohol.

If cholalate of silver be dried at the temperature of the water-bath and then gently warmed with iodide of methyl, the cholalate of methyl (CH_3) $\text{C}_{24}\text{H}_{39}\text{O}_5$ is formed, and may be obtained in the pure state by treating the mass with potassic hydrate solution, filtering, adding ether to the filtrate and allowing to stand. The cholalate of methyl crystallizes in long colorless prisms. The cholalate of ethyl (C_2H_5) $\text{C}_{24}\text{H}_{39}\text{O}_5$ is formed when an alcoholic solution of cholalic acid is saturated with hydrochloric acid gas, allowed to stand for four hours, precipitated by the addition of water, the precipitate shaken with ether and sodic hydrate. As the ether evaporates the cholalate of ethyl is deposited in a crystalline mass, which may be collected, washed with water, dissolved in absolute alcohol and reprecipitated by the addition of ether.

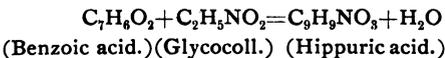
Cholalic acid heated to 200° with glycerine produces a mixture of glycerides. If the cholalate of ammonia (prepared by treating an alcoholic solution of cholalic acid with ammonia gas and adding ether) be heated on the oil-bath until no water is given off, cholamid (NH_2) $\text{C}_{24}\text{H}_{39}\text{O}_4$, remains. If dry cholalic acid be heated, an aromatic substance is given off.

Cholalic acid is often the only bile-acid present in abnormal urine, the glycocholic and taurocholic acids having been decomposed either in the body or in the urine after emission. Even when no traces of bile-acids can be detected in the urine, cholalic acid in quantity may be obtained from the fæces. For this purpose Hoppe-Seyler recommends the following process: The fæces are extracted with alcohol and the extract filtered. To the

filtrate add acetic acid and concentrate to a syrup on the water-bath. The syrup is washed with cold water. To the part insoluble in cold water, add baric hydrate and warm water. Treat this solution with carbonic acid gas until it becomes neutral in reaction, avoiding an excess of carbonic acid. Heat the mixture and filter while boiling. Wash the residue on the filter with hot water as long as any is dissolved. Unite the filtrate and wash-water, and concentrate to a small volume. After cooling, add ether, then hydrochloric acid, agitate, then allow to stand for a short time. Cholalic acid, which is now deposited, is collected on a filter, washed with cold water and dissolved in absolute alcohol. The alcoholic solution is decolorized, if necessary, with animal charcoal, concentrated to a small volume and allowed to stand until the cholalic acid crystallizes. That the crystals are cholalic acid may be demonstrated by applying Pettenkoffer's test for bile-acids, by heating some of the crystals, and observing the peculiar aromatic odor produced, and by their dextrorotary effect upon polarized light.

GLYCOCOLL,— $C_2H_5NO_2$.

In the bile, glycocoll is combined with cholalic and hyocholalic acids forming glycocholic and hyoglycocholic acids. When the bile-acids are decomposed in the intestines and a part, if not all, of the cholalic acid passes out in the fæces, it is supposed that the glycocoll and taurine are returned to the blood. In what condition, whether free or combined, the glycocoll is taken into the circulation is not known. Some glycocoll is constantly excreted in the urine as hippuric acid, which is formed in the body by the combination of glycocoll and benzoic acid.



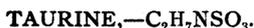
Whether the glycocoll of hippuric acid formerly existed in the bile is not known.

Boil hippuric acid for an hour with hydrochloric acid, dilute and set aside. On cooling, benzoic acid is deposited in crystals. The supernatant fluid is decanted and evaporated on the water-bath to dryness. The residue is dissolved in a little water and

filtered. The filtrate is boiled for five minutes with hydrated oxide of lead, then treated with a current of hydrosulphuric acid gas and filtered. The filtrate is concentrated on the water-bath until crystals of glycocoll are deposited.

The crystals are generally four-sided prisms, soluble in cold water, insoluble in ether and alcohol. The solution of glycocoll has an acid reaction and a sweet taste. If recently precipitated hydrated oxide of copper be boiled with an aqueous solution of glycocoll the former is dissolved. On evaporating the blue solution, thus obtained, the double oxide of copper and glycocoll is deposited in needle-shaped crystals.

If a solution of glycocoll be treated with nitrous acid, the former is converted into glycollic acid, water and nitrogen-gas.

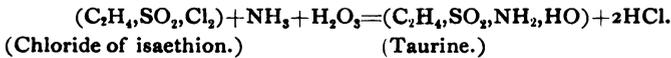


From its composition, especially from the fact that it contains sulphur in complex combination with carbon, nitrogen, hydrogen, and oxygen, it is quite evident that taurine is derived from albumen. In the bile as contained in the gall-bladder, taurine is combined with cholalic acid. And, like glycocoll, it is absorbed from the intestines. Taurine has been found in the muscles and the lung substance of animals (Hoppe-Seyler). The sulphur of this substance is oxidized and appears in the urine as sulphuric acid.

Boil the bile of the dog or of the ox for three hours with dilute hydrochloric acid. Filter, and evaporate the filtrate to dryness on the water-bath. Extract the residue with absolute alcohol. Treat the part insoluble in alcohol with water, filter the aqueous extract. Concentrate the filtrate and allow to cool when taurine crystallizes. In order to purify the taurine, dissolve the crystals in dilute spirits, add acetate of lead, treat with hydrosulphuric acid gas, and filter. Evaporate the filtrate, wash the residue with absolute alcohol. Dissolve the part insoluble in alcohol in water, filter the aqueous solution, concentrate and allow to cool, when pure taurine crystallizes.

The crystals are often very large, colorless, four- or six-sided prisms. They are insoluble in absolute alcohol, soluble in dilute spirits, soluble in both cold and hot water, insoluble in ether. The crystals are not decomposed on being boiled with dilute acids

mixture of ice and salt. The yellowish oily liquid thus obtained, is mixed with water and boiled for some time, then saturated with baric carbonate. The isaethionate of barium crystallizes in six-sided plates. These are treated with a solution of sulphate of potassium, when the sulphate of barium and the isaethionate of potassium are formed. The former is removed by filtration. The filtrate is evaporated when crystals of the isaethionate of potassium are formed. These are dried and distilled with an equivalent of the pentachloride of phosphorus, when the chloride of isaethion is produced. This on being heated with ammonia in hermetically sealed tubes yields taurine.



CHOLESTERINE, $-C_{26}H_{44}O$.

Cholesterine is widely distributed in both the vegetable and animal worlds. It exists in peas, beans, Indian corn, and probably in all seeds. It has been found in the brain, spleen, bile, etc. It also occurs in many pathological formations. It is found in pus, in hydrocele fluid, in the contents of various cysts, in tubercles, and in transudations of every kind. It is present in human milk, in the colorless corpuscles of the blood, and in the seminal fluid. Its existence in the human urine is due to a pathological condition. The faeces of the crocodile contain cholesterine and no uric acid. (Marcet.) It is a normal constituent of human faeces. Cholesterine is best prepared from human gall-stones, in which it exists in large proportion.

Pulverize human gall-stones, wash the powder with water to remove traces of bile; then dissolve in boiling alcohol and filter while hot. As the filtrate cools, crystals of cholesterine are deposited. These are purified by being boiled with an alcoholic solution of potassic hydrate. The solution is allowed to cool, when cholesterine is again deposited: collect, wash with cold alcohol and water, redissolve in boiling alcohol, from which pure cholesterine is deposited on cooling.

Cholesterine crystallizes in two forms. From its solution in chloroform, or benzol, it forms in fine, colorless, needle-shaped crystals. This form contains no water of crystallization. From

its solution in boiling alcohol, cholesterine is deposited in large rhombic tablets. These contain water and are represented by the formula $C_{26}H_{44}O + H_2O$. The tablets generally have a characteristic notch in one corner. Cholesterine is soluble in boiling alcohol, in hot ether, in chloroform, in benzol, in hot glycerine and in solutions of the alkaline glycocholates, taurocholates, and cholalates. It is insoluble in water, cold alcohol, dilute acids and strong alkalis.

Place some cholesterine on a glass slide and add a few drops of concentrated sulphuric acid. A beautiful red color is produced, which, on the addition of water, becomes green. If the crystals of cholesterine be treated with a drop of sulphuric acid and then a little iodine be added, a series of colors, violet, blue, green, red, yellow and brown, is developed. These changes are best observed through a microscope of low power.

Dissolve crystals of cholesterine in chloroform and add an equal volume of concentrated sulphuric acid. The solution becomes violet, then blue, green, and finally yellow. The addition of water decolorizes the solution instantly.

To cholesterine crystals in an evaporating dish, add a few drops of nitric acid and gently heat to dryness. A yellow residue remains, which on the addition of ammonia becomes red. (But is not changed by sodic hydrate, and thus is distinguished from the murexide test.) (Schiff's test.)

To hydrochloric acid add ferric chloride, pour the mixture upon crystals of cholesterine in a porcelain dish and heat. The cholesterine is colored red, then violet, and finally blue. Dissolve cholesterine in acetic acid by the application of heat. Allow to cool, when needle-shaped crystals of the acetate of cholesterine, $C_{26}H_{44}O, C_7H_4O_2$, are deposited. Treat the crystals with alcohol, when cholesterine will be set free and form in tablets. (Hoppe-Seyler.)

Cholesterine is recognized and distinguished from other substances by its crystalline forms and by its reactions with sulphuric acid and with sulphuric acid and iodine.

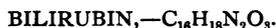
BILE-PIGMENTS.

Besides the acids, which have been described, there are certain

coloring matters which characterize the billiary secretion. In different animals, these coloring principles vary. Thus, in the bile of the ox, biliverdin predominates; while the color of human bile is principally due to the presence of bilirubin. One of these coloring matters can be changed into another by means of oxidizing or reducing agents. Upon this fact depends the various tests for bile-pigment.

Gmelin's Test for Bile-Pigment.—Dilute some bile and spread upon the bottom of a white dish, and add a few drops of fuming nitric acid. A precipitate will form around each drop, and then a green, blue, red, and yellow coloration will extend outward in rings, due to the action of the strong acid upon the coloring matter of the bile. If fuming nitric acid be not at hand, a substitute may be found by adding some strong sulphuric acid to the ordinary nitric acid, and allowing the mixture to stand in the sunlight until red fumes appear. This test is more distinct with human or dog bile than with that of the ox.

Hoppe-Seyler applies Gmelin's test in the following manner: Pour into a test tube some fuming nitric acid. Carefully add, without agitating, dilute bile or the fluid suspected to contain bile. Allow to stand and observe from time to time. Soon colored rings appear. These are, from above downwards, green, blue, violet, red and yellow.



Bilirubin exists free in small quantities in the bile of man and of the carnivora. It is found also in the gall-stones of both men and oxen. Pulverize human gall-stones; wash the powder with boiling water to remove any trace of bile-acids; then with ether to dissolve the fat; next with dilute hydrochloric acid in order to remove any inorganic matter, especially the salts of lime; and at last with hot water. Dry the residue on the water-bath and boil it with chloroform. Filter and distil the chloroform from the filtrate. Wash the residue with absolute alcohol, which removes the bilifuscin. The bilirubin remains as a residue, insoluble in absolute alcohol. Dissolve this in a little chloroform and concentrate the solution until bilirubin begins to separate as an orange-

Expose a solution of bilirubin in sodic hydrate to the air in shallow dishes until the yellow color of the solution is transformed into green. Then add hydrochloric acid, which precipitates the biliverdin. Collect the precipitate on the filter and wash with water until the filtrate ceases to give a test for chlorides; then dissolve in alcohol and evaporate. Biliverdin remains as a dark-green, amorphous powder. Biliverdin is insoluble in water, chloroform and ether; soluble in alcohol and alkalis. The solutions have a green or bluish-green tint. On the application of fuming nitric acid to alkaline solutions of biliverdin, a series of colors, blue, violet, red and yellow, is observed. If an alkaline solution of this coloring principle be warmed and treated with sulphurous acid gas, the green color of the solution is changed to yellow. By the action of the reducing agent, the biliverdin has probably been reduced to bilirubin.

BILIFUSCIN, —C₁₆H₂₀N₂O₄.

Wash pulverized human gall-stones with dilute hydrochloric acid, which dissolves the lime; then with water; next with ether, which removes the fat; finally boil with absolute alcohol, which dissolves the bilifuscin. Filter, evaporate the filtrate to dryness on the water-bath, wash the residue with water, then with ether and chloroform. Redissolve in a little absolute alcohol and evaporate, when bilifuscin, as a glistening, black residue, is observed.

Bilifuscin is insoluble in water and ether; slightly soluble in chloroform, and freely soluble in absolute alcohol. The alcoholic solution is of a yellow color; while the alkaline solutions are reddish-brown. From its alkaline solutions, bilifuscin is precipitated as a brown powder on the addition of dilute hydrochloric acid. Bilifuscin is precipitated from its solution in ammonia by chloride of calcium or barium.

When perfectly free from bilirubin, bilifuscin does not give Gmelin's reaction.

BILIPRASIN, —C₁₆H₂₂N₂O₆.

Wash pulverized human gall-stones successively with ether, chloroform, dilute hydrochloric acid and water. Treat the residue

insoluble in the above reagents with alcohol. Filter, and evaporate to dryness the alcoholic solution. Wash this residue with ether and chloroform. Redissolve in cold alcohol, filter and evaporate, when biliprasin remains.

Biliprasin, as obtained above, is a black, brittle mass which on being pulverized forms a dark-green powder. It is insoluble in water, ether and chloroform; freely soluble in spirits of wine. The alcoholic solution is of a beautiful green tint, which on the addition of an alkali is changed to brown. The brown color of the solution in ammonia enables one to distinguish between biliprasin and bilirubin; for the solution of the latter in ammonia is yellow. From its alkaline solutions, biliprasin is deposited as a green precipitate upon the addition of hydrochloric acid.

BILIARY CALCULI.

Gall-stones vary in size from those just visible to the unaided eye to those which are as large as an English walnut, and in number from a single one to seven thousand. Human gall-stones consist principally of cholesterine, with bile-pigments and salts of lime. They have generally as much as ninety-five per cent. of cholesterine. They are of a brownish color, and round or polyhedral with the faces worn smooth by the constant rubbing of the stones together in the bladder. When first removed, the gall-stones are generally soft and can be crumbled by pressure between the fingers, but on exposure to the air they harden. A nucleus of deeply colored matter, bile-pigment combined with lime, is found in many gall-stones. In others, the nuclei consist of mucus. Rarely one meets with white calculi of pure cholesterine. Again masses of crystalline cholesterine have been found in the gall-bladder. Frequently small, black or deep-red stones, known as pigment calculi, are found in the gall-bladder of the ox, very rarely in that of man. These are poor in cholesterine, rich in bile-pigment combined with lime, and contain traces of copper.

A quantitative analysis of biliary calculi is made as follows: Pulverize the stone, and weigh the powder; wash with boiling water, which removes traces of bile-acids; then treat the powder with a boiling mixture of equal parts of alcohol and ether and filter while boiling. As this filtrate cools, cholesterine is depos-

ited; collect on a weighed filter, wash with cold alcohol, dry at 100° and weigh.

The remainder of the powder is boiled with dilute hydrochloric acid and filtered through a weighed filter, the part insoluble in the acid is collected on the same filter, washed with water, dried at 100° and weighed. This gives the amount of bile-pigments.

The acid solution is carefully evaporated to dryness and the residue weighed. This gives the total amount of inorganic matter. The ash is then redissolved in water, slightly acidified with hydrochloric acid. From this solution, the copper is precipitated as a sulphide and separated by filtration. In the filtrate, the magnesium, calcium and iron may be estimated quantitatively according to the usual methods. The sulphide of copper is redissolved in dilute nitric acid, the excess of acid driven off and the solution diluted with water. From this solution the copper is precipitated by potassic hydrate and estimated as cupric oxide.

PANCREATIC JUICE.

Pancreatic juice, as obtained by means of a temporary fistula, varies in its composition as the animal feeds or fasts. During fasting, the secretion may entirely disappear or a thin, watery fluid may be obtained in small quantity. About six hours after taking a large meal, the secretion is abundantly poured out. This is a clear, colorless, somewhat viscid fluid, which has a decidedly alkaline reaction and a specific gravity varying from 1006 to 1010.

This secretion possesses the following digestive properties: (1.) It readily converts starch into glucose or grape sugar; (2.) it digests fibrine forming peptones, leucine, tyrosine, glutamic acid and asparagic acid; (3.) it emulsifies fats and separates neutral fats into glycerine and their respective acids. Concentrated acids, strong alkalis and many metallic salts arrest the action of the pancreatic juice; but the gastric juice does not contain sufficient acid to materially influence the action of the pancreatic juice, and, moreover, in the intestines, the alkalinity of the bile aids in destroying the acidity of the foods.

If pancreatic juice be kept for some time at the freezing

point, the fluid forms a jelly-like mass, which, on being gently warmed, regains its original fluidity. Heated to 80° , pancreatic juice coagulates.

If this secretion be treated with a stream of chlorine gas, a white precipitate appears and after standing in a warm room becomes rose colored. Alcohol throws down a precipitate which is soluble in water.

The pancreatic juice varies greatly in its composition and properties in different animals. The secretion of this gland in the sheep is thick and viscid; while that of the cat is a thin, watery fluid, is not ropy and contains but little pancreatine. In many fish the pancreatic secretion is acid in reaction, does not possess the power of converting starch into sugar and serves only to aid the gastric secretion in its action upon albuminous food. A similar pancreatic juice is obtained from the cul-de-sac in which the alimentary canal of some insects terminates. The glycerine extract of the honey bee converts cane sugar and starch into grape sugar. (Hofmann.)

The various digestive properties possessed by the pancreatic juice are due to special ferments which have never been obtained in a chemically pure state, and which are imperfectly separated from each other.

Separation of the Ferments.—Heidenhain has discovered a method of partially separating the ferments of the pancreatic juice. His method is essentially as follows: The pancreas is removed from a dog which has been killed six or seven hours after a full meal. Wash the organ with water in order to remove the blood; rub it up with ground glass or sand in a mortar; place the pulp in a beaker; cover with water and keep on the water-bath at a temperature from 20° — 30° , never above the latter, for two hours; filter through linen and press the residue; to the filtrate, which may be either neutral, acid or alkaline in reaction, add an excess of calcined magnesia; allow to stand for fifteen minutes, then filter, first through linen and then through paper. (This filtrate changes starch into sugar and digests albumen, but is without action upon fats, since the ferment peculiar to the fats has been removed by the magnesia.) Pour the filtrate into a flask

capable of holding three times the volume of the filtrate ; pour on the solution, without stirring, a solution of thick collodion equal in amount to one-third of the fluid ; close the flask and shake thoroughly for five minutes ; pour the mixture into a wide beaker and stir constantly in order to hasten the evaporation of the ether. A precipitate is deposited in granules ; bring the precipitate on a linen filter ; treat the filtrate with a solution of collodion as before and bring the second precipitate upon the same filter. The filtrate now contains the starch-ferment, while the albumen-ferment is with the precipitate on the linen. (Set the filtrate aside for further examination.) Wash the precipitate thoroughly with alcohol of from 60 to 70 per cent ; dry the precipitate by pressing it between folds of blotting paper ; suspend it in a mixture of ether and alcohol in order to remove the collodion ; filter and wash the residue on the filter well with ether ; then dissolve in water. This solution digests fibrine, but is without action upon starch or fats.

Concentrate the solution containing the starch-ferment in vacuo to one-sixth its volume ; add a large excess of absolute alcohol and allow to stand for three days. Collect the precipitated ferment on a filter and wash it with absolute alcohol ; treat it with a mixture of two parts of alcohol and one part of water : this dissolves the ferment from the albuminous substances which have continued with it so far. Filter the solution and evaporate it to dryness in vacuo ; dissolve the residue in a small quantity of water. This solution acts energetically upon starch in either neutral, alkaline, or acid solutions ; it also possesses slight digestive action upon fibrine, all of the albumen-ferment not having been removed. This aqueous solution also contains traces of leucine, tyrosine and inorganic salts, from which it may be freed by dialysis.

Pancreatine.—This is the name proposed by Heidenhain for that ferment which enables the pancreatic juice to digest fibrine. This substance does not exist preformed in the pancreas ; for if this organ be taken directly from the animal after death, cut into fine pieces and covered with glycerine, and allowed to stand for a month, only a trace of pancreatine is extracted. Heidenhain thinks that it is probable that in the pancreas the pancreatine is com-

bined with some albuminous substances; this compound is inert and only after it has been decomposed by macerating the organ in warm water, or by the addition of an acid, can the active pancreatine be obtained artificially. This inert compound he designates as *zymogen*. The amount of this substance contained in the pancreas varies inversely with the amount of pancreatine in the secretion. Consequently while the latter is most abundant during the sixth and seventh hours after a meal, the inert compound is stored up in greatest quantity in the organ from the eighteenth to the thirtieth hour after a meal. The secreting cell of the pancreas of the fasting animal consists of an outer and inner zone; the former is readily stained by carmine, while the latter is stained with difficulty, or not at all. The inner zone is granular, while the outer is homogeneous or striated. When the animal fasts the inner zone widens, and the wider this zone the more zymogen can be extracted. During digestion the inner zone grows smaller, while the outer increases in size. It seems that the zymogen is furnished by the inner zone, which, in turn, obtains the zymogen or the substance from which the zymogen is formed, from the outer zone; while the outer obtains the material from the blood. During digestion the zymogen is poured out from the inner zone, while the outer continues to receive material; consequently the explanation why the former decreases and the latter increases is evident.

Extraction of Zymogen.—Remove the pancreas from an animal which has been without food for thirty hours; cover with glycerine immediately; cut into fine pieces and allow to stand for three days. This glycerine extract is without action upon fibrine; but if it be diluted with warm water or with water acidulated with acetic acid, pancreatine is formed and will digest fibrine in an alkaline solution.

Properties of Pancreatine.—Pancreatine is a yellowish-white powder, soluble in water and glycerine, insoluble in alcohol. The aqueous solution is without action or acts very slowly upon fibrine; but upon the addition of a .1 per cent. solution of sodic carbonate, fibrine is digested rapidly. The activity is lessened and finally destroyed by the farther addition of an alkali. Thus pan-

creatine differs from pepsine inasmuch as the former requires an alkaline, while the latter demands an acid reaction for the manifestation of its activity. Pancreatine is most active when freely supplied with oxygen; whether this be due to more rapid conversion of zymogen into pancreatine, or whether the action of the latter consists in oxidizing, are questions not positively settled. Kühne designates this substance by the name *trypsiene*.

Amyolytic Ferment.—The partial separation of this ferment from pancreatine has been given. It seems to be identical with the ptyaline of the salivary glands. The action of this ferment is, however, much more intense than that of ptyaline. At from 37° to 40° it instantly converts starch into sugar. At 12°, it transforms glycogen into glucose.

The Fat Ferment.—This has not been obtained free from the others. An active extract may be obtained when a perfectly fresh pancreas is covered with a mixture of nine parts of glycerine and one part of a one per cent. solution of sodic carbonate, the whole rubbed up with sand or powdered glass, and allowed to stand for four days. This extract emulsifies fats and divides neutral fats into glycerine and fatty acids. As soon as sufficient fatty acid has been freed to neutralize the sodic carbonate, the action is arrested, since it can continue only in an alkaline solution. Shake some of this extract with olive oil, place upon the water-bath at 40° and allow to remain for some time. The reaction, ascertained by placing a drop on a strip of blue litmus paper, will soon become acid.

INTESTINAL JUICE.

From the duodenum a ferment can be obtained by extraction with glycerine, which bears a close resemblance to, and probably is identical in composition with the *succus pyloricus*. It is strongly alkaline, and on being acidified with hydrochloric acid, digests fibrine. Intestinal juice also possesses two other ferments, by means of which it converts starch and cane sugar into grape sugar. The secretion of the large intestines is also alkaline, is tenacious, turbid, contains albumen and is, outside of

the body at least, without action upon starch, albumen or fat. It is true that the food after it enters the large intestine generally possesses an acid reaction; but this acidity is due to fermentative changes in the food and not to the secretion of this part of the alimentary canal. That fermentation does go on, is shown by the nature of the intestinal gases. These vary greatly in kind and amount with the nature of the food; the gas from carnivora is rich in nitrogen, while the intestinal gas obtained from herbivora contains as much as fifty per cent. of methane, CH_4 . It is probable that these fermentative changes may fit some parts of the food for absorption; this is most likely to be the case in herbivora. Evidently, food has been partially absorbed when administered per rectum and that this food is fitted for absorption by fermentation, is probable.

The secretion of the large intestine of the herbivora decomposes amygdaline in the same way that emulsine does. (Hofmann.) The rapidity of this conversion is a matter of some importance; because, as is well known, emulsine converts amygdaline, the principal constituent of bitter almonds, into hydrocyanic acid, oil of bitter almonds and sugar. Moreover, it has been stated (U. S. Dispensatory, thirteenth edition, p. 117,) that "Amygdaline appears not to be poisonous when taken pure into the stomach; since there is nothing in the system capable of taking the part of emulsine."

Preparation of Intestinal Juice.—Remove the small intestines from the animal as soon as it dies. Ligate one extremity and force water into the open end until the intestine is filled; temporarily fasten the open extremity and agitate the contents by moving through the hand and gently pressing upon the intestine. Remove the temporary fastening and pour out the water. Repeat the process of filling, agitating and emptying, until the wash-water is transparent or but slightly turbid. Lay open the canal from one extremity to the other; remove the muscular layer from the mucous membrane; cut the latter into fine pieces; rub these up with powdered glass; cover with water; allow to stand for three hours and filter, first through cloth and then through paper. This solution converts starch and cane sugar into grape sugar.

THE FÆCES.

The faeces consist of undigested parts of the food more or less changed by the various physical and chemical agents to which they have been subjected and mixed with the secretions and transudations. The proportion of the faeces to the food varies with the kind of the latter, the condition of the digestive fluids and the movements of the intestines. Normally, the faeces of man are equal in weight to about one-eighth of the food.

The color of the faeces varies with the food and with the action of the liver. After a meal consisting of flesh, the faeces are dark-brown; while after an exclusive milk-diet, they are yellow. When the biliary secretion is arrested, the faeces are light colored and when this secretion is excessive, they may be dark or green. In many diseases, the color of the excrement is decidedly changed; thus in diarrhœa, the stools are light colored and, in obstruction of the bile duct, may be perfectly colorless. From the faeces, traces of unchanged bile-pigments may often be obtained. Hæmatine is frequently present and in carnivora is often from the flesh of the food and, in any animal, may be present from bleeding of the walls of the alimentary canal. Only when the bleeding is in the lower part of the large intestine, is unchanged blood present in the stools.

The odor of the stools is due to indol, valerianic or butyric acid or hydric sulphide. Normally, the odor is due to indol, C_8H_7N . This substance is obtained as a final product in the reduction of indigo; it is a weak base, forms large colorless crystalline plates, which are freely soluble in hot, sparingly, in cold water. Indol is often formed during pancreatic digestion, but is supposed to be due to the presence of bacteria, since its formation is arrested if the pancreatic digestion be carried on in the presence of salicylic acid. Indol is recognized by its odor and by its reaction with nitrous acid. With this acid even very dilute solutions of indol produce a red color.

Normally, the reaction of the faeces is neutral or alkaline; but in certain diseased conditions, it is acid. The amount of solids varies from 174 to 317 parts in a thousand. The solids consist of earthy salts, excretine, taurine, cholalic acid dyslysin,

fats, sometimes acetic, lactic, butyric, and valerianic acids; while urea, haematine, haemaglobine, albumen, bile-acids and bile-pigments are occasionally found.

Excretine.—This substance, which was discovered by Marcet, exists in human faeces, but not in those of the dog. It is non-nitrogenous, is represented by the formula, $C_{78}H_{156}O_2S$, and is prepared as follows: Extract the faeces with alcohol and filter; to the concentrated filtrate, add milk of lime; collect and dry the precipitate which forms; extract the dried precipitate with a mixture of equal volumes of ether and alcohol; set the extract aside in a cool place. After six or eight days usually, excretine forms in fine, needle-shaped crystals; it can be purified by dissolving in boiling alcohol, from which it recrystallizes on cooling. Excretine melts at 92° , is insoluble in water, soluble in hot alcohol and ether.

Flint's stercorine is impure cholesterine. (Hofmann.)

Analysis of Normal Faeces.—Hoppe-Seyler recommends the following process for the examination of normal faeces: First extract the mass with hot alcohol and filter. This filtrate contains fatty acids free or combined with alkalis, bile-acids, bile-pigments, traces of cholesterine and some inorganic salts. The fat may be detected by allowing some of the alcoholic solution to evaporate to dryness, adding a little water to the residue and examining under the microscope. The bile-acids and the bile-pigments may be recognized by evaporating the alcohol, dissolving the residue in water and applying Pettenkoffer's and Gmelin's tests. The cholesterine may be recognized by its crystalline form.

The mass insoluble in alcohol is now extracted with ether. The ethereal solution contains fats. The remaining residue is extracted with ether, acidified with hydrochloric acid; this dissolves out the palmitic and stearic acids which were combined with lime.

A quantitative estimation of the fats of the faeces is often desirable for diagnostic as well as experimental purposes. This is best accomplished in the following manner: Extract a weighed portion of the excrement first with a mixture of alcohol and ether and then with ether acidified with hydrochloric acid. Fin-

ally, wash well with ether. Unite these filtered extracts, add carbonate of sodium and evaporate the mixture to dryness on the water-bath; wash the residue with water into a flask, add ether and shake thoroughly; allow to stand and draw off the ethereal layer with a pipette; shake again with ether and repeat this process as long as the ether takes up anything; this is known by allowing a few drops of the ethereal solution to evaporate and observing whether any residue remains or not. The united ethereal solutions are poured into a weighed beaker, evaporated to dryness, and dried at the temperature of the water-bath and weighed. If the fats which have combined with the sodium are to be estimated, acidify the watery solution with a few drops of hydrochloric acid, shake with ether, evaporate the ethereal solution, dry and weigh as before.

Urea.—This substance is often abundant in cholera-stools and is obtained as follows: Extract the faeces with cold water; filter; evaporate the filtrate to dryness on the water-bath; extract this residue with absolute alcohol; filter; again evaporate to dryness on the water-bath; dissolve the residue in a little water and add an equal volume of nitric acid. After some minutes, nitrate of urea forms in rhombic tablets.

Albumen.—This is frequently present in the stools of diarrhoea. Filter the liquid faeces and test the filtrate for albumen with heat and nitric acid.

Haematine.—Extract the faeces with cold alcohol; boil the insoluble part with alcohol to which a few drops of sulphuric acid has been added, and filter. Concentrate this filtrate and examine through the spectroscope (see haematin.) Whether this evidence be positive or negative, proceed as follows: Saturate the solution with sodic hydrate and filter; evaporate the filtrate to dryness at a gentle heat; wash the residue with dilute nitric acid. Pure haematine now remains and may be recognized by its spectroscopic appearance and by the detection of iron in the ash. (Hoppe-Seyler).

Meconium.—The excrement which is passed by the newborn infant and which has accumulated during intra-uterine life, is of a dark-green or black color and consists of intestinal mucus

and epithelium, with bile-acids and -pigments and traces of cholesterine. The bile-acids and cholesterine may be extracted with boiling alcohol, from which the latter crystallizes on cooling; while the bile-acids may be obtained by evaporating the alcohol, dissolving the residue in a little water and applying Pettenkoffer's test. This excrement has an acid reaction, is odorless and exhibits, under the microscope, cylindrical epithelium, fat globules, and plates of cholesterine.

Intestinal Calculi.—These are frequently found in herbivorous animals, seldom in man. They are especially likely to occur in horses which have been fed upon bran. These calculi consist principally of magnesian and ammonio-magnesian phosphates, consequently are soluble in acetic acid and reprecipitated from this solution on the addition of ammoniac hydrate. When burned the odor of ammonia is given off. In horses, intestinal calculi have been found which weighed as much as fifteen pounds. Sometimes the very large calculi found in oxen and horses are light, of a grayish color and consist of grass and parts of plants held together by earthy phosphates.

Intestinal calculi have been found in persons who have lived principally upon oat meal, and coarse breadstuffs. These are seldom as large as a hazelnut and consist of phosphates, fat and bran.

Ambergris, which is found floating upon the sea, or cast on the shore by the waves, or in the bodies of diseased and dead whales, is an intestinal concretion formed in the spermaceti whale and perhaps in other fish. It is generally found in small pieces, but sometimes in masses weighing from fifty to one hundred pounds. It has the consistency of wax and is of various tints, gray, brownish, yellow and dark. Ambergris contains a crystallizable, non-nitrogenous substance which is known as ambreine and closely resembles cholesterine.

Boil ambergris with alcohol and filter while boiling; on cooling, ambreine forms in needle-shaped crystals which may be purified by collecting on a filter and redissolving in boiling alcohol. As thus obtained, ambreine forms in tufts of delicate needles, which are tasteless and odorless, melt at 35° and sublime

unchanged at 100° . Ambreine is insoluble in water, soluble in hot alcohol, ether and nitric acid.

Dissolve ambreine in hot nitric acid and evaporate the solution to dryness; wash the residue with cold water; then boil with water to which a little carbonate of lead has been added. Collect the mixture upon a filter paper and wash with cold water as long as the filtrate contains lead. Dissolve the residue in boiling alcohol, from which *ambreic acid* crystallizes in small, yellowish-white tablets, on standing. (Pelletier.)

Bezoare, which was formerly used for its supposed medicinal virtues consists of concretions formed in the stomach and intestines of various herbivorous animals, as the antelope and goat. The variety most esteemed is brought from Persia and Arabia and is known as *oriental bezoare*. It melts when heated and gives off a rich, aromatic odor and consists principally of *lithofellic acid*, $C_{20}H_{36}O_4$.

Boil pulverized oriental bezoare with alcohol and filter while hot. To the filtrate, add an excess of sodic carbonate and evaporate the mixture to dryness on the water-bath; extract the residue with boiling absolute alcohol; filter and again evaporate the filtrate to dryness; dissolve this residue in water and precipitate with baric chloride; collect the precipitate upon a filter and dissolve in boiling water. To the concentrated filtrate add acetic acid as long as a precipitate forms, avoiding an excess of the acid; collect the precipitate upon a filter; wash with cold water and dissolve in hot alcohol. As this solution cools, pure *lithofellic acid* forms in fine rhombic plates with rounded sides.

Lithofellic acid melts at 205° ; when heated above this temperature it forms an amorphous, electric mass. When crystallized it is insoluble in water, sparingly soluble in ether and cold alcohol, freely soluble in hot alcohol. It unites with bases forming salts: the compound with the alkalis being freely soluble in water. The baric salt crystallizes from its solution in hot water in needle-shaped crystals. If ammonio-argentic nitrate be added to an alcoholic solution of lithofellic acid and the flocculent precipitate which forms be collected and dissolved in hot alcohol and this

solution be concentrated, needle-shaped crystals, which blacken on exposure to the air, appear.

BLOOD.

HÆMOGLOBINE.

Synonyms: Simon's *Haematoglobuline*; Lehmann's *Haematocrystalline*; Stoke's *Scarlet Cruorine*; Berlin's *Chromatine*; also the *Oxyhaemoglobine* and *Erythrocruorine* of various authors.

Haemoglobine is the principal constituent of the red corpuscles of the blood of vertebrate animals. In man, the dog, pig, ox and many other animals, the red corpuscles are almost pure haemoglobine, only traces of other substances being present: while in birds, this coloring matter is associated with an albuminous substance. Healthy human blood contains, on an average, twelve per cent. of haemoglobine; but it must be remembered that the amount varies at different times of the day and with other circumstances influencing the normal periodic changes of the individual. Arterial blood contains a somewhat larger amount than venous blood. In a person suffering with cholera, the blood, on account of its concentration, contains a much larger per cent. of haemoglobine than is normal; while in leucocythaemia the per cent. is decreased. (Hofmann.) Haemoglobine is more abundant in carnivora than in herbivora, in the adult than in the young, and in the fasting than in the recently fed animal.

The following table, taken from Hofmann's *Zoochemie*, shows the per cent. of this coloring matter in some of the domestic animals: One hundred grammes of blood contain

In the rabbit—	8.4	grammes of haemoglobine.
“ “ sheep—	11.2	“ “ “
“ “ ox —	12.3	“ “ “
“ “ pig —	13.2	“ “ “
“ “ hog —	13.8	“ “ “
“ “ cock —	8.5	“ “ “
“ “ duck —	8.1	“ “ “

Haemoglobine exists not only in the blood corpuscle, but also in some muscles and in solution in the blood of some invertebrates, as, for instance, in the angle-worm. Amorphous haemo-

globine can be separated from the blood of man, crystals of haemoglobine are obtained with difficulty; while the blood of the dog, cat, rat, goose and many other animals, readily yields the crystalline form.

Preparation.—Stir fresh blood for ten or fifteen minutes with a piece of whale-bone or a bundle of glass rods, and filter through calico or linen, which has been freed from starch by having been previously washed and dried. To the filtrate, add ten times its volume of a mixture of one volume of a saturated solution of sodic chloride and nine volumes of water; place the beaker containing this mixture in a cool place, at or below 0° , and allow to stand for two days. During this time, the greater part of the blood corpuscles will have fallen to the bottom. Decant the supernatant fluid; stir up the corpuscles with a dilute solution of sodic chloride; allow them to subside and again decant the supernatant fluid; repeat this operation two or three times. By means of a little water, transfer the corpuscles thus freed from serum to a small beaker; add a large excess of ether; shake well; allow to stand and remove the ethereal layer which contains lecithine and cholesterine. Filter the red aqueous solution, from which the ether has been removed, through a fast filter; cool the filtrate to 0° , then add one-fourth its volume of alcohol which has also been cooled to 0° ; allow this mixture to stand at a temperature of from -5° to -10° for a few days, when haemoglobine will be deposited in either the crystalline or amorphous form. If the blood used were that of the dog, rat, squirrel, or hedgehog, crystals will form so fast on shaking the corpuscles with ether, that the greater part of the haemoglobine will rest in the crystalline form on the filter in the subsequent filtration. These crystals may be used for microscopic examination and then dissolved by digesting with a little water at 35° on the water-bath. Filter this solution; cool the filtrate to 0° ; add one-fourth its volume of alcohol, previously cooled to 0° , and allow to stand as recommended above. In this way, crystals of pure haemoglobine are obtained. (Hoppe-Seyler).

It is sometimes desirable to obtain crystals of haemoglobine from coagulated blood. Place a piece of the coagulum in a small

beaker or test tube and set in a cold place, below 0° , and allow to stand for three days. To the blood add a few drops of water, place a drop of the mixture on a glass slide, cover with a thin glass and leave for some hours in a cold place, when crystals will form and may be detected by the microscope.

To some blood of the guinea-pig, add half its volume of water, shake well and allow to stand in a cool place, when a crystalline sediment forms. If a larger proportion of water is added, the crystals will not appear, or will be decomposed and replaced by an amorphous deposit.

To 5 c. c. of defibrinated blood add, drop by drop, water until a clear solution is obtained. To this add one-fourth its volume of alcohol and place the mixture in a platinum dish in a freezing mixture, when haemoglobine crystallizes and may be recognized by microscopic examination. (Hofmann.)

Properties.—The crystals of haemoglobine are mostly rhombic prisms, but vary in form and composition with the species of animal from which the blood is taken. Blood from the dog yields haemoglobine, which forms in four-sided prisms; from the squirrel, in six-sided plates; from the goose, in rhombic tablets; from turkeys, in cubes and octohedrons; from the horse and man (when obtained in the crystalline form), in rhombic tablets and prisms.

The crystals of haemoglobine vary also in the per cent. of water of crystallization which they contain and in the relative amount of each element represented in the molecule. The following table, taken from Hoppe-Seyler's Handbuch, S. 252, shows these variations:

	Per cent. of						
	Water.	C.	H.	N.	O.	S.	Fe.P ₂ O ₅ .
Crystals from the dog.....	3.4	53.85	7.32	16.17	21.84	0.39	0.43
Crystals from the goose.....	7	54.26	7.10	16.21	20.69	0.54	0.43
Crystals from the guinea-pig...	6	54.12	7.36	16.78	20.68	0.58	0.48
Crystals from the squirrel.....	9.4	54.09	7.39	16.09	21.44	0.40	0.59

It will be seen from this table that of the animals there represented, the dog furnishes haemoglobine poorest, and the squirrel, richest in water of crystallization.

Again, the crystals vary in the degree of solubility in water;

those obtained from the blood of birds are most sparingly, while those from the dog are most freely soluble in this menstruum. In direct proportion to their solubility, the crystals vary in the readiness with which they absorb water from the atmosphere; thus haemoglobine from the blood of the raven is very sparingly soluble in water and is not at all hygroscopic. (Hofmann.)

If the crystals be dried at 0° in vacuo over sulphuric acid, a brick-dust deposit remains. If the temperature rises above the freezing point during the process of drying, the haemoglobine partially decomposes and a black residue remains.

The crystals of haemoglobine, as well as an aqueous solution of the same, have the bright red color of arterial blood. The aqueous solution gives a feebly acid reaction, and is decomposed, with the formation of an albuminous substance which coagulates, on being heated to 65° .

The crystals or the aqueous solutions of haemoglobine contain oxygen, which is loosely held in combination and which may be removed by means of the air-pump or by various reducing agents. This oxygen is not reckoned in the ultimate analysis of this coloring matter, which has already been given. The term, *Oxyhaemoglobine*, is often used to designate this substance as it holds the oxygen, and in contradistinction to the haemoglobine from which this oxygen has been removed. After the removal of the oxygen, the coloring matter dissolves more readily in water, but does not recrystallize or does so with great difficulty. The amount of this loosely combined oxygen which may be freed is constant; thus, measured at a pressure of one metre, the oxygen given off from one gram. of pure crystals occupies 1.34 c.c.

If a dilute solution of oxyhaemoglobine be examined with the spectroscope, a very characteristic spectrum will be observed. A small portion of the red, and a larger portion of the blue end will be absorbed, while between the solar lines D and E will appear two bands. Of these, the one nearer D is the smaller, darker and more sharply defined; the other lies close to the line E and is less intense. These two bands appear in very dilute solutions, being plainly visible in a thickness of 1 cm. of a solution of 1 gram of dry oxyhaemoglobine in 10,000 c. c. of water. On

further dilution these bands finally disappear; the one nearer E being the first to become invisible. If, on the other hand, more concentrated solutions be used, the spectrum varies with the degree of concentration. With a .5 per cent. solution, the absorption of the blue end extends to the red-ward side of G; while of the two bands between D and E, the one nearer the former covers that solar line. With an .8 per cent. solution, the two bands unite, and the only rays which pass through lie to the red-ward side of D.

If now an aqueous solution of oxyhaemoglobine be treated with a current of nitrogen or hydrogen gas, the brilliant hue of the solution is replaced by a purple color; the loosely combined oxygen has been removed and *reduced haemoglobine* remains. The same effect is produced by adding to the solution of oxyhaemoglobine reducing agents, as the alkaline sulphides, ammonical solutions of tartrates (as tartaric acid added to a solution of ferrous sulphate, until a precipitate no longer occurs on the addition of sodic hydrate, and then the whole made alkaline with ammonic hydrate), finely divided tin or other metals.

Spectroscopic examination of a solution of reduced haemoglobine reveals a spectrum entirely different from that of the oxyhaemoglobine: the two bands between D and E are replaced by a single broad band which is less distinct than either of the other two. Moreover, less of the blue end of the spectrum is absorbed; while the brightest part lies between B and C: thus, the red and blue rays pass through a solution of reduced haemoglobine, consequently its color is purple.

If a solution of reduced haemoglobine be shaken with air, oxygen is reabsorbed, oxyhaemoglobine is formed and will be recognized by the change in color from purple to scarlet and by the reappearance of the two bands in the spectrum. A concentrated solution of oxyhaemoglobine presents a dark spectrum with the exception of a red band between C and D; if a drop of a solution of sodic sulphide be added to the oxyhaemoglobine the light between C and D will be excluded and a bright band will appear between B and C. Now shake the solution with air and the spectrum of oxyhaemoglobine will reappear.

• *Physiology.*—By studying the chemical properties of haemo-

globine or the red corpuscle, a more exact knowledge of its physiology has been obtained than could have been secured in any other way. Vain conjectures, wild fancies, and strange theories have been proposed concerning the change of arterial into venous blood. For a long while, honest, earnest workers have endeavored to ascertain the chemical properties of the red corpuscle. Brande, Gmelin, Lehmann and others worked diligently in this field and with partial success; and finally, Hoppe-Seyler succeeded in preparing haemoglobine in its pure state. It is true that every particular concerning the red corpuscle is not yet understood and it may be that future years of investigation will yield more than the present possesses; but it becomes us to appreciate as fully as possible the benefits arising from a clear understanding of these facts. The light arising from the discovery of the chemical properties of haemoglobine not only illuminates many hitherto dark corners of physiological science, but extends in all directions through the various departments of pathology.

Arterial blood contains much oxyhaemoglobine and but little reduced haemoglobine; while venous blood is poor in the former and rich in the latter. It is true that if diluted venous blood be examined with the spectroscope the two bands which have already been described as characteristic of oxyhaemoglobine will appear: this is due to the fact that these bands are much more sharply defined than the one of reduced haemoglobine. Consequently, in a mixture of these two substances, the oxyhaemoglobine, though it may be present in very small proportion, will be recognized on spectroscopic examination. If all of the oxyhaemoglobine should disappear from the blood, death would follow: this happens in the last stages of asphyxia and then the two characteristic bands cannot be obtained (Foster.) As the blood leaves the left ventricle for all parts of the body, it contains much loosely combined oxygen; nearly all of the haemoglobine exists in the oxidized condition. During its passage through the arteries and capillaries, the blood performs its great function, that of an oxygen carrier; recently received material from the alimentary canal must undergo certain chemical changes which are essential to the maintainance of animal heat, to the exercise of muscular activity, to the repair

of various tissues, and to the production of thought. Waste material must be removed, solid tissue must be dissolved or converted into gases, organic matter must be changed into inorganic, poisons introduced from without and poisons generated within must be rendered inert and fitted for excretion. The greater part of these changes are produced by the chemical activity of the loosely combined oxygen of the haemoglobine and during its passage through the capillaries, this substance is deprived of a part of the oxygen and as reduced haemoglobine is returned through the veins to the heart and lungs. As the venous blood passes through the lungs, the greater tension of the oxygen contained in the air cells over that of the blood causes the passage of the former into the capillaries and the reduced haemoglobine is again oxidized and sent forth on its mission.

It has been stated that these facts illustrate some pathological conditions; for instance, in a case of phthisis an insufficient amount of oxygen is absorbed, oxyhaemoglobine is deficient and consequently many of the normal transformations of the body are completely or partially arrested. In such a case, large quantities of oxalate of lime will be found in the urine; the carbon of the food and of the waste material from the tissues is only partially oxidized and that which should have been exhaled from the lungs as carbonic acid, is excreted by the kidneys as oxalic acid. Again, in the condition of venous stasis arising from feeble action of the heart, the blood stagnates in the veins, becomes loaded with poisons, is not carried to the lungs with due rapidity and those nitrogenous parts of food and tissue, which normally are converted into and excreted as urea, appear in the urine as uric acid free or combined.

Compounds.—Besides oxygen, haemoglobine takes up some other substances in a similar manner. It must be remembered that the association or dissociation of oxygen does not affect the molecular arrangement of the haemoglobine itself. It is true that this combination is a chemical one, but the oxygen is held so loosely that it is replaced without injury to the structure of the haemoglobine molecule; thus the red corpuscle receives its oxygen in the lungs and loses the same in the systemic capillaries

and is not itself materially changed. Carbonic oxide (C O) has the power of freeing oxygen from oxyhaemoglobine and of forming carbonic oxide-haemoglobine.

Treat a warm concentrated aqueous solution of oxyhaemoglobine for a short time with a current of carbonic oxide (C O); the oxygen will be liberated and an equal volume of the other gas will be taken up. Cool the solution to 0° ; add one-fourth its volume of cold alcohol; allow to stand for twenty-four hours, exposed to a temperature at or below the freezing point, when beautiful, purple colored, four-sided prisms of this compound will appear. These crystals are more permanent and less freely soluble in water than those of oxyhaemoglobine. The spectrum of this compound is very similar to that of oxyhaemoglobine, however the two lines are less distinct and regular and on accurate measurement will be found a little farther toward the violet end; also more blue light passes through. This spectrum is not so readily destroyed as that of oxyhaemoglobine, the addition of ammoniac sulphide causing the disappearance of the bands only after several hours. Carbonic oxide-haemoglobine is decomposed by arseniatted hydrogen gas.

The combination of carbonic oxide with haemoglobine is stronger than that of oxygen with the same; thus, while oxygen is readily removed from oxyhaemoglobine by a current of carbonic oxide, the latter is but slowly freed from its compound by being treated with oxygen gas. Continued agitation with oxygen converts carbonic oxide-haemoglobine into oxyhaemoglobine; probably the carbonic oxide (C O) is first changed into carbonic acid (C O₂).

A study of the properties of carbonic oxide-haemoglobine explains the poisonous effects of inhaled carbonic oxide. When this gas is taken into the lungs, it combines with the reduced haemoglobine, gives the blood a bright cherry-red color and destroys its function as an oxygen carrier. Moreover, since this gas is but slowly displaced by oxygen, the animal dies of suffocation. The blood of an animal poisoned with this gas will often hold its color for days or weeks. It also manifests a different reaction with sodic hydrate from that of normal blood. If the

latter be defibrinated and treated with twice its volume of a solution of caustic soda (specific gravity 1.3), a brown gelatinous mass separates and when this is spread upon a clean porcelain surface, it presents a dirty, greenish-brown tint. If blood from an animal poisoned with carbonic oxide be treated in the same way, a cherry-red coagulum (which if spread upon a porcelain surface will present a red, slowly changing into a dark-brown color) appears.

Render a concentrated aqueous solution of oxyhaemoglobine feebly alkaline with baric hydrate and treat it with pure nitrous dioxide gas ($N O_2$). This compound is more permanent than the corresponding one with carbonic oxide. Blood containing nitrous dioxide-haemoglobine is of a bright red color and without the purple tint of the carbonic oxide compound. Spectroscopic examination reveals two bands, identical in position but different in appearance from those of oxyhaemoglobine. The bands of the nitrous dioxide compound are at first very faint, gradually growing darker, but never becoming so dark and distinct as those of oxyhaemoglobine. (Hofmann.)

An insoluble form of haemoglobine is sometimes found in cysts. It appears as a brick-red deposit, consisting of corpuscles which are insoluble in water and alcohol, permanent at ordinary temperature; but are decomposed by acids or alkalis in the same way as haemoglobine is decomposed. The ash of these corpuscles contains as much iron as that of haemoglobine; besides iron, carbonate of lime is found in the ash. (Hoppe-Seyler.)

- *Detection of Hæmoglobine.*—Since this coloring matter differs from most others of the animal world in not being precipitated by basic acetate of lead, nor by this reagent in the presence of ammonia, it is easily separated from any mixture. In making this separation, the basic acetate of lead should be added as long as the precipitate increases; but an excess should be avoided, because methæmoglobine and other substances which may be present are soluble in an excess of the basic acetate solution, and moreover, such an excess may cause the decomposition of the hæmoglobine. This separation should be made with the substance under examination and the reagents subjected to a tem-

perature of, or as near 0° as possible. After other coloring matters and impurities have been removed by precipitation with the basic acetate of lead, and filtration, the filtrate is tested for the presence or absence of haemoglobine with the spectroscope. As a confirmatory test, the change of color and spectroscopic appearance on the successive additions of reducing agents and oxygen may be observed. Finally, if a portion of the solution be evaporated on the water-bath at 40° to 45° and to the residue on a watch crystal, a drop of a dilute solution of sodic chloride and a few drops of glacial acetic acid be added, and the acid be evaporated to dryness on the water-bath, crystals of haemine will form and may be recognized on microscopic examination.

Decomposition.—The products of the decomposition of haemoglobine have not been satisfactorily studied. Evidently it can be broken up with the formation of at least one other coloring matter and one or more albuminous substances. If an aqueous solution of oxyhæmoglobine be allowed to stand for some hours at ordinary temperature, and then be examined with the spectroscope, an absorption band will appear between C and D, nearer the former, and it will also be found that a brownish precipitate will be formed on the addition of a few drops of a solution of basic acetate of lead. Moreover, a brownish substance, soluble in water and giving the above spectroscopic appearance and reaction with the lead solution, is not unfrequently found in cysts of various kinds into which blood had previously extravasated. This substance has been named *met-haemoglobine* by Hoppe-Seyler, who recommends the following method for its detection: If the absorption band between C and D is not sufficiently distinct, add to the solution basic acetate of lead as long as a precipitate forms; collect this precipitate upon a filter; suspend it in water; add to the mixture carbonate of soda until the coloring matter is completely dissolved and the lead precipitated as a carbonate; filter, and examine the filtrate with a spectroscope. Besides the spectroscopic appearance, the formation of haemine crystals, according to the method already given, and the detection of iron in the ash may be employed as confirmatory tests.

Methaemoglobine contains an albuminous substance which

on further decomposition is set free and *haematine*, a non-albuminous coloring matter, remains.

Quantitative Estimation of Haemoglobine.—The quantity of haemoglobine contained in blood may be estimated either by ascertaining the amount of iron present, by comparing the intensity of the color with that of an aqueous solution of a known quantity of haemoglobine, or by ascertaining by means of the spectroscope the degree of dilution necessary to allow the transmission of the red rays only.

(1) By estimating the amount of iron in a weighed or measured quantity of blood, the amount of haemoglobine may be calculated. In this method it is assumed that all of the iron obtained from the ash came from the haemoglobine. It is known that haemoglobine contains .42 per cent. of iron, which is equivalent to .60 per cent. of ferric oxide (Fe_2O_3); consequently, if the amount of iron be ascertained and its equivalent of ferric oxide be calculated, and the amount of the latter be multiplied by 166.7, the result will represent the quantity of haemoglobine. A weighed or measured quantity of the blood is evaporated to dryness; the residue is deprived of all organic matter by heat; the ash is dissolved in pure dilute hydrochloric acid; the solution filtered and boiled with small pieces of pure zinc until it becomes colorless, or all of the iron is reduced to the condition of a ferrous compound. In the whole or a measured portion of this solution, the amount of iron is estimated volumetrically with a standard solution of permanganate of potash.

The standard solution of permanganate of potash should be graduated with the greatest care. For this purpose weigh out .7 grammes of pure double sulphate of iron and ammonia. This salt contains one-seventh of its weight of iron, consequently .7 grammes contain .1 gramme of metallic iron. Dissolve this weighed portion of the salt in water acidified with hydrochloric acid and dilute the solution to 50 c. c. To this solution in a beaker, add from a burette, a solution of permanganate of potash of indefinite strength drop by drop (constantly stirring the mixture) until a pale rose color appears and remains on stirring. Note the number of c. c. of the permanganate required and which represents

.1 gramme of iron. From this, the value of each c. c. of the permanganate solution is calculated and marked upon the bottle. Suppose that 20 c. c. of the permanganate solution were required to produce the rose color, then 20 c. c. are equivalent to .1 gramme of iron and 1 c. c. will represent .005 gramme.

The whole or a measured portion of the solution of blood-ash is now diluted to 50 c. c., and to this in a beaker, the permanganate solution is added as above until the pale rose-color remains; the number of c. c. of the standard solution used are noted, and from this the amounts of iron, of ferric oxide and of haemoglobine are calculated from the relations between these substances as already given.

(2) Hoppe-Seyler estimates the amount of haemoglobine by comparing the intensity of the color with that of a normal solution of the pure crystals, according to the following method which is taken from the Handbuch: Crystals of haemoglobine are prepared from the blood of the dog, goose, or guinea-pig, preferably of the latter, purified as already directed (p. 64) and dissolved in water at 0° and filtered. Exactly 50 c. c. of this solution are poured into a porcelain crucible (the weight of which is known) evaporated to dryness on the water-bath, dried at 110°, cooled over sulphuric acid and weighed. From this the amount of haemoglobine in each c. c. of the solution of crystals is estimated. From this solution (which is to be kept in a clean, corked flask) 10 c. c. are taken and diluted with from 10 c. c. to 60 c. c. of water and this is known as the dilute normal solution.

Dilute a small weighed quantity (not exceeding 20 grammes) of the blood to be examined, previously defibrinated, to 400 c. c. by the addition of distilled water. For the comparison of the color, two similar cylindrical flasks may be used; but it is better to have two vessels, each of which is made of two parallel plates of glass, which are 1 centimetre apart, and whose edges on three sides are united by metallic strips, thus forming a deep, thin vessel, the bottom and two sides of which are 1 centimetre broad and made of metallic strips, while the remaining two sides are formed by the plates of glass. Such an instrument is made and known as a haematinometer. Fill one of the glass cases or

cylinders with the dilute normal solution; into the other pour 10 c. c. of the dilute blood solution. Both vessels are placed side by side on white paper, and so that the light will pass through. The blood solution will always be darker than the normal. To the former add water, a c. c. at a time, stirring, until the color of the two solutions is the same. Note the amount of water which has been added to the 10 c. c. of dilute blood. It is necessary to make one or more confirmatory tests, using different dilutions of the normal solution. In order to do this, pour the contents from each of the vessels and cleanse the same thoroughly. Into one, pour 20 c. c. of the dilute normal solution and add 10 c. c. of water; into the other, pour 10 c. c. of the dilute blood solution and add water as before until the color of the two is the same. Then dilute the normal solution by the farther addition of 30 c. c. of water, and add water to the blood until the color is again the same. By these repeated experiments the chances of error are diminished. When the color of the two solutions is the same, equal volumes of the two solutions contain the same amount of haemoglobine, and from this the per cent. of this coloring matter in the blood may be calculated.

To illustrate this, suppose that 100 c. c. of the dilute normal solution contain .12 grammes of haemoglobine, and that it required the addition of 30 c. c. of water to reduce 10 c. c. of the dilute blood solution to the same color as that of the dilute normal solution; then each 10 c. c. of the 400 c. c. would require dilution to 40 c. c.; or for every 10 c. c., 30 c. c. of water must be added, or 1200 c. c. of water must be added to the 400 c. c.; consequently, 1600 c. c., the amount which would be if the whole of the blood were reduced to the color of the dilute normal solution, contain 1.92 grammes of haemoglobine. Suppose that the quantity of blood weighed for this estimation was 15 grammes, then in this case 15 grammes of blood would contain 1.92 grammes of haemoglobine, which is 12.8 per cent.

This method is easy of application and the normal solution may be kept for a week without decomposition; but the formation of the crystals is accomplished with facility only in cold weather.

(3) The third method of estimating the per cent. of haemoglobine contained in blood is known as that of *Preyer*, and is as follows: Place a concentrated aqueous solution of haemoglobine crystals in a haematinometer in front of the slit of a spectroscope; the light used being that of a petroleum lamp. To this, distilled water is added, with constant stirring, from a pipette graduated to one-hundredth of a c. c. as long as only red rays are transmitted, or until the green begins to appear. The per cent. of haemoglobine in this solution is estimated by evaporating a measured portion to dryness, drying and weighing as given in the preceding method. In this way the per cent. of haemoglobine required to allow the transmission of the red rays and the faint appearance of the green is ascertained.

The fresh blood, to be examined, is defibrinated by whipping, but is not filtered; a certain measured portion (perhaps .5 c. c.) is taken up with a pipette and placed in the haematinometer, care being taken that the position of the lamp is the same as when examining the solution of crystals. Water is now added from the finely divided pipette as before until the green begins to appear. If now we represent the per cent. of haemoglobine required in the solution of crystals by k , the volume of the blood placed in the hamatinometer by b , and the volume of water added to the blood by w ; then x , the per cent. of haemoglobine in the blood will be found by the following equation:

$$x = \frac{k(w+b)}{b}.$$



This substance, which is the chloride of haematin, does not exist preformed in the blood, but is prepared from haemoglobine.

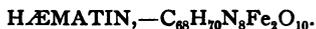
Preparation.—Sufficient crystals of haemin (known also as Teichmann's crystals) for microscopical examination may be obtained as follows: A small quantity of dry blood is rubbed up with a few crystals of sodic chloride; the powder is placed on a glass slide; a fine thread or a hair is laid through the powder across the slide so as to afford a means of escape to the bubbles of gas; a few drops of glacial acetic acid are added, and the whole is covered with a thin glass. The slide is now placed on

the water-bath and gently heated as long as air bubbles pass off. It is then removed, and the remaining acid is allowed to evaporate spontaneously. On examining this slide with the microscope, reddish-brown crystals of haemin with a metallic lustre will be observed. Other objects, as colorless crystals of sodic chloride and acetate with threads of coagulated albumen, will be seen; but the color of the haemin crystals will render their identification easy.

Haemin in quantity may be prepared as follows: The corpuscles of defibrinated blood are freed from serum according to the method given for the preparation of haemoglobine. The pulp of the corpuscles is transferred to a flask with a little water and shaken with half its volume of ether. After standing for a while, the ether is removed and discarded; the aqueous solution of the coloring matter is filtered and allowed to stand in a shallow dish at 50° until it acquires a syrupy consistency. This is shaken with from 10 to 20 volumes of glacial acetic acid, and the mixture heated on the water-bath for two hours. By this time the coloring matter will be converted into haemin crystals, and the albumen will be partially dissolved. The deposit is stirred up and the whole is transferred to a large beaker and three volumes of water are added. After two or three days, the supernatant liquid is decanted from the crystalline deposit; the latter is washed repeatedly with water by decantation, and then heated for several hours with glacial acetic acid, which dissolves remaining traces of albumen. The crystalline deposit is again washed with water by decantation, then collected upon a small filter and washed first with alcohol and then with ether.

The crystals of haemin have a reddish-brown or bluish-black color, a metallic tint, and are odorless and tasteless. When rubbed up, they form a yellow-ish brown powder. The powder and crystals are permanent at ordinary temperature and exposure; however, if the air contains a great excess of ammonia, haemin is gradually decomposed on exposure, with the formation of ammoniac chloride and ammoniacal haematin. The crystals form in rhombic plates with variations of many kinds; but those from the blood of various species of animals have no char-

acteristic form, or the form of the crystals is not determined by the animal from which the blood came. Haemin is insoluble in water and very sparingly soluble in hot alcohol or ether, soluble with decomposition in alkalis, forming an alkaline chloride and haematin. It may be heated to 200° without decomposition but when the temperature is raised above this point, the haemin is destroyed, hydrocyanic acid being given off and ferric oxide remaining as a residue. Compounds similar to haemin are formed by the action of hydriodic and hydrobromic acids upon haemoglobine. The iodide of haematin is a little darker than the chloride and the former has a violet tint; while the bromide is of a lighter red color than either of the other two.



Haematin is frequently found in old blood extravasations and in the intestines. In the former instance, it comes from the decomposition of haemoglobine; in the latter, from the action of the gastric juice upon the blood contained in the food, or it may have already existed as haematin in the food. For the reasons just given, haematin is frequently found in the faeces of the carnivora. It appears in the urine in certain diseased conditions of the kidney and in cases of arsenic poisoning.

Preparation.—Boil haemin crystals with glacial acetic acid, then wash them well with water, then with alcohol and ether. Dissolve the crystals in pure dilute potassic hydrate and filter; to the filtrate add dilute sulphuric acid; collect the brown precipitate which forms and wash this with water until the filtrate no longer gives a test for chlorine on the addition of nitrate of silver. The haematin now freed from chlorine is warmed at first gently and then heated to from 120° to 150° until dry.

Haematin is an amorphous, bluish-black substance, with a metallic luster and forms a dark-brown powder, insoluble in water, alcohol, ether and chloroform; soluble in dilute alkalis. It is soluble in acidified alcohol, but insoluble in slightly acidified water. Haematin may be heated to 180° without decomposition, but when the temperature is raised much above this point, it is decomposed, hydrocyanic acid being given off and pure ferric

oxide remaining as a residue. The amount of ferric oxide which remains is 12.6 per cent. of the weight of the haematin.

An alkaline solution of haematin, when examined in thin layers by transmitted light, presents a beautiful red color; when the light passes through thicker layers of the same solution, an olive-green tint is observed. Acid solutions have a brown color which is not influenced by variations in the volume of fluid through which the light passes. Both solutions absorb the violet end most notably and the extreme red end the least. A solution containing .015 gramme of haematin, one centimetre thick, presents an illy-defined absorption band between C and D, covering the latter. Haematin dissolved in alcohol acidified with sulphuric acid and placed before the spectroscope gives a band near C, between that line and D; another, less sharply defined, much broader and disappearing sooner on dilution, between D and F. This last band, by careful dilution, is broken into two bands of unequal distinctness; the one near F being the darker, the brightest interval being between E and b. A very much smaller band appears on dilution between D and E, near D. After treatment with ammoniac sulphide, tartrate of zinc, or other reducing agents, the solution of haematin changes its color, and on spectroscopic examination presents a small, dark, sharply-defined band between D and E, nearer the former, and a paler band which covers the lines E and b, the band of haemochromogen. (Hoppe-Seyler.)

If cyanide of potassium be added to an alkaline solution of haematin, the color immediately becomes reddish-brown, and on spectroscopic examination, a broad, illy-defined band similar to that of reduced haemoglobine is observed between D and E. This broad band is divided into small ones upon the addition of ammoniac sulphide or other reducing agents to the solution.

Compounds and Derivatives.—Haematin is precipitated from alkaline solutions on the addition of the chloride of either barium or calcium: the exact nature of the precipitate is not known. The most important compound of haematin is the chloride or haemin, the preparation and properties of which have already been described. Haematin is carried down mechanically with a pre-

$C_{34}H_{38}N_5FeO_5$. It readily takes up oxygen and is converted into haematin.

PLASMA.

We have seen that the principal office of the red corpuscle is to serve as a vehicle for carrying oxygen to the various tissues of the body ; but there must be some agent to convey the corpuscle, to bring to the tissues material for repair and to remove the debris. Oxygen alone can not support life : there must be something to combine with the oxygen in order to produce animal heat. Moreover, this combustion must go on in every part of the body ; even if it be true that the solid tissues enter but little into those chemical changes whereby life is supported, it is still necessary that combustion should take place in every organ. Let us suppose that the blood as it leaves the heart contains all of the oxygen and all of the material to be consumed, still life could not be maintained did this oxidation become complete immediately, or take place in one organ only ; the muscles of the arm and of every other part of the body alike need the production of heat within themselves before they can contract and relax ; the brain requires combustion within its substance, whether of its substance or not, before it can act. The plasma serves as the channel for the transmission of material which supports life and of that which is the product of decay. It is, as Bernard said, the internal medium which bears the same relation to the tissues as the external medium, the world, does to the individual. The composition of the plasma is necessarily very variable : at one time it may be bearing that which strengthens the body and elevates the mind ; at another time it may contain poisons which injure both body and mind.

In order to obtain a large quantity of plasma the following method may be used : Allow the blood from a vein of a horse to fall into a tall, narrow beaker which is surrounded by a freezing mixture. After two or three hours, three layers will be observed ; the lowest one is colored red and consists of the red corpuscles ; above this and occupying not more than one-twentieth the space, is a layer of white corpuscles, while the upper part of

the cylinder contains the plasma, which may be drawn off into another cooled cylinder.

Plasma kept at a temperature below 0° is a somewhat viscid, yellowish, strongly alkaline fluid. When the temperature is allowed to rise above 0° , the plasma is transformed into a jelly-like mass which gradually contracts and presses out a fluid resembling plasma in appearance and known as *serum*.

Coagulation.—When blood is drawn from a vein and subjected to ordinary temperature, it is soon transformed into the jelly-like mass mentioned above. The coagulation of blood may be hastened or retarded by many agents; thus the higher the temperature, within certain limits, the more rapid the coagulation; while at or below 0° coagulation does not take place; and again by the addition of large quantities of some neutral salts, as magnesian sulphate, the formation of the clot may be prevented. In the latter instance, coagulation takes place after the addition of a sufficient quantity of water.

The less oxygen and the more carbonic acid blood contains, the more slowly will it coagulate; for this reason arterial blood coagulates more rapidly than venous. This process goes on more rapidly in blood which is poor in morphological elements than in that which is rich in the same constituents; for this reason the blood of a hydræmic person coagulates rapidly.

The causes of coagulation and the chemical changes which take place in the blood during the formation of the clot are not fully understood; but the labors of Denis, Schmidt and others have been of great value and justify us in entertaining the belief that soon the mystery of this process will be removed by chemical investigation.

Plasmine.—Prevent the coagulation of plasma by cold and remove it from the corpuscles according to the method already given; or gently mix the blood as it flows from the vein with about one-third its volume of a saturated solution of magnesian sulphate, allow to stand until the corpuscles sink, and then remove the supernatant fluid. In either case, plasma free from corpuscles is obtained; by the first method coagulation is prevented by the cold, and would take place if the temperature were raised;

by the second method coagulation is prevented by the presence of the neutral salt, and would take place if the solution were diluted. To plasma obtained by either of these methods, add sodic chloride to saturation, when a white precipitate will appear. Wash this precipitate with an aqueous saturated solution of sodic chloride, then dissolve it in a little water and filter through a fast filter. Allow the filtrate to stand exposed to an ordinary temperature, and soon it will be observed to coagulate just as the plasma did. It is evident from this that the coagulation of plasma is dependent upon this substance which has been precipitated by the sodic chloride, and which is called *plasmine* by its discoverer, Denis.

Is this plasmine a compound body, and if so, what are its components? Serum nor hydrocele fluid either clots when kept separately; but if the two be mixed, coagulation occurs just as it does in plasma. Thus if some filtered hydrocele fluid be kept at from 38° to 40° , no coagulum appears, and the fluid will remain clear until decomposition takes place; but if a little serum be added, the mixture soon clots. This seems to be an answer to the question. *Plasmine* is a compound containing at least two substances, one of which is present in serum and the other in hydrocele fluid. By the labors of A. Schmidt each of these has been isolated, and the one from serum is known as *fibrinoplastin*, *paraglobulin*, or *fibrinoplastic globulin*; while the one from hydrocele fluid is known as *fibrinogen*. Both of these are present in plasma and the plasmine of Denis is a mixture of fibrinoplastin and fibrinogen.

Fibrinoplastin.—Dilute serum from the blood of the horse or ox with ten times its volume of water, add a few drops of dilute acetic acid, not sufficient to destroy the alkalinity, and then treat with a current of carbonic acid gas. Wash the granular precipitate of fibrinoplastin, which falls, with water, until the wash-water no longer contains chlorides (tested for with argentic nitrate) or albumen (tested for with acetic acid and ferrocyanide of potassium.)

Fibrinoplastin is insoluble in pure water, soluble in water containing much oxygen, soluble in dilute solutions of sodic chlo-

ride, sodic phosphate, and some other neutral salts. Dissolved in the above solutions, fibrinoplastin retains its active properties; for instance, if such a solution be added to hydrocele fluid, coagulation will take place. It is also soluble in acetic acid, but solution by this solvent destroys the activity of fibrinoplastin. The following table, taken from Hofmann's *Zoochemie*, shows the amounts of various substances required in 100 c. c. of water in order to dissolve 1 gramme of paraglobulin :

0.002	grammes of sodic hydrate.
0.017	“ “ “ carbonate.
0.034	“ “ “ bicarbonate.
0.046	“ “ “ acetic acid.
0.092	“ “ “ phosphate.
1.974	“ “ “ chloride.

Besides the method already given for its preparation, fibrinoplastin may be obtained by saturating serum with sodic chloride, when it falls as a flaky precipitate. If this precipitate be collected and treated with distilled water, it dissolves; because, when prepared in this way, the precipitate holds sufficient sodic chloride to cause the distilled water to act as a dilute solution of that salt. It is thus seen that while a saturated solution of common salt precipitates fibrinoplastin, a dilute solution of this substance dissolves this fibrine factor.

A filtered solution of fibrinoplastin in a dilute solution of sodic chloride does not clot: but if such a solution be added to hydrocele fluid, coagulation takes place. If the serum from which the fibrinoplastin has been removed be added to hydrocele fluid, no coagulum appears; thus it seems evident that one of the fibrine-factors is in excess in the plasma, and that this excess remains in the serum and may be extracted by the methods given.

Fibrinogen.—To a specimen of hydrocele fluid, which has been found to coagulate serum, carefully add finely pulverized sodic chloride to saturation. As soon as this point is reached, fibrinogen falls as a flaky precipitate and may be collected upon a filter, washed with a saturated solution of sodic chloride and dissolved in a little distilled water. This solution by itself does

not clot; but when added to serum causes coagulation. If the hydrocele fluid, from which the fibrinogen has been removed, be added to serum, coagulation does not occur. Again if a solution of fibrinoplastin, obtained by saturation with sodic chloride from serum, be added to a solution of fibrinogen, obtained by the same method from hydrocele fluid, coagulation takes place in a normal manner; while if the serum freed from its fibrinoplastin and the hydrocele fluid freed from its fibrinogen be mixed, no clot forms.

If instead of the saturation method, fibrinoplastin and fibrinogen be prepared by precipitation with carbonic acid, a mixture of the solutions of the two does not clot at all or does so imperfectly. This has given reason to suspect that either the fibrinoplastin or fibrinogen, as prepared by the saturation method, is a mixture of one or more substances. A. Schmidt has succeeded in isolating a third substance which he calls *fibrin-ferment*.

Fibrin-ferment.—To some serum add 20 times its volume of alcohol and allow the precipitate which forms to stand under alcohol for about three months. Dry the hardened precipitate over sulphuric acid, pulverize it and extract with water. Treat the aqueous solution with a current of carbonic acid gas and filter; repeat this as long as the solution gives any reaction for albumen. If this solution freed from proteids be added to a mixture of (fibrinoplastin and fibrinogen,) prepared by the carbonic oxide method, coagulation occurs readily.)

Fibrine.—The product of coagulation is fibrine. According to the views of Schmidt, it arises from the combination of fibrinoplastin and fibrinogen in the presence of the ferment. Hammarsten thinks that fibrine is simply converted fibrinogen and that fibrinoplastin does not enter into the composition of fibrine, but acts as a ferment rendering the transformation of the fibrinogen possible.

Whip freshly drawn blood with a piece of whalebone or with a bundle of glass rods and collect the coagulated fibrine on a filter; or take the clot from blood which has coagulated spontaneously; cut the fibrine into small pieces; place these in a linen sack; press and rub under water, changing the water as soon as it becomes colored, until the color is no longer imparted to the

fluid. Then place the sack containing the fibrine in a two per cent. solution of chloride of sodium and allow to stand, with frequent agitation, for two days. In this way, traces of globulins are removed. Then place the fibrine in distilled water for 12 days, changing the water daily; cover the fibrine with alcohol and allow to harden; cut into fine pieces and extract with ether in order to remove any fat. Even when prepared in this way, fibrine is not perfectly pure, but contains traces of fat, white corpuscles and inorganic salts.

Fibrine is insoluble in water, alcohol and ether, soluble in dilute alkalis forming albuminates. When digested with a two per cent. solution of HCl, fibrine is transformed into a semi-transparent, jelly-like mass. By the action of gastric juice, fibrine is converted into peptones, the change being a chemical one and not one of simple solution. If the gastric juice contains but little pepsine the products of the digestion of fibrine with this fluid will be precipitated by neutralizing the solution. By the action of pancreatic juice, fibrine is transformed into peptones, tyrosine and leucine. Fibrine contains 52.6 per cent. of C, 17.4 of N, 21.8 of O, 7.0 of H, and 1.2 of S.

It is sometimes desirable to estimate the amount of fibrine in a specimen of blood. This is best done by the following method which is taken from Hoppe-Seyler's Handbuch: A small beaker with a rubber cap is needed; through a small opening in the center of the cap is passed a piece of whalebone shaped like an oar. This should be of such a length that when the cap is placed on the beaker, the tip of the wide end of the bone should just touch the bottom of the beaker, while the other end should project above the cap sufficiently to be grasped and moved easily with the hand. The apparatus is carefully cleansed, dried and weighed. The cap is removed and from 30 to 40 c. c. of the blood to be examined are placed in the beaker. (If plasma is to be examined, it is removed with a pipette from the beaker surrounded by the freezing mixture.) The cap is now replaced and the blood stirred vigorously for 10 minutes with the bone. The apparatus with its contents is now weighed. The difference between the two weights now found will be the weight of the

blood taken. After weighing, the beaker is filled with water, the contents well mixed and then allowed to stand. As soon as the fibrine has completely subsided, the supernatant fluid is decanted into another beaker. By means of a dilute solution of sodic chloride, the fibrine is transferred to a small weighed filter and washed with distilled water until the filtrate is colorless. With a small, clean pair of pincers, any pieces of fibrine which may be found clinging to the whale-bone are removed and placed upon the filter. Finally, wash the fibrine several times with boiling alcohol in order to remove any fat. Then dry at from 110° to 120° in the air-bath, cool over sulphuric acid and weigh.

If it be desired, the decanted fluid and the wash-water may be mixed and the amount of haemoglobine which they contain, estimated according to one of the methods given for the estimation of this coloring matter.

The addition of a little chloride of sodium to the wash-water, as recommended above, dissolves out any fibrinoplastin that may adhere to the fibrine. In case of experimenting upon the blood of mammals, this addition of sodic chloride is of advantage only as it causes the fluid to filter more rapidly; for the amount of fibrinoplastin, which would remain undissolved with the fibrine in from 30 to 40 c.c. of blood, would not be sufficient to materially influence the weight. However, if the blood under examination be that of a bird or an amphibian, the fibrine should be well washed with a solution containing from one to three per cent. of sodic chloride; then with water and alcohol.

In order for this estimation to be exact and easy of performance, it is necessary that the fibrine should be washed by decantation until the supernatant fluid is perfectly clear; for if the fibrine be brought upon the filter, before it has been well washed, the fluid will filter so slowly that the fibrine will partially decompose before it can be weighed.

- *Serum*.—It has already been mentioned that after plasma coagulates and as the clot contracts, a clear fluid separates and surrounds the coagulum. This is serum and is equivalent to the plasma minus the fibrine. It is colored partly by small quantities

of dissolved haemoglobin and partly by a coloring matter peculiar to itself. When examined with the spectroscope, the lines characteristic of haemoglobine are observed. Serum has a specific gravity of from 1027 to 1030, has a more decidedly alkaline reaction than plasma and is coagulated on being boiled with mineral acids and many dilute metallic salts.

The various transudations, contents of cysts and synovial fluid resemble serum and the methods to be given for the examination of the latter will apply to all serous fluids. These, with an occasional exception, are more or less alkaline in reaction and vary in specific gravity from 1002 to 1030. Some are viscid and can be drawn out into threads; while others are thin and contain much water. Some are perfectly clear, while others are colored with blood or bile, or are rendered turbid by the presence of epithelial scales, pus corpuscles, fat, threads of fibrine or crystals of cholesteroline. Some of these morphological elements can be removed by decantation or filtration; while finely divided fat can be removed only by repeated agitation with ether. These fluids contain albuminous substances, fats, extractives and organic salts. Upon microscopical examination, most serous fluids will be found to contain cytoïd corpuscles. The corpuscles of Gluge are not unfrequently found in the fluid contents of various tumors; these are larger than the white corpuscles of the blood, are granular in appearance and consist of coherent granules of fat, deprived of the cell wall. After removal from the body, some serous fluids coagulate spontaneously but slowly; while others do not coagulate at all. Of those of the latter class, coagulation may be caused in some by the addition of fibrin-ferment and in others by the addition of fibrinoplastin.

The *pericardial fluid* is of a yellowish color and, if it be removed immediately after the death of the animal, coagulates; but if it is not removed until some hours after death it does not coagulate spontaneously, but will do so after fibrinoplastin, prepared by the saturation method, has been added. It is very rich in fibrinogen and contains about five per cent of solids. Of the solid constituents, one-fourth is albuminous, and in structural diseases of the kidneys, this amount is increased. In cases of exces-

sive accumulation of this fluid, it has been found to contain crystals of cholesterine, uric acid and urea.

4. *Hydrocele fluid* is an abnormal substance which sometimes accumulates in the serous sac of the testis. It is of a greenish-yellow tint, varies in specific gravity generally from 1010 to 1025 and contains from 5 to 50 per cent. of solids. Hydrocele fluid is rich in fibrinogen and seldom or never clots spontaneously, but does so after the addition of fibrinoplastin. In some specimens, a peculiar kind of fibrinogen, which forms an easily soluble fibrine, is found. Sugar, urea and uric acid have been found in hydrocele fluid.

Peritoneal fluid is found in that serous sac known as the peritoneum. Normally, the amount of this fluid is very small: but in ascites the accumulation is often considerable. The following remarks apply only to the pathological fluid: in appearance this fluid varies very much; sometimes it is clear and colorless, and at other times, it will be found milky and containing much finely divided fat. It may contain urea, uric acid, xanthine, creatine, cholesterine, lecithine, fat, and albuminous substances. In some cases, small moving parasites are observed. The specific gravity varies from 1005 to 1020.

Pleural fluid, arising from certain pathological conditions, is either alkaline or acid in reaction; the acid fluid always contains pus and the acidity is probably due to the decomposition of the pus corpuscles. The specific gravity varies from 1005 to 1030; the specimens which contain pus are of greater specific gravity than those free from that morphological constituent. The gas, which collects in pneumothorax, is composed of CO_2 , O , and N , with H_2S gas as an occasional constituent.

Cerebrospinal fluid is clear, strongly alkaline and contains but a small amount of solids: consequently the specific gravity is low, from 1002 to 1007. It contains cholesterine, urea and mucine, also a substance which reduces copper, but which has not yet been isolated. Of the solids, only traces are organic; while the inorganic salts are represented by the chlorides, sulphates and phosphates of sodium and potassium.

Aqueous humor is clear, of feebly alkaline reaction and does

not coagulate either spontaneously or when heated; because it contains no fibrinogen and only traces of fibrinoplastin. Dr. Bence Jones found that after the administration of quinia and many other therapeutic agents, these could be detected in the humors of the eye. (See Lectures on Pathology and Therapeutics, p. 12 et seq.)

Synovial fluid has a faintly yellow tint, is alkaline and contains mucine, albumen, extractives, fats and inorganic salts. The proportion of the constituents varies as the animal, from which the fluid is taken, has been quiet or moving. In the following table, taken from Hofmann's Zoochemie, the first column gives the composition of synovial fluid as taken from an ox which had been confined in a stall, and the second, of that taken from an ox which had been driven constantly:

	1.	2.
Water.....	969.9	948.5
Solids.....	30.1	51.5
Mucine.....	2.4	5.6
Albumen and Extractives.....	15.7	35.1
Fats.....	0.6	0.7
Salts.....	11.3	9.9

It will be seen from this table that the proportion of water is decreased by exercise.

The Amniotic fluid of the human subject is of a yellowish or brownish color, with a stale odor and a feebly alkaline reaction. This fluid is frequently turbid and on standing deposits a white flaky sediment, which on microscopical examination is seen to consist of epithelial scales. The specific gravity is variable, ranging from 1002 to 1030. It contains serum-albumen, fibrinoplastin, urea, creatinine and occasionally sugar and ammoniac carbonate; the latter probably arises from the decomposition of urea.

These various serous fluids have been mentioned in this place, because the methods to be given for the examination of serum will apply to the other serous fluids and in this way unnecessary repetition may be avoided.

Examination of the Albuminous Substances in Serous Fluids.—The following methods are taken from Hoppe-Seyler's Handbuch. Besides serum-albumen, other albuminous sub-

stances, especially one or both fibrine-factors, are frequently found. In ovarian cysts, a caseous substance is present.

If both fibrinogen and fibrinoplastin be present and the temperature, reaction and contained salts be favorable, the fluid will, sooner or later after its removal from the body, partially or completely coagulate: the fibrine thus formed will have the properties already described.

Whether coagulation takes place or not, it is necessary to test for the presence of globulins. Dilute a portion of the fluid with from 10 to 20 times its volume of water and add, drop by drop, very dilute acetic acid as long as the precipitate increases; or it is better to treat the fluid, after the addition of water and dilute acetic acid, with a stream of carbonic acid gas and allow to stand for some time. If the fluid becomes turbid on dilution and deposits a flaky precipitate on the addition of the acid, then it contains some substance which belongs to the albuminates or globulins.

Decant the supernatant fluid and heat a portion in a test tube; if it coagulates the fluid contains *serum-albumen*.

Suspend the precipitate, from which the greater part of the supernatant fluid has been decanted, in the remaining fluid and divide into two parts; to one of these add a few drops of a concentrated solution of chloride of sodium; if the precipitate dissolves, the fluid under examination contains *fibrine-factors* or *myosine*. If the precipitate does not dissolve on the addition of the salt solution, it consists of *casein*.

To the second portion of the suspended precipitate add twice its volume of a one-tenth per cent. solution of HCl; if the precipitate dissolves it consists of *fibrine-factors*, *myosine* or *casein*.

To another portion of the fluid under examination, add a few drops of serum pressed from a recently formed clot, shake and set aside in a warm place for a day, observing from time to time whether coagulation takes place or not. Should a coagulum form, it is evidence that the fluid contained *fibrinogen*.

To still another portion of the original fluid or to some of the precipitate (which has been thrown down on dilution and treatment with a current of carbonic acid gas) redissolved in dilute

sodic hydrate, add some hydrocele fluid and allow to stand for one day. If a coagulum forms, *fibrinoplastin* is contained in the fluid.

Synovial, and some other fluids owe their viscosity to mucine or paralbumen. If mucine be present, the addition of acetic acid will throw down a precipitate which is not soluble in an excess of the acid, nor in a solution of sodic chloride. If paralbumen be present, the addition of acetic acid causes a turbidity which disappears on the further addition of the acid. As a confirmatory test for paralbumen, precipitate the fluid by the addition of three times its volume of alcohol, filter and dissolve the precipitate in water; if paralbumen were present, the aqueous solution would be viscid and would pass through a filter slowly.

Test for Sugar in Serous Fluids.—Dilute the fluid with an equal volume of water, acidify with acetic acid, boil, remove the coagulum, which forms, by filtration and test the filtrate for sugar with Fehling's solution. If it be desired, the per cent. of sugar contained in the fluid may be ascertained as follows: Dilute a weighed portion of the fluid with water, acidify with acetic acid, boil, remove the coagulum by filtration, concentrate the filtrate on the water-bath to a syrup, extract this with boiling alcohol, filter, evaporate the alcoholic solution to dryness on the water-bath, dissolve the residue with water, and estimate the amount of sugar in the aqueous solution with Fehling's solution according to the method given for the estimation of sugar in the urine.

Test for Urea.—Agitate a measured or weighed portion of the serum or other fluid with three times its volume of strong alcohol; collect the precipitate upon a filter; wash well with cold alcohol; evaporate the united wash-water and filtrate at a gentle heat on the water-bath; extract the residue with cold absolute alcohol and filter; evaporate this filtrate as before; dissolve this residue in a little water, and estimate the amount of urea in the aqueous extract with a standard solution of mercuric nitrate as directed for the quantitative estimation of urea in the urine. In order to confirm this estimation, collect the precipitate formed by the mercuric nitrate, on a small filter; wash with water; suspend the precipitate in water and treat with a current of H_2S gas;

remove the precipitated sulphide of mercury by filtration ; concentrate the filtrate on the water-bath to a small volume ; place this concentrated solution in a strong tube, add a few crystals of pure baric chloride, and then a small quantity of a solution of baric chloride rendered alkaline with ammonia ; the tube, which should not be more than half full, is now hermetically closed and heated on an oil-bath at 200° for four hours. The heat decomposes the urea, forming ammonic carbonate which precipitates the barium. The tube is now opened with a file, and the baric carbonate collected upon a filter, and well washed with water. The filtrate and wash-water are now discarded and the precipitate dissolved in dilute HCl. The barium is precipitated from this solution as a sulphate by the addition of dilute sulphuric acid. The mixture is heated and filtered while hot. The filter with its contents is dried, the baric sulphate and filter-ash placed in a weighed crucible, heated to redness, cooled over sulphuric acid and weighed ; each part of baric sulphate represents .2574 parts of urea. (Hoppe-Seyler.)

Test for Creatine.—Dilute some of the fluid, acidify with acetic acid, boil and filter. To the filtrate, add basic acetate of lead as long as the precipitate increases, but avoiding an excess of this reagent, and again filter. From the filtrate remove the lead by precipitation with H_2S gas and filtration. Concentrate the filtrate to a small volume and allow to stand for some days, when creatine crystallizes. (For the appearance and further examination of the crystals, see under creatine.) This substance is abundant in the serum of typhus patients.

Test for Uric Acid.—Remove the albumen by boiling and filtering through cloth ; evaporate the filtrate to dryness ; treat the residue with boiling water and filter while hot ; concentrate this filtrate to a small volume ; acidify strongly with acetic acid and allow to stand for a day or two when crystals of uric acid will be deposited and may be recognized by microscopic appearance and by the application of the murexide test. (See uric acid.) Urates are in excess in the serum in instances of deficient oxidation, arthritis and valvular disease of the heart.

Test for Tyrosine and Leucine.—To some of the fluid, add

basic acetate of lead as long as a precipitate forms, but avoiding an excess, and filter; from the filtrate remove the lead by H_2S gas and filtration; concentrate this filtrate on the water-bath to a syrup and extract the residue with alcohol, and filter. The alcohol dissolves the greater part of the impure leucine and but traces of tyrosine. Evaporate the alcoholic solution and extract the residue with ammoniac hydrate. To the ammoniacal solution add plumbic acetate as long as a precipitate forms. Suspend the precipitate, which consists of the oxide of lead and leucine in water; treat with H_2S gas and filter. Concentrate the filtrate when leucine crystallizes. In the residue, insoluble in alcohol, tyrosine forms in needle-shaped crystals. (See tyrosine and leucine.) These substances are found in the serum, and transudates in cases of structural diseases of the liver.

Test for Bile Acids.—Remove the albumen with acetic acid and heat. Evaporate the filtrate to a syrup on the water-bath, extract with absolute alcohol and filter. Evaporate the filtrate; extract the residue with water to which a few drops of a dilute solution of sodic hydrate have been added; filter and to this filtrate apply Pettenkoffer's test. (See p. 34.)

Estimation of the Amount of Albumen.—To 100 c. c. of boiling water acidified with acetic acid, add gradually a weighed or measured portion of the fluid under examination. As the fluid is added the reaction of the boiling water should be tested from time to time, and if it becomes neutral or alkaline, a few drops of acetic acid should be added. Collect the albumen upon a weighed filter, wash with water, and then with boiling alcohol. Dry the filter with its contents at 100° , heat to 120° , cool over sulphuric acid and weigh. Repeat the heating, cooling and weighing until the weight remains constant.

Estimation of the Fat.—Evaporate a measured or weighed portion of the serous fluid to dryness. Pulverize the residue and extract repeatedly with ether. Place the united ethereal solutions in a weighed dish, evaporate to dryness, cool and weigh.

Estimation of the Amount of Solids.—A measured or weighed portion of the fluid is placed in a small weighed dish or

crucible and evaporated to dryness at the temperature of the water-bath. The residue is kept for 12 hours at 100° , then heated to 110° , cooled over sulphuric acid and weighed. The heating, cooling and weighing are repeated until the weight remains constant. From this the per cent. of both the water and the solids may be calculated.

Estimation of the Inorganic Salts.—If the residue, obtained as above and containing all the solids, be deprived of organic matter by burning and the ash be cooled and weighed, the per cent. of inorganic salts may be calculated. If it be desired to ascertain the amount of soluble and insoluble salts separately, the latter may be obtained by precipitating the albumen in a measured portion of the fluid, collecting the coagulum upon a Swedish filter paper (which has been deprived of inorganic matter by being first washed with dilute HCl and then with water until the wash-water has no longer an acid reaction), burning the filter with its contents and weighing the ash.

THE CORPUSCLES.

Some reliable method of estimating the amount of corpuscles contained in specimens of blood has long been needed. Moreover, it is desirable to know the weight of the corpuscles in the moist, and not in the dry state. We wish to know the amount of the corpuscles as they exist in the blood and any method, by which the weight of dried corpuscles or of those changed either physically or chemically is obtained, is not the method desired. The efforts made in this direction by some of the most noted chemists have been more or less successful as will be seen from the following processes:

(1) To Hoppe-Seyler belongs the honor of introducing the first method here given for estimating the amount of the moist corpuscles. It is known that the corpuscles of the blood of mammals contain a trace of fibrine-forming substances, but the amount is so small that it may be overlooked and we may consider that the fibrine is furnished wholly by the plasma. Now, if the fibrine in a weighed portion of blood and that in a weighed portion of plasma from the same blood be estimated, the weight of the plasma contained in a certain amount of blood may be

calculated and this weight subtracted from the weight of the blood will give the weight of the corpuscles. Thus, suppose that b grammes of blood yield c grammes of fibrine and that b grammes of plasma from the same blood yield d grammes of fibrine; then b grammes of blood contain $\frac{c}{d}$ parts of b grammes of plasma and if we represent the weight of the corpuscles contained in b grammes of blood by x , its value is found from the following equation :

$$x = b - \frac{bc}{d}$$

This method is applicable only to those specimens of blood which coagulate slowly and in which the corpuscles sink rapidly, as the normal blood of the horse, and that of men suffering with inflammatory disease.

The method is applied as follows: Draw a considerable portion of the blood from the vein into a cylinder surrounded by a mixture of ice and salt and another smaller portion, from 30 to 40 c.c., into the beaker for estimating fibrine (see p. 86), agitate, collect, wash, dry and weigh the fibrine as already recommended. After the corpuscles of the first portion have completely subsided, a small quantity of the plasma is transferred, by means of a cooled pipette, to the beaker, weighed and the amount of fibrine, which it contains, estimated.

It is necessary that the fibrine be estimated with the greatest care; for the amount of this substance obtained from blood is so small that a slight error in the estimation of it would be greatly magnified when the amount of plasma is calculated.

(2) This method consists in estimating the moist corpuscles from the amount of albumen and haemoglobine which they contain. In this, four things are necessary; (*a*) the total amount of albumen and haemoglobine in a certain portion of the blood must be ascertained; (*b*) the amount of albumen and haemoglobine in the corpuscles is found; (*c*) the quantity of albumen contained in a given amount of serum must be known; (*d*) the weight of the fibrine that can be obtained from a weighed portion of the blood is to be noted.

(*a*) Collect from 30 to 40 c.c. of the blood in a weighed dish or crucible, cover with a weighed watch-crystal, and weigh; then evaporate to dryness on the water-bath, transfer the residue to a mortar, washing the dish with alcohol and adding the washings to the contents of the mortar; rub up the mixture well and place in a beaker, washing all traces of the blood from the mortar into the beaker with alcohol; boil the mixture and collect the coagulum upon a small weighed filter, which has been freed from ash. Boil several successive portions of alcohol in the beaker and pour upon the same filter. The contents of the filter are washed with ether, then with distilled water and finally with boiling absolute alcohol. The filter with its contents is now dried at 100° , then heated to 120° for a short time, cooled over sulphuric acid and weighed. The heating, cooling and weighing are repeated until the weight remains constant. This (— the weight of the filter) gives the weight of the albumen + the haemoglobine + the insoluble salts. The filter with its contents is now placed in a small, weighed, open dish and heated until all the organic matter is destroyed. The ash is cooled over sulphuric acid and weighed. By subtracting the weight of the ash from that of the albumen, haemoglobine and ash, the weight of the first two in the blood is ascertained.

(*b*) A second portion of blood, from 30 to 40 c.c., is received in the fibrine apparatus, stirred, weighed, filtered through calico, diluted with 10 times its volume of a solution of sodic chloride, (made by mixing one volume of saturated sodic chloride solution with nine volumes of water). Allow to stand for 24 hours, or until the corpuscles have completely subsided; decant the supernatant fluid; wash the corpuscles once or twice by decantation with the salt solution; finally, transfer the corpuscles with a little water to a small dish; evaporate to dryness on the water-bath; rub the residue in a mortar with alcohol, and ascertain the amount of albumen and haemoglobine as given under (*a*).

(*c*) A third portion of the blood, from 80 to 100 c.c., is allowed to coagulate in a porcelain capsule; the separated serum is poured into a second dish, weighed, evaporated, dried, rubbed with alcohol and the amount of albumen estimated as in (*a*).

(*a*) The fourth portion, from 30 to 40 c.c., is collected in the fibrine apparatus, stirred, weighed, and the fibrine is estimated by the method already given.

In (*a*), we have found the weight of the albumen and haemoglobine contained in the blood; in (*b*), the weight of the albumen and haemoglobine in the corpuscles; in (*c*), the proportion of albumen in the serum; in (*d*), the amount of fibrine in the blood. It is now necessary to calculate each of these for the same weight of blood (100 grammes). After having done this, it is easy to understand and apply the following principles: (1) the albumen + the haemoglobine of the blood — the albumen + the haemoglobine of the corpuscles = the albumen of the serum; (2) that after the proportion of albumen contained in a weighed portion of serum has been ascertained as in (*c*), the quantity of serum represented by the albumen calculated in (1) may be found; (3) that the fibrine + the serum = the plasma; (4) that the blood — the plasma = the moist corpuscles.

(3) There have been various methods proposed for numbering the corpuscles in a given volume of blood and thus ascertaining whether the proportion be normal or not. While this work deals with chemical and not with microscopical processes, it will not be amiss in this place to mention the most reliable method for the enumeration of blood corpuscles. This consists in the use of Dr. Gower's modification of the *Haemacytometer* of MM. Hayem and Nacet. The following is taken from Dr. Gower's description of the instrument, which has been furnished the author by the maker, Mr. Hawksley, of London:

"The Haemacytometer consists of (1) a small pipette, which, when filled to the mark on its stem, holds exactly 995 cubic millimetres. It is furnished with an India rubber tube and mouth-piece to facilitate filling and emptying. (2) A capillary tube marked to contain exactly 5 cubic millimetres, with India rubber tube for filling, etc. (3) A small glass jar in which the dilution is made. (4) A glass stirrer for mixing the blood and solution in the jar. (5) A brass stage plate, carrying a glass slip, on which is a cell, one-fifth of a millimetre deep. The bottom of this is divided into one-tenth millimetre squares. Upon the top

of the cell rests the cover glass, which is kept in its place by the pressure of two springs proceeding from the ends of the stage plate.

“ Various diluting fluids have been recommended in order to change as little as possible the aspect of the corpuscles. It is not well, however, to observe the characters of the corpuscles during the numeration. Whatever solution be employed, the corpuscles are more or less changed by it. One which answers very well is a solution of sulphate of soda in distilled water, of a specific gravity of 1025.

“ The mode of proceeding is extremely simple. Nine hundred and ninety-nine cubic millimetres of the solution are placed in the mixing jar ; five cubic millimetres of blood are drawn into the capillary tube from a puncture in the finger, and then blown into the solution. The two fluids are well mixed by rotating the stirrer between the thumb and finger, and a small drop of this dilution is placed in the center of the cell, the covering glass gently put upon the cell, and secured by the two springs, and the plate placed upon the stage of the microscope. The lens is then focussed for the squares. In a few minutes the corpuscles have sunk to the bottom of the cell, and are seen at rest on the squares. The number in ten squares is then counted, and this multiplied by 10,000 gives the number in a cubic millimetre of blood.

“ The average of healthy blood was decided by Vierordt and Welcker to be 5,000,000 per cubic millimetre, and later results agree with this sufficiently nearly to justify the adoption of this number as the standard, it being remembered that in a healthy adult man the number may be a little higher, in a woman a little lower. The number per cubic millimetre is the common mode of stating the corpuscular richness of the blood ; but by employing this dilution, and squares of this size, a much more convenient mode of statement is obtained. Taking 5,000,000 as the average per cubic millimetre for healthy blood, the average number in two squares of the cell is 100. These two squares contain .00002 cubic millimetre of blood, and it is proposed to take this quantity as the ‘haemic unit.’ The number per haemic unit, i. e., in two

squares (ascertained by counting a larger number, 10 or 20, and taking the mean) thus expresses the percentage proportion of the corpuscles to that of health, or made into a two-place decimal, the proportion which the corpuscular richness of the blood examined bears to healthy blood taken as unity. This is a much more simple method than any hitherto used. The proportion of white corpuscles to the red, or their number per haemic unit, is best ascertained by observing the number of squares visible in the field of the microscope, and noting the number of white corpuscles in a series of ten or twenty fields. The number of red corpuscles corresponding to the ten or twenty fields is easily computed, and thus the proportion of white to red is ascertained. The normal *maximum* of white per two squares (haemic unit) is .3." (For further details see Practitioner, August, 1878.)

WHITE CORPUSCLES.

The proportion of white to red corpuscles varies as the animal fasts or eats. The author found that when his meals were taken at 8 A. M., 1 P. M. and 6 P. M., and no food was taken between meals, that the greatest scarcity of white corpuscles (ascertained from numeration of the corpuscles in a drop of blood from the finger) was apparent about 7 A. M. or just before breakfast, when the proportion of white to red was as 1 to 1800. The white were observed to be most abundant from 2 to 4 P. M., when the proportion was frequently 1 white to 200 red. In the blood of hibernating animals, examined about the close of the period of hibernation, the proportion has been observed to be one white, to many thousand red corpuscles.

The proportion between the white and red corpuscles varies in blood taken from different parts of the body. Hirt found in the arterial blood of the spleen 1 white to 2179 red corpuscles, and in the venous blood from the same organ 1 white to 70 red. In the hepatic veins the proportion is generally about 1 white to 180 red corpuscles. When the spleen is enlarged, the proportion of white corpuscles is generally greatly increased. I found in a case of this kind that the blood taken directly from the spleen, by means of a hypodermic syringe, two hours after a meal contained 1 white corpuscle to 15 red ones. (See Mich. Med. News, March

25, 1878.) The exact relation of the spleen to the white corpuscle is not understood; for it is well known that excision of that organ does not permanently influence either the absolute or relative number of either the white or red corpuscles.

In leucocythemia, besides the great abundance of white corpuscles, crystals consisting of double pyramids are not unfrequently observed. These may be mistaken for oxalate of lime, are insoluble in water, alcohol, ether and chloroform, soluble in acetic, tartaric and phosphoric acids and in alkalis. In dilute mineral acids, these crystals are very soluble, but in the concentrated acids they do not dissolve but lose their firmness and can be flattened by pressure on the thin glass cover. In some instances, the points of the pyramids are drawn out into a fine line. These are known as the crystals of *Charcot-Neumann*, and their relation to the white corpuscles or their pathological significance is not known. In the case of enlarged spleen above referred to and in which I found the proportion of white to red corpuscles as 1 to 15 in the blood from the spleen, the same blood contained a great number of these crystals.

Chemistry of the White Corpuscles.—The colorless corpuscles consist of a membrane enclosing a semi-fluid mass. On account of the facility with which many fluids pass through this membrane, the corpuscles swell, lose their granular appearance and often burst upon the addition of water or dilute acids. On the contrary, solutions of the caustic alkalis, alkaline salts, bile, and sodic taurocholate and glycocholate cause the corpuscles to contract and finally disappear. Fat is a constituent of the white corpuscle. Blood in leucocythemia is very rich in lecithine and the excess of this constituent is contained in the white corpuscles; for the serum from such blood contains only traces of lecithine.

THE BLOOD IN DISEASE.

It is necessary for the physician to know something of the condition of the blood in disease; for in this way, he acquires a more thorough knowledge of the nature of the disease and will be better able to base his treatment upon rational principles. It is true that this subject has not received the attention due it, but the intelligent physician will avail himself of what is already

known, and endeavor to increase the number of facts by his original investigations. In cholera, the amount of the water of the blood is diminished by transudation from the capillaries of the intestines; moreover many inorganic salts pass out with the water; consequently we find the blood of the cholera patient poor in water and inorganic salts, and rich in corpuscles, albumen, fat and urea, and containing carbonate of ammonia as an abnormal constituent arising from the decomposition of the retained urea. The quantity of fibrine is increased in inflammatory diseases, and in some cases as much as ten parts per thousand have been obtained. In some structural diseases of the liver, especially in the so-called yellow atrophy of that organ, tyrosine and leucine are contained in the blood, and may be obtained from the serum by the method already given. In puerperal fever, free lactic acid and bile-pigments are found in the blood; while of the normal constituents, the corpuscles, fibrine and albumen are increased. In diabetes, an excess of sugar is found in the blood; the sugar is not consumed, and when it accumulates in the blood, the excess escapes through the kidneys. In structural disease of the kidney, urea is found in the blood in excessive quantity.

The condition of the blood in various diseases is here represented in tabular form. The table slightly modified is taken from the *Lehrbuch* of Gorup-Besanez. The sign + represents an increase, and the sign - represents a decrease, while 0 is used when there is no characteristic variation in the constituent:

CONSTITUENTS OF THE BLOOD.

DISEASES.	ABNORMAL CONSTITUENTS.	Water.	Fibrine.	Corpuscles.	Albumen.	Urea.	Sugar.	Fat.	Salts.
Inflam. Diseases	o	o	+	—	—	+	o	+	o
Acute Exanth'm	o	o	o	—	o	o	o	+	+
Malaria	Bile-Pigment	o	—	+	—	+	o	—	+
Morbus Brighti	o	o	+	!	—	+	o	+	+
Plethora	o	o	o	+	+	o	o	o	o
Chlorosis	o	+	+	—	o	o	o	o	o
Hydræmia	o	+	—	—	—	o	o	o	o
Puerperal Fever	Bile-Pigment and free Lactic Acid	o	+	—	—	o	o	o	o
Pyæmia	o	o	—	white+	—	o	o	o	o
Cholera	Carbonate of Ammon.	—	o	+	+	+	+	+	—
Dysentery	o	o	+	—	—	o	o	o	+
Atroph. of Liver	Tyrosine and Leucine	o	o	o	o	o	+	+	+
Arthritis	Uric Acid	o	o	o	o	+	o	o	o
Diabetes	o	+	o	o	o	o	+	o	o
TYPHUS :									
1. First stages	o	—	+	+	+	o	o	o	o
2. Later stages	o	+	o	—	—	o	o	o	+
Uremia	Carbonate of Ammon.	o	o	o	o	+	o	o	o
Yellow Fever	o	o	o	o	o	+	o	o	o
Scurvy	o	+	+	—	o	o	o	o	+
Chyluria	o	+	+	o	o	o	+	+	o
Icterus	Bile-Acids and Pig'nts,	o	o	o	o	o	o	+	o
Cancerous Dys-thetica	o	o	+	o	o	o	o	o	o
Leucocythemia	Uric Acid, Hypoxanthine, Leucine, Lactic and Acetic Acids and Crystals of Charcot-Neumann	o	o	white+	o	o	o	o	o

EXAMINATION OF BLOOD STAINS.

The reputation, and in some instances the life of an individual depend upon the decision as to whether certain stains are produced by blood or by other coloring matters. Upon this subject several questions may arise and be of legal importance. These are (1) is the stain that of blood? (2) is it the blood of man or of some of the lower animals? (3) is it menstrual blood or not? These stains may be upon some article of clothing, upon wood, iron, dirt, grass, etc. In some instances the stains will afford abundance of material for examination; while in others only traces may be present, and these may be mixed with or covered by some other substances; thus a stain which has been upon

iron for a long time may be partially or wholly covered with rust.

Haemin Test.—The most reliable test for blood in stains consists in an examination for the crystals of haemin. This test is made as follows: The stains are first separated from the material on which they are deposited; if on iron or stone by scraping, if on wood by a sharp knife, if on cloth by rubbing with a little cold water. The dry stains or the residue obtained by evaporating the solution, (if cold water has been used to remove the stain) are covered with a small quantity of glacial acetic acid, boiled gently for a short time, then transferred to a watch-crystal and concentrated on the water-bath. If blood be present in quantities not too small, crystals of haemin appear when the solution has been evaporated nearly to dryness. Under the microscope these appear as rhombic tablets of a reddish-yellow or brown color. These crystals are insoluble in water, alcohol, ether, chloroform, dilute hydrochloric, acetic, and phosphoric acids. In potassic hydrate they dissolve slowly, forming a brown solution which on standing becomes purple.

✧ It is necessary to remember that in order to obtain the crystals of haemin the blood must contain a small quantity of sodic chloride; now this salt is removed from dried blood with warm water; consequently if the stain has been washed with warm water before examination, it is necessary to add a small quantity of sodic chloride with the glacial acetic acid.

If the stain be upon cloth which has been washed with warm water, it is better to cut out a small piece covered by the stain, place it in a test tube, add glacial acetic acid and sodic chloride boil, decant, or filter, and evaporate the solution on the water-bath, when, if blood were present, crystals of haemin will be obtained. If the piece of cloth be thin and transparent, it may be placed on a glass-slide, a few grains of salt and a few drops of glacial acetic acid added, the whole covered with a thin glass, heated on the water-bath, and examined from time to time under the microscope. Should this test fail at first, more acid is added and the process repeated several times.

Crystals of haemin after once being seen can hardly be mis-

taken for anything else. Cloth colored with indigo, when treated with glacial acetic acid as recommended above, yields crystals; but these are colored blue always, and are not at all similar in appearance to the crystals of haemin. The greatest objections to the haemin test for blood in stains are: (1) the crystals cannot be obtained when any substance is present which forms an insoluble compound with haematin; thus, they can not be obtained when the stain has long been covered with iron rust; (2) they can not be obtained when the stain is mixed with any decomposing matter, as with excrement. If a drop of blood be deposited upon a clean surface, haemin crystals may be obtained from it many years afterwards; thus Scriba prepared crystals of haemin in 1860 from blood-stains deposited on paper in 1820.

Spectroscopic Test.—Digest the stain on the cloth, wood, iron or stone with water to which a few drops of ammoniac hydrate have been added. Bring the solution, filtered if necessary, before the spectroscope. If the characteristic lines of haemoglobine appear, the presence of blood is certain. However, the non-appearance of these lines is not evidence sufficient of the absence of blood. It then remains to examine for haematin, and this examination is made as follows: To the ammoniacal solution, add glacial acetic acid sufficient to produce an acid reaction. Pour the solution into a graduated jar, add an equal volume of ether, shake well, and then allow the ether to separate. Should the ether not separate readily, add a little more of the glacial acetic acid. Remove the ethereal solution, which is of a brown color, and examine with the spectroscope; when if the stains were those of blood, the lines of haematin in an acid solution will be observed. These lines consist of a dark, sharply defined one in the red, and a less distinct one in the green.

Test for Soluble Albuminates.—Treat the stains with cold distilled water, when the coloring matter of the blood will be imparted to the water. Not unfrequently microscopical examination will reveal in the water fibres of undissolved fibrine. Upon heating some of the filtered aqueous solution, a cloudiness from the coagulated albumen appears. Another portion of the filtrate is precipitated upon the addition of nitric acid. Chlorine water

at first colors the solution green ; but the further addition of chlorine destroys all the color and deposits a white flaky precipitate. If this precipitate be removed by filtration, and the filtrate be concentrated to a small volume, the test for iron will be obtained on the addition of sulphocyanide of potassium. If some of the albumen, which has been coagulated by heat in the solution from the stains, be boiled with Millon's reagent, a brick-red color is produced.

If the stain has been washed with hot water, the albumen is coagulated, rendered insoluble, and fails to respond to the above test. If such a stain be washed with dilute sodic hydrate, the albumen is dissolved, and may be precipitated from the solution by the addition of nitric acid. The coloring matter is not imparted to the alkaline solution, consequently, if a stain from which the coagulated albumen has been removed by sodic hydrate be washed with hydrochloric acid, the coloring matter is taken up, and the concentrated acid solution responds to the test for iron when treated with the sulphocyanide of potassium.

Blood stains dissolve in boiling alcohol, which has been acidified with sulphuric acid, forming a solution which appears green by transmitted and red by reflected light. The residue obtained by the evaporation of this solution also gives the test for iron, after the removal of organic matter by burning.

If to a mixture of ozonized turpentine (turpentine which has been exposed to the air for some time and which decolorizes water containing traces of indigo) and tincture of guaiacum of equal volumes, some particles from blood stains be added, the mixture is colored blue and a precipitate of the same color is deposited.

Formation of a Ferro-cyanide.—If some blood stains or an aqueous or alkaline solution of the same be evaporated to dryness with pure carbonate of potash and the dry residue be placed in a glass tube, more carbonate of potassium added in the solid state, the tube be hermetically sealed and the contents heated to redness, cyanide of potassium is formed. If now the tube be opened with a file and the fused mass be boiled with water and iron filings, the solution filtered and treated with a few drops of a

solution of ferric chloride, Prussian blue will be produced. The cyanide of potassium has been converted into the ferro-cyanide by being boiled with water and iron filings. This test is of value only as a confirmatory one and can not be used when the stain is deposited upon leather or cloth. It is of most value when the stain is upon iron and mixed with rust; in this case, the addition of the iron filings is unnecessary.

It is impossible to distinguish between stains produced by blood from man and those produced by the blood of some of the domestic animals. It is true that corpuscles of the blood of birds may be distinguished from those of the blood of man by microscopical examination; but the blood of the ox or dog can not be distinguished with that certainty necessary in criminal prosecution from that of man. Moreover, in stains the corpuscles are frequently so altered that they can no longer be recognized by the microscope. If recent stains be moistened with a one per cent. solution of sodic chloride and a drop of the solution examined under the microscope, the corpuscles will be observed. *M. Barruel* is able to distinguish the animal from which a specimen of blood was taken by the odor obtained by warming the specimen with sulphuric acid. This is a degree of proficiency in the development of the sense of smell which but few can hope to attain and upon which none should wish to decide as to the guilt or innocence of a fellow being.

Menstrual blood can sometimes be detected by the presence of vaginal epithelium.

Pfaff claims to have discovered a means of ascertaining the relative age of blood stains. He depends upon the readiness with which the stains dissolve in a solution of arsenious acid (6 centigrammes of the acid in 8 grammes of water). Fresh stains dissolve immediately when treated with this solution. Stains which have been made for from one to two days require fifteen minutes for solution; while those from four to six months old require from three to four hours; and those over one year old, dissolve in from four to eight hours.

EPITHELIAL TISSUE.

KERATIN.

The epidermis, epithelium, horn, hair, nails and feathers constitute the class here known as epithelial tissue. All of these consist principally of a substance known as keratin. If some finely divided horn be washed first with boiling water, then with alcohol, ether, dilute hydrochloric acid and finally with water until the filtrate has no longer an acid reaction, keratin will remain. This is not a simple chemical compound, but probably contains several substances.

Keratin is insoluble in alcohol and ether and is freed from fat by being washed with these reagents. In hot water, it swells but does not dissolve. When burnt, it gives off a characteristic odor, that of burning feathers. When heated with water or acetic acid in closed tubes to 200° , keratin dissolves and liberates H_2S gas; on cooling, the solution forms a jelly-like mass. If hair, nails or horn-shavings be heated to 120° in closed tubes with glacial acetic acid, these substances are dissolved. This solution becomes turbid on the addition of water, is precipitated by neutralization with sodic hydrate and the precipitate is not soluble in an excess of the alkali.

Nitric acid colors most of epithelial tissues yellow and, on the application of heat, dissolves them forming yellow solutions and evolving nitrogen oxide. These solutions are changed to a brown color on neutralization with ammonia. By continued action of nitric acid upon epithelial tissue, oxalic acid is produced and may be obtained by neutralization and precipitation with some soluble salt of lime, as the chloride.

Hydrochloric acid colors epithelial tissue violet, which after prolonged boiling is changed to brown, the tissue being dissolved. Hair, treated with concentrated hydrochloric acid, takes a purple color and dissolves after continued maceration in the cold.

By the action of warm concentrated sulphuric acid, most epithelial tissues are converted into a slimy mass, which on microscopical examination is seen to be composed of cells. On boiling this mixture, the tissue is dissolved. After prolonged boiling with dilute sulphuric acid, tyrosine and leucine are produced.

If horn-shavings be digested for a long time with concentrated potassic hydrate, a gelatinous mass is formed and is insoluble in the strong alkali in the cold. If the excess of alkali be removed by washing with water, the jelly dissolves in the dilute alkali. If acetic acid be added to this solution a white precipitate forms. By boiling epithelial tissue with alkalis, the sulphur of the tissue combines with the base forming an alkaline sulphide, and if to this, some hydrochloric acid be added hydric sulphide will be given off and may be recognized by its odor and by blackening silver.

In hair a granular pigment has been observed and these granules are wanting in white hair; this has led to the belief that the various colors of hair are due to physical and not to chemical properties. Be this as it may, pigments have been extracted from the colored feathers of some birds. Church has extracted the pigment from the feathers of the *Touracos*. The feathers are washed with ether and alcohol and then extracted with water containing one five-hundredth of an alkali. From this alkaline solution the coloring matter is precipitated on the addition of hydrochloric acid. The precipitate forms in layers of a deep-violet color; this coloring matter is not changed at a temperature of 100°, but above this point it melts, becomes dark-green and gives off violet fumes. It is known as *turacin* from the name of the bird from which it was obtained, and contains from 5 to 8 per cent. of copper. For Church's original paper on turacin, see Chem. News, 19, 265.

“The beautiful blue-violet wing-feathers of the *Touracos* lose their color when the bird gets wet and then give a red stain. On drying, they recover their original color or acquire a blue color if the bird has died in the interval. In the dead bird the coloring matter has become insoluble in water. When the feathers are soaked in ammonia-water and the filtrate is precipitated with acetic acid, the pigment is obtained as a red powder.” (Gmelin's Handbook.)

TYROSINE,— $C_9H_{16}NO_3$.

Tyrosine is a product of the oxidation of the less complex animal tissues. It is prepared with facility from horn, nails,

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hair and the skin. It is found preformed in the substance of the liver, spleen, kidneys, suprarenal capsules, thyroid and salivary glands in various degenerations of these organs. It is also found in diseased epidermis, thickened nails, and atheromatous cysts. Tyrosine appears in the urine only from degeneration of the liver or kidney. It is a normal constituent of some insects. If cochineal be treated with boiling water, an amount of tyrosine equal to one-third of one per cent. of the cochineal is dissolved and crystallizes as the solution cools. Tyrosine represents a low state of organization and if the substance of any organ be unduly transformed into tyrosine, the function of such an organ can not long be performed. Consequently, tyrosine in the urine is indicative of changes of a very serious nature in the liver or kidney, especially the former. All proteids can, by the action of oxidizing agents, yield tyrosine; but it is generally prepared from horn or hair.

Boil two parts of horn-shavings with five parts of sulphuric acid and twelve parts of water for twenty-four hours. From time to time add water to replace that evaporated. While hot, dilute with water and saturate with marble dust or chalk. Filter, wash the precipitate with boiling water, in order to dissolve any tyrosine that it may contain, and unite the filtrate and wash-water. Concentrate on the water-bath, when tyrosine crystallizes in fine needles. In order to purify the tyrosine, redissolve in water, boil with hydrated oxide of lead and filter. (The compound of tyrosine and lead is soluble.) Treat the filtrate with H_2S gas and remove the sulphide of lead by filtration. Render the filtrate acid with acetic acid and concentrate, when tyrosine crystallizes.

Tyrosine can be prepared from albumen, flesh, fibrine, and hair in the same manner as from horn. The crystals are fine, needle-shaped, often arranged in bundles. They are freely soluble in ammoniac hydrate and in the dilute mineral acids, insoluble in acetic acid, alcohol and ether, very sparingly soluble in cold, more freely in hot water. These crystals are tasteless and odorless, but when burnt the odor of burning feathers is given off.

Heat tyrosine with an acid solution of mercuric nitrate, a rose color is produced, and a reddish precipitate is thrown down,

slowly. Boil finely cut horn-shavings with the same reagent and observe that a similar color appears. (Hoffmann's test.)

Dissolve tyrosine in concentrated sulphuric acid, warm on the water-bath, dilute with water, neutralize with carbonate of lime and filter. To the filtrate, concentrated if necessary, add neutral ferric chloride, when the solution is colored violet. (Piria's test.)

To some tyrosine on platinum foil, add two or three drops of nitric acid and gently heat to dryness. A bright yellow residue remains and dissolves in sodic hydrate forming a reddish-yellow solution. (Scherer's test.)

Dissolve tyrosine in strong nitric acid and set aside until a yellow precipitate, nitrate of tyrosine, forms. This compound dissolves in sodic hydrate, forming a reddish solution. Dissolve tyrosine in strong hydrochloric acid and allow to stand until the chloride of tyrosine is deposited as an amorphous powder or in needle-shaped crystals. The chloride of tyrosine is soluble in alcohol.

To a solution of tyrosine in ammonia add nitrate of silver, then neutralize with nitric acid, when argento-tyrosine, $C_9H_{10}AgNO_3$, is deposited. To a saturated solution of boric or calcic hydrate add tyrosine, warm and set aside when crystals, needles of the boric or calcic compound, are formed. Boil tyrosine with nitric acid for some time, neutralize and test the solution for oxalic acid. Tyrosine is easily converted by oxidizing agents into oxalic acid. -

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LEUCINE, $-C_6H_{13}NO_2$.

Leucine is a constant product of decomposing albumen. It can be easily produced with tyrosine from the various substances mentioned in describing the latter. Leucine is formed with tyrosine in the method given for the preparation of the latter from horn.

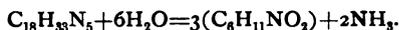
After the horn has been boiled the required time with sulphuric acid and water, the solution diluted, saturated with marble dust, filtered and concentrated, both tyrosine and leucine are deposited. For the separation of the leucine from the tyrosine and the subsequent purification of the former, Hlasiwetz and Habermann, as quoted by Hoppe-Seyler, give the following method: Boil the mixture with water to which a little ammonia has been added.

To the hot solution add basic acetate of lead until a white precipitate forms and remains on being agitated. Filter, heat the filtrate to boiling, and saturate the ammonia with dilute sulphuric acid which also precipitates the excess of lead. Filter through a fast filter. As this solution cools, the tyrosine is deposited. Decant the supernatant fluid and treat it with H_2S gas to remove any traces of lead. Filter, to the boiling filtrate add an excess of recently precipitated hydrated oxide of copper and continue the boiling for a few minutes. A precipitate is formed and contains a part of the leucine. This precipitate is collected, suspended in hot water, treated with H_2S gas, acetic acid added and the sulphide of copper removed by filtration. The filtrate is decolorized with animal charcoal and concentrated to a small volume. On standing, pure leucine is deposited. The other part of the leucine is in the blue solution which had been boiled with the oxide of copper. On evaporating this solution a blue colored compound of copper and leucine remains. If this compound be dissolved in water, the solution treated with H_2S gas and filtered, and the filtrate concentrated, leucine forms in needle-shaped crystals.

Leucine exists preformed in the pancreas and is found in the liver, kidneys and spleen in certain diseased states. In order to obtain the leucine, when preformed in any of these organs, the following process is used. Cut the organ into very fine pieces and rub the finely divided parts with ground glass in a mortar. Stir the pulp with much water, allow to stand for four hours, filter through a cloth and press the residue. Again wash with water and press. It is better if all the water can be removed by pressure in a screw-press. Unite the filtrate and wash-water, acidify with acetic acid, boil and filter in order to remove the albumen. Evaporate the filtrate to dryness, redissolve in a small volume of water. Separate from tyrosine and purify according to the method already given.

When pure leucine is wanted, it is best prepared synthetically, as follows: Boil in a retort a mixture of two parts of valerian-ammonia, one part of hydrocyanic acid and an excess of dilute hydrochloric acid until the oily compound disappears. At first

crystals having the formula, $C_{18}H_{33}N_5$, appear: then these take up water and are converted into leucine:



Evaporate to dryness on the water-bath. Treat the residue with a little water and neutralize with ammonia; when some leucine is precipitated; filter, collecting the precipitated leucine, evaporate the filtrate to dryness; treat the residue with dilute hydrochloric acid; concentrate on the water-bath and again neutralize with ammonia, when more leucine is deposited. Repeat the process as long as any leucine is deposited on neutralizing the solution with ammonia.

When pure, leucine crystallizes either in fine needle-shaped crystals arranged in bundles, or more commonly in thin, colorless rhombic plates. From solutions containing impurities, especially coloring matters, leucine is deposited, on concentration, in brownish balls or discs. In this form it is found in the urine, and the balls resemble urates, being distinguished from the latter by the weak refractive power of the leucine. The globules or balls resemble fat, from which they are distinguished by the insolubility of the leucine in ether. In some cases, the balls and discs will be seen to be composed of radiating needles; in others of thin plates, and in others they will appear perfectly smooth.

Leucine is soluble in 27 parts of cold, more freely soluble in hot water. When the leucine is impure or when the water contains animal coloring matter, leucine is still more freely soluble. Thus, it is readily soluble in the urine. It is soluble in 1040 parts of cold, or in 800, of hot alcohol. When impure, it is freely soluble in spirits of wine. It is insoluble in chloroform and ether. Leucine dissolves freely in both alkalis and dilute acids: it dissolves in concentrated hydrochloric or sulphuric acid, without decomposition. From its solutions in acids, leucine is precipitated by neutralization.

Dissolve leucine to saturation in nitric acid and allow to stand. Fine needle-shaped crystals are deposited. The compound of leucine and hydrochloric acid is represented by the formula, $C_6H_{11}ClNO_2$, and forms in colorless plates. These compounds are freely soluble in water. Boil a solution of leucine with an

excess of the hydrated oxide of copper; remove any undissolved copper, that may remain, by filtration; allow the filtrate to cool, when beautiful violet scales of $3(C_6H_{13}NO_2) + 2CuO$ are deposited. The corresponding compound of leucine and mercury forms in white granules; while the lead compound appears in glistening white scales.

Put some leucine into a perfectly dry test tube and gently heat. It is vaporized and deposited upon the upper and cool part of the tube in thin plates often arranged in rosettes. If leucine be heated above 170° in a retort, a yellow, oily liquid is distilled over. On standing, this distillate is covered with crystals of carbonate of ammonia. The leucine has been decomposed into amylamine and carbonic acid; later, the amylamine gives off ammonia which combines with the carbonic acid.

To some leucine, or the substance under examination and suspected to be leucine, on platinum foil, add a few drops of nitric acid and gently heat to dryness. If the substance be pure leucine an almost invisible residue remains. Warm this residue with a few drops of sodic hydrate, a more or less yellow color, according to the purity of the leucine, is produced. On farther concentration of the yellow sodic hydrate solution, an oily globule is produced and rolls about upon the foil without adhesion. (Scherer's test.)

Put some of the suspected substance into a dry test tube and gently heat, the peculiar odor of amylamine is given off, if leucine be present. On farther heating, the leucine is vaporized and deposited in crystalline plates upon the upper part of the tube.

Treat a solution of leucine in hot water slightly acidified with nitric acid, with nitrous acid gas. A part of the leucine is converted into leucic acid. Evaporate to a syrup, extract the syrup with ether; evaporate the ethereal solution; dissolve the residue in water; filter; precipitate the filtrate with the acetate of zinc; collect the precipitate; wash with cold water; suspend in water and treat with H_2S gas. Remove the precipitated zinc by filtration, evaporate the filtrate to a syrup and allow to stand when pure leucic acid is deposited in glistening, needle-shaped crystals.

Leucic acid is freely soluble in ether, alcohol and water. Its solutions have a decidedly acid reaction. Dissolve some leucic acid in water and distribute the solution in six test tubes. To the first, add acetate of copper; to the second, chloride of barium; to the third, acetate of zinc. These reagents throw down precipitates which, on standing, form in glistening crystalline scales. To the fourth, add chloride of lime; to the fifth, nitrate of silver. The compounds produced with lime and silver crystallize in needles. To the sixth test tube add acetate of lead, when a white flocculent precipitate of the leucate of lead is formed.

Leucic acid melts at 73° and is sublimed unchanged under 100° . If some leucic acid be heated in a glass dish, on the water-bath, the sides of the dish, on cooling, will be covered with crystals. Leucic acid bears the same relation to leucine that glycollic acid bears to glycocoll and is represented by the formula, $C_6H_{12}O_3$.

ELASTIC AND CONNECTIVE TISSUE.

ELASTIN.

The basis of elastic tissue is an albuminous substance known as elastin and is prepared as follows: Remove the cellular tissue from the *ligamentum nuchæ* of an ox or a horse. Boil the finely divided ligamentum for some time with a mixture of alcohol and ether in equal parts; decant the fluid and boil the pieces for 24 hours with water; then for another 24 hours with acetic acid and then with water until the rinsings are no longer acid. Now boil the substance with dilute potassic hydrate until the pieces begin to swell; decant the fluid; add to the residue water acidified with acetic acid and boil; again decant the fluid and wash well with water; add cold hydrochloric acid to the residue and allow to stand for 24 hours; pour off the acid and wash the residue with water as long as the wash-water leaves any residue on evaporation. The substance which remains insoluble, after being treated as above, is elastin. The fat has been removed by the alcohol and ether, and the collagen and inorganic substances by the water, alkali and acids.

Elastin prepared as above is of a yellowish-white color, elastic when moist, but brittle after drying. Examined under the micro-

scope, the fibres are distinctly seen. It swells when boiled with water or acetic acid, but is insoluble in these reagents and in alcohol and ether. When heated with a concentrated solution of an alkali, elastin dissolves, forming a brownish solution, in which no precipitate is produced upon the addition of sulphuric acid. With pure concentrated nitric acid, elastin is colored yellow and converted into a jelly which upon the addition of ammonia becomes yellowish-red. When boiled for a long time with dilute sulphuric acid, elastin yields leucine and tyrosine, the former in larger quantity than the latter.

COLLAGEN.

The basis of ordinary connective tissue is collagen and is prepared as follows: Wash finely divided tendons with cold water: then cover with baric or calcic hydrate and allow to stand for some days: then wash with water acidified with acetic acid and finally with water as long as the water dissolves anything.

Collagen is insoluble in cold water, but in boiling water it is converted into gelatin, and forms a jelly-like mass on cooling. Dilute acids and alkalis hasten the conversion of collagen into gelatin; thus, if collagen be placed in dilute acid or alkali until it begins to swell and then be placed in water at 40°, it will dissolve. In strong acetic acid, collagen swells and the fibres become indistinct, but reappear when the acid has been washed out with water or been neutralized with an alkali.

GELATIN.

Boil collagen prepared as above, and allow the solution to cool, when gelatin will form; or, pure gelatin is best prepared by dissolving clean white pieces of isin-glass in dilute hydrochloric acid and removing the inorganic salts from this solution by dialysis, when pure gelatin remains.

Pure gelatin is an amorphous, transparent, yellowish-white, tasteless and odorless substance. In cold water it swells, but does not dissolve; in hot water it dissolves and is deposited in a jelly-like mass on cooling. It readily undergoes putrefaction and then gives off the odor of ammonia; putrefaction is prevented by carbolic acid. Gelatin heated in the flame swells, evolves the odor of burning feathers and burns with a pale flame.

From solutions in hot water, gelatin is not precipitated by nitric acid nor by acetic acid and ferro-cyanide of potassium; but it is thrown down by chlorine gas, mercuric chloride and tannic acid. If an aqueous solution of gelatin be treated with a current of chlorine gas, it is precipitated in white, strong threads which contain chlorine and dissolve in the alkalis, forming chlorides. This precipitate evolves chlorine when treated with sulphuric acid. Alkaline solutions of gelatin give a violet color with Fehling's solution on boiling.

If gelatin be heated for a long time with water in sealed tubes at 140° , it is so modified as to be soluble in cold water. Neither the form soluble in cold water, nor that insoluble in the same menstruum is diffusible through animal membranes. Fuming nitric acid dissolves gelatin, evolving nitrogen and forming oxalic and malic acids and fat. By prolonged boiling with dilute sulphuric acid or with alkalis, gelatin is decomposed and yields leucine and glyccoll.

CARTILAGE.

There are both histological and chemical differences between true or hyaline cartilage, and the fibrous variety or fibro-cartilage. The corpuscles of the former lie imbedded in a smooth, semi-transparent base; while the structure of the latter is distinctly fibrous: the basis of hyaline cartilage is *chondrogen*, while that of fibro-cartilage is *collagen*.

Chondrogen is changed by boiling water into a soluble substance which resembles gelatin in some respects and which is known as chondrin. It must be borne in mind that the organic basis of true cartilage is chondrogen, and that during the process of extraction this is changed into chondrin. Chondrin is prepared as follows: Boil costal cartilages from man or from calves for half an hour with water; remove with a knife the loosened perichondrium; macerate the cartilage in cold water for some time; then boil for four hours in Papin's digester at a temperature of 120° , or for 48 hours in an open vessel; filter the solution while boiling; to the filtrate add acetic acid, which throws down the chondrin; collect the precipitate and wash, first with ether and then with boiling alcohol, in order to remove the fat.

Dried chondrin is a glassy, transparent, yellowish substance, which is insoluble in alcohol and ether. In cold water it swells but does not dissolve, while in hot water it dissolves and separates as a jelly-like mass on cooling. It is also soluble in alkalis. Like gelatin, chondrin if heated in closed tubes for some time at 140° is so modified as to be soluble in cold water.

From its solutions, chondrin is precipitated (1) by dilute mineral acids, (2) by organic acids, (3) by many metallic salts. The precipitate produced by dilute mineral acids is soluble in an excess of the precipitant; the precipitates thrown down by strong sulphuric, arsenious and pyrophosphoric acids forming exceptions to this rule. Most of the organic acids precipitate chondrin from its solutions; tannic acid causes only a faint opalescence. In the majority of cases the chondrin precipitated by organic acids is insoluble in an excess of the precipitant; that produced by acetic acid is sparingly soluble on being boiled with an excess of the acid. The fact that chondrin is precipitated by acetic acid affords an easy method of distinguishing between and separating chondrin from gelatin; for, as has been stated elsewhere, the latter is not precipitated by this acid. Moreover, the chondrin, precipitated by acetic acid, is soluble in either the ferro-cyanide or ferricyanide of potassium and in this way may be distinguished from albumen. Soluble salts of iron, copper, lead, silver and mercury precipitate chondrin from its solutions; the precipitate being soluble in an excess of either the precipitant or of the solution of chondrin. The deposit thrown down by the acetate of lead is insoluble; mercuric chloride only produces a faint cloudiness in solutions of chondrin.

Chondroglucose.—It is a fact of no little interest that sugar can be obtained from cartilage; this sugar is lævorotatory, but differs both from dextroglucose and from lævoglucose. (De Bary.) It is prepared as follows: Cover finely divided pieces of rib-cartilage with cold dilute hydrochloric acid and allow to stand for some time, then pour off the acid; add more dilute acid and continue washing the cartilage with the acid until the inorganic matter is removed. Now boil the cartilage for some hours with concentrated hydrochloric acid; add to the mixture some recently

precipitated oxide of lead; boil again for a few minutes and remove the precipitated chloride of lead by filtration. To the filtrate rendered alkaline by ammonia, add basic acetate of lead and collect the precipitate, which forms and contains the sugar, upon the filter; suspend the lead precipitate in water and treat with hydrosulphuric acid gas; remove the precipitated sulphide of lead by filtration and concentrate the filtrate, which contains the sugar, to a syrup.

Cartilage sugar readily reduces copper; but it is only partially fermentable. It seems very probable that two kinds of sugar are present; for before it is allowed to ferment at all, the solution turns the light—46.5° and after fermentation is completed the solution still reduces copper but turns the light only half as far to the left as it did previous to fermentation.

The resemblance between chondrin and gelatin is so close that the following table, taken from Hofmann's Zoöchemie and which points out the differences between these two substances, is inserted:

GELATIN.

C=50.0 H= 6.7
N=18.1 O=24.6

- (1) Not precipitated by acetic acid.
- (2) Soluble in mineral acids.
- (3) Not precipitated by acetate of lead.
- (4) Precipitated by tannic acid and mercuric chloride.
- (5) Yields leucine and glycocoll by putrefaction.
- (6) Yields no sugar on being boiled with hydrochloric acid.

CHONDRIN.

C=50.0 H= 6.6
N=14.4 O=29.0

- (1) Precipitated by acetic acid.
- (2) Precipitated by mineral acids.
- (3) Precipitated by acetate of lead and by most salts of the heavy metals.
- (4) Only rendered turbid by tannic acid and mercuric chloride.
- (5) Yields leucine but no glycocoll by putrefaction.
- (6) Yields chondroglucose on being boiled with hydrochloric acid.

Besides chondrin, cartilage contains water, fat and inorganic salts: the latter consisting of the phosphate and sulphate of lime, the phosphate of magnesium and the chloride, carbonate, phosphate and sulphate of sodium. It is an interesting fact, first

observed by Von Bibra, that the salts of potash are not found in cartilage. The per cent. of water contained in cartilage varies from 50 to 70. The per cent. of inorganic salts varies from 3 to 7 and seems to depend upon the age of the animal from which the cartilage is taken. The following table, taken from the Lehrbuch of Gorup-Besanez, shows the per cent. of ash found by Von Bibra in the costal cartilages of persons of different ages :

A child of 6 months of age.....	2.24
A child of 3 years of age.....	3.00
A girl of 19 years of age.....	7.29
A woman of 25 years of age.....	3.92
A man of 20 years of age.....	3.40
A man of 40 years of age.....	6.10

Of the inorganic salts, the sulphate of lime is the most abundant, constituting from 50 to 80 per cent. of the ash ; the second salt in regard to quantity is the phosphate of lime, which varies from 5 to 20 per cent. of the ash.

OSSEOUS TISSUE.

Bones consist of organic and inorganic matter and these can be separated by various means. Free bones as completely as possible from periosteum, blood-vessels and the contents of the medullary canal ; crush into a coarse powder : extract with alcohol and ether in order to remove the fat ; extract repeatedly with dilute hydrochloric acid (1 part of the acid to 9 of water) until the acid ceases to remove anything ; wash the residue with water until the wash-water no longer has an acid reaction ; boil the pieces thus freed from inorganic salts, with water for 24 hours ; filter while boiling through a fast filter, wash any residue with boiling water ; concentrate the united filtrate and wash-water to a small volume on the water-bath and allow to cool, when bone-gelatin is deposited. This substance will be found insoluble in cold, soluble in hot water and, in short, will manifest the properties already described as those of gelatin ; while, should any chondrin be present it may be distinguished from the gelatin by precipitation of the former with acetic acid.

If bones be placed in dilute hydrochloric acid (1 part of the acid to 9 of water) and the acid be frequently changed, all the

inorganic salts will be removed. The bone will still possess its original form but is pliable and, if a long one, may be bent double or tied into a knot. On the other hand, if bones be kept at a red heat for some time, all the organic matter will be removed. The bone will maintain its original form, but will be brittle. In the bones of children the organic matter predominates and consequently their bones are not so easily broken.

The bones of the embryo even to the latest period of intra-uterine life contains no bone-gelatin or ossein but chondrogen; while after complete ossification, the bone contains no trace of chondrogen. Fremy found that the organic basis of some fish-bones and of the bones of certain water-fowls after being boiled with water, deposited no gelatin and consequently differs from ossein.

Fossil bones contain that modification of collagen which is soluble in cold water and together with this, in some cases, the ordinary form, i. e., that soluble in hot water and forming a jelly on cooling; the latter may be entirely replaced by the former. In very old fossil bones, the organic basis has entirely disappeared; also parts of the bone are replaced by silica and alumina, forming a petrification. Fresh bones when completely freed from blood and marrow contain no iron, but this element is often found in considerable quantity in buried bones. Haidinger found the medullary canal of the bones of a human skeleton containing crystals of vivianite.

The fat contained in bones has not been very thoroughly studied, but consists principally of triolein and tripalmitin. If it be desired, the amount of fat contained in bone may be estimated. For this purpose, extract a weighed portion of the dried bone-powder with ether; evaporate the ethereal solution at a low temperature or allow to evaporate spontaneously; again extract with ether, filter, evaporate the filtrate, dry the residue at 100° and weigh.

The marrow of the long bones consists of collagen containing fats. The cellular tissue of the spongy bones contains a soft, reddish substance which consists of albumen, free acid and extractive matters. Whether the free acid be lactic, as claimed by Berzelius, is not yet positively known. Cholesterine is not

unfrequently present in marrow, and hypoxanthine has been found in cases of leucocythæmia.

The inorganic constituents of bone are calcic chloride, CaCl_2 , calcic fluoride, CaFl_2 , calcic carbonate, CaCO_3 , calcic phosphate, $\text{Ca}_3(\text{PO}_4)_2$, and magnesian phosphate, $\text{Mg}_3(\text{PO}_4)_2$. From a great number of analyses made by *Zalesky*, it seems that there are certain variations in the proportion of organic and inorganic constituents also of the various inorganic salts which are constant in different animals. The following table of some of the analyses made by *Zalesky* is taken from the *Lehrbuch of Gorup-Besanez* :

IN 100 PARTS.	Man.	Ox.	Tortoise Testudo graeca.	Guinea-pig.
Inorganic.....	65.44	67.98	63.05	65.30
Organic.....	34.56	32.02	36.95	34.70
Calcic phosphate.....	83.89	86.09	85.98	87.38
Magnesian phosphate.....	1.04	1.02	1.36	1.05
Calcic fluoride, chloride and carbonate.....	7.65	7.36	6.32	7.03
Carbonic acid.....	5.73	6.20	5.27	—
Chlorine.....	0.18	0.20	—	0.13
Fluorine.....	0.23	0.30	0.20	—

In some diseased states, the proportion between the organic and inorganic constituents of bone may be very different from the normal and indeed not unfrequently the per cent. of the two is reversed; thus *Marchand* found a femur in rhachitis to contain 79.40 per cent. of organic and 20.60 per cent. of inorganic matter; *Lehmann* found a tibia, in the same disease, consisting of 66.36 per cent. of organic and 33.64 per cent. of inorganic matter; *Ragsky* obtained 81.12 per cent. of inorganic matter from a rhachitic humerus; while *Schloosberger* ascertained that the amount of organic matter contained in the bones in three cases of craniotabes varied from 51.50 to 52.32 per cent., while the amount of inorganic salts in the same cases varied from 48.50 to 47.68 per cent.

In osteomalacia, not only is the proportion between the

organic and inorganic salts abnormal, but the organic part is often radically changed so that after having been boiled with water it fails to deposit gelatin on cooling. Not unfrequently the bones in osteomalacia impart an acid reaction to water in which they are placed or with which they are washed. Schmidt and Weber claim to have detected free lactic acid in three cases of osteomalacia. Interesting in this connection is the assertion of Heitzmann, that by the continued incorporation of lactic acid in the food of dogs and cats, rhachitis and later, osteomalacia could be produced. However, these experiments have been repeated by Heiss and the above results are not confirmed. (Gorup-Besanez.)

It is well known that the cavities of the bones of birds contain air and that the per cent. of inorganic salts, especially of the phosphate of lime is greatly increased in these bones. On the other hand, the bones of fish are poor in inorganic salts and are rich in fat. Fish bones also contain salts of sodium and potassium, especially the sulphates and chlorides of these bases. The bones of amphibians contain less inorganic matter than those of mammals and more than those of fish.

The scales of fish have a composition similar to that of bones, the only difference consisting in a greater proportion of organic matter. The organic basis of fish scales is soluble in boiling water and forms a jelly on cooling. The so-called essence of pearl which is obtained from the scales of the white fish and which is used for the manufacture of artificial pearls consists, according to the analyses of Barreswil and Voit, of the carbonate of lime and guanine. The scales of amphibians are essentially different from those of fish and belong both chemically and histologically to epithelial tissue. (Gorup-Besanez.)

How bones are formed and in what way they grow is a question of no little importance and one which is not yet fully understood. It seems that the chondrogen of the fœtus is not transformed into ossein or collagen, but is replaced by it. We know but little more concerning the inorganic part of the bone. It has been proven that the chick as it escapes from the shell contains more lime than the interior of the egg and that the shell has, during the period of incubation, lost an equal amount of lime.

How the lime is transferred from the shell to the embryo is not known. "The inner membrane of the shell, the interior parts of the embryo and in one case also the liquor amnii exhibited an acid reaction after fourteen days of incubation." (Lehmann.) As the result of a number of experiments, it was found that the average amount of lime in one fully developed chick is five and a half times that found in the interior of one fresh egg. (Bills and Vaughan.)

Dr. Geo. G. Groff suggests that the solution of the carbonic acid, given off by the developing chick, in the fluids of the egg, might possibly form a solvent for the shell; since calcic carbonate is soluble in water containing carbonic acid gas.

TEETH.

In the teeth three distinct structures exist; these are the dentine, cement and enamel. The first two of these contain the same inorganic constituents as bone and also yield an organic basis which dissolves in hot water and forms gelatin on cooling. The proportion between the organic and inorganic constituents of dentine is as 28 to 72.

The enamel is the poorest in water and richest in inorganic salts of any part of the body. The organic part of the enamel, when separated from the inorganic by solution of the latter in hydrochloric acid, appears as four- or six-sided prisms, which on being boiled with water do not form gelatin and which behave as epithelial tissue. The enamel of the growing teeth contains more organic matter than that of the fully developed tooth. The fluid which surrounds the tooth as it is enclosed in the dental sac is strongly alkaline in reaction and contains albumen. The watery extract of the enamel itself contains no trace of albumen; but if the inorganic salts be removed by nitric acid, the residue yields an albuminous substance which is precipitable by acids. (Gorup-Besanez.)

Saliva containing an excess of albumen or of other organic matter is very destructive to the teeth. The organic matter will collect to a greater or less extent between and around the teeth, where it undergoes an acid fermentation whereby the teeth are

destroyed. In a case where the teeth were badly decayed, I found as much as 20 parts of albuminous matter per thousand in the saliva. Three or four hours after each meal, the contents of the mouth were slightly acid.

FAT.

Fat is an important constituent of many plants and animals. As an article of food its value can hardly be overestimated. The fat which is contained in animals is derived partially from the fat of the food and partially from the carbohydrates and the albumen of the food: thus the honey bee, when fed entirely upon sugar, is able to produce wax, a substance closely related to fat in its chemical composition and physical properties; animals fed upon grain, potatoes, etc., substances rich in carbohydrates, soon gain more fat than is contained as such in the food; the carnivora often gain fat when food containing some starch is furnished them. This transformation of starchy substances into fat, or rather the derivation of the latter from the former, may take place outside of the animal body; thus Pastuer found that glycerine, a component of neutral fats, is produced together with alcohol and carbonic acid when cane sugar undergoes the alcoholic fermentation. Thus it is evident that carbohydrates supply fatty material as the result of chemical changes.

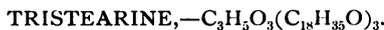
That fat results from certain changes in the albuminous molecule is evident; by the action of the pancreatic juice, leucine is produced from parapeptones; the unused muscle contains an excess of fat; in the aged and in certain diseased states various organs undergo fatty degeneration. Wetherill has shown that the dead body is converted into fats.

The small amount of oxygen contained in the fatty molecule and the fact that the hydrogen and carbon are converted into water and carbonic acid gas explain the value of fatty food in sustaining animal heat and show why it is so extensively used by the inhabitants of cold countries. Fat is a normal constituent of all the principal fluids of the body, with the exception of the urine, existing in a finely divided condition in the chyle, blood, milk, etc. In the solid tissue it is well distributed in the healthy state and in pathological conditions it may exist in excessive quantity in any or

every organ. It represents a low state of organization and when the tissue of the liver, heart or other organ becomes unduly transformed into fat, that organ will soon cease to perform its function normally. The fat which accumulates pathologically is identical with that which, in smaller quantity, is a normal constituent of the tissues.

Fatty globules, even when present in small quantity, may be recognized by their microscopic appearance. They consist of a thin membrane enclosing a fluid; in the dead body the contents of the membrane are sometimes found crystallized, in consequence of the removal of the heat of the body. These crystals generally appear in needles arranged in bundles or in rosettes. The perfect oil globule is spherical, floats upon water and is colorless or of a faintly yellow tint.

Some of the fats of the body are fluid and others solid at ordinary temperature. They give a neutral reaction, since they consist of fatty acids combined with glycerine forming neutral compounds. They are insoluble in water, sparingly soluble in cold, more freely in hot alcohol, and soluble in ether, chloroform and volatile oils; also soluble to some extent in each other, thus olive oil is a solution of tripalmitine and tristearine in trioleine. Water containing albumen or bile-acid will hold fat in a finely divided state and will appear milky, while if fat be added to water alone the globules will float upon the surface. Upon being boiled with an alkali, the fats are broken up into glycerine and fatty acids, the latter combining with the alkali to form a soap. If the fats, for instance butter, be allowed to stand exposed to the air, it sooner or later becomes rancid, volatile oils being formed. The most important of the fats of the animal body are *tristearine*, *trioleine* and *tripalmitine*.



It will be seen from the formula that tristearine is formed by the combination of three molecules of the monobasic stearic acid with one of glycerine. Tristearine is prepared as follows: Extract mutton or beef tallow with cold ether, which dissolves only traces of tristearine; extract the residue insoluble in cold ether with hot ether and allow this extract to cool when tristearine

is deposited in rectangular tablets or rarely in rhombic prisms. These crystals are very sparingly soluble in alcohol; they melt at 63° .

If tristearine, the melting point of which is 63° , be heated to 64° and then the heat be removed it solidifies at 61° and before it can again be melted, must be heated to 66° . Again if tristearine, the melting point of which is 63° , be heated to 70° and the heat be removed it solidifies at 51° and, when again heated, melts at 52° . There seem to be three modifications, the melting points of which are 52° , 63° and 66° . (Hofmann.)

Stearic acid, $\text{HO}(\text{C}_{18}\text{H}_{35}\text{O})$.—If tristearine be boiled with sodic hydrate and the solution be diluted with 10 times its volume of water, or if ordinary soda soap be dissolved in hot water and then largely diluted with cold water, a precipitate will fall and will consist of the acid stearate of soda, mixed with the acid palmitate of soda, if soap has been used. This precipitate is treated with boiling alcohol and the solution decanted; when the solution cools, the acid stearate is again deposited and should be washed with cold alcohol and then treated with dilute hydrochloric acid. Chloride of sodium is formed and the stearic acid set free; the former in solution is decanted and the latter is redissolved in boiling alcohol from which it crystallizes on cooling.

Stearic acid forms in thin plates, some of which are rectangular while others are oval. They are insoluble in water and cold alcohol; soluble in hot alcohol, ether, chloroform and benzol. They melt when pure at 69.2° , when mixed with palmitic acid, at a lower temperature.

TRIOLEINE, $-\text{C}_3\text{H}_5\text{O}_3(\text{C}_{18}\text{H}_{35}\text{O})_3$.

Pure trioleine is at ordinary temperature a colorless fluid, which on exposure to the air takes up oxygen and becomes more or less yellow. It is insoluble in water, slightly soluble in cold dilute alcohol, freely soluble in ether and absolute alcohol. It readily dissolves both tristearine and tripalmitine.

If olive oil be kept at or below 0° for 24 hours, a crystalline precipitate consisting of tripalmitine will form. The supernatant oily fluid may be decanted, dissolved in alcohol and again left for 24 hours at 0° , when the remainder of the tripalmitine is depos-

ited. If now the alcoholic solution be poured off and diluted with water, trioleine separates in globules, which crystallize in needles after being kept for some time at a temperature of -5° .

Oleic acid, $\text{HO}(\text{C}_{18}\text{H}_{33}\text{O})$.—Olive oil is freed from palmitine by being kept for 24 hours at 0° and the fluid oil is poured off, mixed with a small quantity of the oxide of lead and the mixture heated for some hours at 100° . The oleate of lead, which is formed, is now dissolved in ether, while the other salts of lead remain insoluble in this menstruum. The ethereal solution is treated with a few drops of hydrochloric acid and shaken. The chloride of lead is formed and upon standing sinks to the bottom. The supernatant ether containing the oleic acid is removed and evaporated at a gentle heat. The residue, which is impure oleic acid, is dissolved in ammoniac hydrate and precipitated from the ammoniacal solution by baric chloride, as baric oleate. This precipitate is dissolved in warm absolute alcohol, from which baric oleate crystallizes on cooling. These crystals excluded from the air are treated with tartaric acid which frees oleic acid. The fatty acid is washed quickly with water and kept in an atmosphere of carbonic acid until dry; this precaution is quite necessary, because oleic acid readily takes up oxygen from the atmosphere.

Oleic acid is a colorless, odorless and tasteless fluid, which when kept at a temperature of -4° , crystallizes in thin plates. It is insoluble in water, freely soluble in ether, alcohol and chloroform. (Hoffman.)

TRIPALMITINE, $-\text{C}_3\text{H}_5\text{O}_3(\text{C}_{16}\text{H}_{31}\text{O})_3$. *C. 7. 1895 O.*

It has already been stated that when olive oil is kept for some time at a temperature of 0° , tripalmitine is deposited in a crystalline form; these crystals, after the supernatant oil has been poured off, are dissolved in boiling alcohol from which they separate on cooling. They are slightly soluble in cold, freely soluble in hot alcohol and ether. From a saturated solution in hot alcohol, tripalmitine forms in needles as the solution cools. If stearine be also present the mixture not unfrequently forms in balls which consist of radiating needles or fine plates; this

mixture has been mistaken for a fourth fat and designated by the name *margarine*. The crystals of tripalmitine melt at 62° .

DETECTION OF FATS.

On account of their insolubility in water and solubility in ether, fats are easily separated from other substances when proper caution is used. Fats suspended in fluids may be removed by agitating the fluid with ether, allowing to stand for a short time when the ethereal layer containing the fat will rise to the top and may be removed with a pipette. If it be desired to remove all the fat, the fluid may be repeatedly shaken with ether as long as the latter dissolves any fat; this is ascertained by allowing a few drops of the ethereal solution, placed on a glass slide, to evaporate, adding a drop of water to the residue and examining under the microscope for oil globules.

From emulsions, for example milk, fat is best removed by agitation with ether as above, after the addition of a few drops of sodic hydrate.

From fatty tissue or from solutions, fat is extracted as follows: Heat the tissue or solution at the temperature of the water-bath until all the water is driven off; rub up the residue with ether and remove the ethereal solution; boil the part insoluble in ether with alcohol; filter the alcoholic solution and evaporate it to dryness on the water-bath; extract this residue with ether; unite and concentrate the ethereal extracts which may contain besides neutral fats, fatty acids, cholesterine and coloring matters. In order to remove the fatty acids, evaporate the ethereal solution to dryness on the water-bath; add to the residue a small volume of a concentrated solution of sodic carbonate and again evaporate to dryness. The sodic carbonate does not saponify the neutral fats and these with cholesterine are removed by dissolving the residue in a little water, shaking this solution with ether and removing the ethereal layer. In order to separate the cholesterine from the fat, evaporate the ethereal solution at a gentle heat or allow it to evaporate spontaneously; heat the residue on the water-bath with an alcoholic solution of potassic hydrate and evaporate the alcohol; dissolve the residue in much water, shake with ether and remove the ethereal layer, which, if sufficient water had been

added, contains only cholesterine. Heat the aqueous solution which contains the soap formed by the action of potassic hydrate on the fat, on the water-bath until all traces of any remaining ether are evaporated; slightly acidify the solution of soap with dilute sulphuric acid, and allow to stand for a short time when the fatty acids are precipitated; filter, when the fatty acids remain upon the filter and the filtrate contains glycerine and traces of sulphates. Neutralize the filtrate with ammonia; concentrate to a small volume on the water-bath; extract with alcohol; filter and evaporate the alcoholic solution; rub up the residue with some oxide of lead; suspend the mixture in water; treat with hydrosulphuric acid gas and filter. Evaporate the filtrate to a syrup when glycerine remains and may be recognized by its taste and by its dissolving the oxide of copper. (Hoppe-Seyler.)

MUSCULAR TISSUE.

A chemical analysis of muscle is attended with many difficulties owing to the changes produced by various causes: thus, muscle at rest manifests a neutral or an alkaline reaction, while the tetanized muscle gives a distinctly acid reaction. Again as long as the muscle is contractile and living, it contains a fluid resembling the plasma of blood; while in the dead muscle, coagulation of this fluid has taken place. So long as the muscle is contractile, its plasma is transparent; while after the supply of blood has been cut off, the muscle becomes shorter, thicker, less elastic and less transparent. Besides albuminous substances, muscle contains many other organic and inorganic constituents.

MUSCLE-PLASMA.

Keep the contractile muscle of a frog, freed from blood by the injection of a one-half per cent. solution of sodic chloride, at from -7° to -10° until it freezes; then cut it into fine pieces and rub these up in a mortar with snow containing one per cent. of chloride of sodium. Soon the mass melts at a temperature of about -3° into a cloudy, alkaline fluid which filters slowly at a temperature below 0° . This opalescent fluid is muscle-plasma and when exposed to an ordinary temperature is transformed into a jelly-

like mass which gradually contracts and presses out a fluid, muscle-serum.

MYOSIN.

Myosin corresponds to the fibrine produced by the coagulation of the blood and, like fibrine, it is supposed to have its antecedents which exist in the plasma of the muscle. Myosin is not a constituent of living muscle, but is formed after death.

Preparation.—Drop muscle-plasma, which has been kept in the cold, into water. As each drop falls, a fine white precipitate of myosin forms. This should be collected and washed with water. The myosin prepared in this way is quite pure.

From dead muscle, the ready formed myosin is separated as follows: The muscle, freed from blood, tendon, fat, fascia and connective tissue, is cut into fine pieces and washed with water until the wash-water no longer contains albuminous substances. The pieces are then rubbed up with a ten per cent. solution of sodic chloride and the viscid fluid, which forms, is filtered through linen. If now the filtrate be allowed to fall drop by drop into a large volume of distilled water, the myosin will be precipitated and may be collected and washed as above, or the mixture may be allowed to stand for several days when the myosin will have fallen to the bottom and may be freed from the supernatant fluid by decantation.

Myosin forms in transparent flakes and is not at all fibrous. It forms very rapidly from muscle-plasma when the latter is subjected to a temperature of from 35° to 40° . Myosin is insoluble in water, soluble in dilute solutions of sodic chloride and does not separate from these solutions on standing. From its solutions myosin is precipitated unchanged on the addition of much water. It is also precipitated by boiling, and by alcohol; but in these cases, it has been changed into albumen and dissolves in alkalis forming albuminates.

The fluid which separates after the coagulation of muscle-plasma and which is known as muscle-serum, is of a faintly yellow color, is neutral when kept at or below 0° ; but at ordinary temperature, it soon becomes acid owing to the development of paralactic acid. It contains an albuminate of potash, an albumen which coagu-

lates at 75° and another which coagulates at 45° and various extractives.

CREATINE,— $C_4H_9N_3O_2$.

Creatine is found in varying proportions in the muscles of all vertebrates and of some invertebrates. According to Hofmann, the amount of creatine in human muscle varies from 0.14 to 0.49 per cent. About the same amount is found in the muscles of the ox, dog and cat. A somewhat larger per cent. is present in the flesh of the domestic fowl and of the frog. Creatine exists normally in small quantities in the brain, in blood, in the urine, and in various transudations.

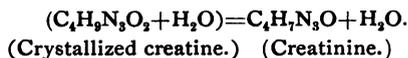
Preparation.—Cut five pounds of muscle, freed from fat, into very fine pieces. Cover with water. Stir frequently for four hours and then filter through cloth. Wash and press the residue. Unite the filtrate and wash-water and boil quickly. Remove the coagulated albumen by filtration through cloth. To the filtrate add baric hydrate as long as a precipitate is produced. Remove the precipitated phosphate and sulphate of barium by filtration. Treat the filtrate with a current of carbonic acid gas. Again filter, in order to remove the excess of barium which has been precipitated as a carbonate. Evaporate the filtrate to a syrup on the water-bath: if a pellicle forms on evaporation, it must be removed. Set the syrup aside for a few days, when creatine separates in rhombic prisms.

Dilute Liebig's extract of meat. Remove the phosphates and sulphates by precipitation with baric hydrate and filtration and proceed as above.

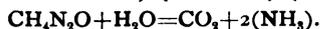
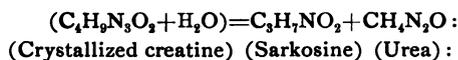
Creatine crystallizes in beautiful prisms with many modifications. These contain one molecule of water of crystallization and are represented by the formula, $C_4H_9N_3O_2 + H_2O$. The crystals are sparingly soluble in cold, freely soluble in hot water. From a saturated solution in hot water, creatine is deposited in fine needles on cooling. It is insoluble in cold alcohol and ether, soluble in hot dilute spirits of wine. Its solutions are neutral to litmus and have a bitter, irritating taste. If crystals of creatine be heated to 100°, they lose their water of crystallization and become opaque.

Dissolve creatine in dilute acid and allow the solution to evaporate spontaneously, when creatine crystallizes unchanged.

Dissolve creatine in strong hydrochloric, nitric or sulphuric acid and gently evaporate the solution. Crystals of a *creatinine* salt are formed; the creatine has given off water and been converted into creatinine:



Boil creatine with baric hydrate and observe that ammonia is given off. The ammonia may be recognized by the odor; and also by the production of a white cloud of vapor, if a rod moistened with hydrochloric acid be held over the boiling mixture. As soon as the ammonia is given off freely, cool the mixture, remove the barium with a stream of carbonic acid gas and subsequent filtration; evaporate the filtrate on the water-bath, when urea will remain and may be recognized by the formation of nitrate of urea on the addition of a drop of nitric acid. The creatine has been converted into sarkosine and urea; while the latter has been decomposed into ammonia and carbonic acid.



Creatine is so easily converted into creatinine, that it is not certain whether the latter exists preformed in muscle or not. The small amount of creatinine which has been obtained by some chemists from flesh might have been produced from creatine during the process of separation. (Creatinine is a constant constituent of normal urine.) It is best prepared from creatine. Boil creatine for an hour with dilute hydrochloric acid, evaporate to dryness on the water-bath, and redissolve the residue, which consists of the chloride of creatinine, in water. To this aqueous solution add some hydrated oxide of lead; boil, filter, and again evaporate to dryness on the water-bath. Extract the residue with alcohol and evaporate the alcoholic solution on the water-bath, when pure creatinine remains.

Creatinine may be obtained from creatine by the action of

other acids. Heat creatine with dilute sulphuric acid on the water-bath for one hour. Neutralize the solution with baric carbonate, filter and evaporate the filtrate until creatinine crystallizes.

Creatinine forms in prisms which belong to the monoclinometric system. It is more freely soluble in water than creatine is; creatinine requiring only 11.5 parts of cold water for solution. It is sparingly soluble in cold alcohol, freely soluble in hot alcohol. From its solution in hot alcohol, creatinine crystallizes on cooling. Its solutions have a caustic taste resembling that of ammonia and give a decidedly alkaline reaction. Creatinine is a true animal alkaloid, combines with acids forming salts and liberates ammonia from its combinations.

To a moderately concentrated solution of argentic nitrate, add creatinine; a dense precipitate of fine acicular crystals is formed. Boil the mixture, when the precipitate dissolves; but again separates on cooling. The precipitate consists of creatinine-silver nitrate. A similar compound is formed by the addition of creatinine to a solution of mercuric chloride.

To an alcoholic solution of creatinine add a few drops of a neutral, concentrated solution of chloride of zinc. A precipitate of the double chloride of creatinine and zinc, $(C_4H_7N_3O)_2 Zn Cl_2$, is produced. This precipitate forms either in fine needle-shaped crystals, or in warty granules. Often, microscopic examination will show that the granules are composed of radiating needles. This compound is insoluble in cold water and alcohol, soluble in hot water and the mineral acids. If this salt be decomposed by the sulphide of ammonia, a part or all of the creatinine is transformed into creatine.

Creatinine may be obtained from the urine and the amount daily excreted estimated by the following process which is known as Neubauer's method: To 300 c.c. of urine add milk of lime until an alkaline reaction is produced; then add chloride of lime as long as precipitation continues. Allow to stand for two hours; filter; wash the precipitate with water; unite the filtrate and wash-water, and evaporate to dryness on the water-bath. Mix the residue with strong alcohol (absolute or 95 per cent). Pour

the mixture into a clean beaker which has been rinsed with alcohol; allow to stand for six hours; at the expiration of this time a precipitate will have formed; filter the supernatant fluid; then collect the precipitate upon the same filter and wash with a small quantity of alcohol; unite the filtrate and washings. If these measure more than 50 c.c., concentrate to that amount with gentle heat on the water-bath. To the concentrated fluid add .5 c.c. of an alcoholic solution of perfectly neutral zinc chloride, of sp. g. 1.2. Stir the mixture vigorously until a cloudiness appears, then cover it with a glass plate and set aside in a cool place for four days; collect the crystals of creatinine-zinc chloride on a weighed filter; wash with alcohol until a colorless filtrate appears and no longer gives the reaction for chlorine; dry the crystals on the filter at 100° and weigh. The normal amount of creatinine excreted daily in the urine varies, according to Neubauer, from 0.6 to 1.3 grammes. (Hoppe-Seyler.)

It will be seen from a study of the sources of creatine and creatinine, that the amount of these substances present in the body and in the excretions will vary greatly with the kind of food. Liebig found that a dog, while being fed upon muscle, excreted large quantities of creatine and creatinine and but little kynuric acid; while when the animal subsisted upon fatty food, the proportion of these substances was reversed. Since muscle contains creatine, it is evident that an increased consumption of this article of food will augment the amount of creatinine excreted in the urine.

SARKOSINE, $-C_5H_7NO_2$.

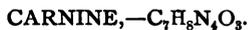
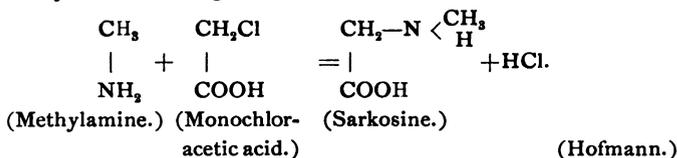
The formation of this substance from creatine has already been referred to, and the reaction by means of which sarkosine and urea are produced from creatine has been written. It is not itself a constituent of muscle and is of interest in this connection on account of its derivation.

Preparation.—Boil a saturated solution of creatine with 10 times its volume of baric hydrate as long as ammonia is given off and baric carbonate is formed. (If it is necessary more baric hydrate may be added from time to time.) As soon as ammonia is no longer given off, filter, treat the filtrate with a current of

carbonic acid gas and remove the precipitated carbonate by filtering again; concentrate this filtrate on the water-bath to a syrup and allow to stand for some days, when sarkosine forms in crystals. In order to purify the crystals, dissolve them in dilute sulphuric acid, filter and concentrate this filtrate to a syrup on the water-bath. Wash this syrup with alcohol, then dissolve it in water, add baric carbonate and heat as long as carbonic acid is given off; remove the sulphate of barium by filtration; concentrate the filtrate to a syrup on the water-bath and allow to stand for 24 hours when pure sarkosine crystallizes. (Hofmann.)

Sarkosine forms in large, colorless, rhombic prisms, which are soluble in alcohol and in water, not soluble in ether. It acts as a base uniting with acids forming salts. With chloride of gold it forms a double salt which is freely soluble in alcohol and hot water, but very sparingly soluble in cold water. From its solution in hot water, this salt forms in rhombic tablets on cooling. With chloride of platinum, sarkosine forms a double salt which crystallizes in large, yellow octohedrons.

Sarkosine is methyl glycooll and can be formed synthetically by adding an excess of monochloracetic acid to an aqueous solution of methylamine and keeping the mixture at about 130° for some time; then removing the chlorine by oxide of silver, decolorizing the solution with animal charcoal, concentrating to a syrup and allowing to stand for a few days, when sarkosine crystallizes. The formation of sarkosine synthetically is represented by the following formula:



This substance has been found, as yet, only in the prepared meat extracts, in which it exists in as great a proportion as one per cent.

Preparation.—To Liebig's extract, add six times its weight of warm water; to this solution add a saturated solution of baric

hydrate as long as the precipitate increases and then filter through linen; to the filtrate add basic acetate of lead and collect the precipitate which forms and consists of inorganic salts of lead, especially the chloride, and a double salt of lead and carnine; wash this precipitate with hot water, which dissolves all the carnine compound and only traces of the inorganic salts; treat the filtrate, while yet hot, with hydrosulphuric acid gas and remove the precipitated sulphide of lead by filtration; concentrate the filtrate and add to it a concentrated solution of nitrate of silver. This forms a precipitate which consists of the chloride of silver and a double nitrate of silver and carnine. Collect this precipitate and wash it, first with water and then with a small quantity of ammoniac hydrate. The ammonia dissolves the chloride of silver, while the nitrate of silver and carnine remains. Suspend this remaining precipitate in water and treat with a current of hydrosulphuric acid gas; heat the mixture and filter while hot; concentrate the filtrate and allow it to cool when carnine, more or less colored with impurities, crystallizes. It may be purified by solution in hot water and filtration of the hot solution through animal charcoal; but a part of the carnine will remain in the charcoal.

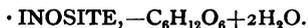
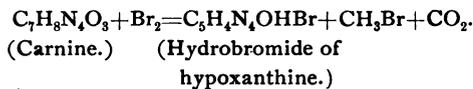
Carnine forms in fine, irregular crystals, which are very sparingly soluble in cold, more freely in hot water, insoluble in ether and alcohol. Its hot aqueous solution is neutral in reaction and is not precipitated by the neutral acetate of lead; indeed the presence of the neutral acetate will prevent the precipitation of carnine by the basic acetate of that metal. When heated to 100° , carnine gives up its water of crystallization and is transformed into an amorphous mass.

If carnine be dissolved in warm hydrochloric acid, crystals of the chloride of carnine form in needles as the solution cools. This compound is formed by the simple combination of the acid with the base and has the formula, $C_7H_8N_4O_3HCl$. If chloride of platinum be added to a solution of the chloride of carnine, a double salt is formed and deposited in a yellow crystalline powder. Nitrate of silver precipitates carnine, forming a white flocculent

mass which has the formula, $(C_7H_8N_4O_3)_2AgNO_3$, and is insoluble in both ammonia and nitric acid.

If a small quantity of carnine be treated with fresh chlorine water and a trace of nitric acid and the mixture, after gas has ceased to be given off, be heated to dryness on the water-bath, a white residue remains. If now the dish containing this residue be placed under a bell jar which has been filled with vapor of ammonia, the white residue will gradually become dark-red. If this experiment be performed in the laboratory where there is considerable ammonia in the atmosphere, the red color will frequently appear as soon as the chlorine water and acid have been evaporated. Hypoxanthine gives this same test, and a similar one, known as the murexide test, is given by uric acid.

If a hot aqueous solution of carnine be treated with a saturated solution of bromine water, gas will be given off and the brown color of the mixture will shortly disappear. If more bromine water be added until the color is permanent and the mixture be concentrated and allowed to cool, the hydrobromide of hypoxanthine will form in needle-shaped crystals. This change is represented by the following equation :



Inosite, also known as muscle-sugar, is found not only in muscle, but also in the vegetable world especially in green fruits and grains. It is present in the urine in diabetes mellitus, and in some forms of albuminuria. The muscular tissue of those long accustomed to the excessive use of alcohol, contains more inosite than that of healthier persons.

Preparation.—Inosite is best prepared from the muscles of the heart. Cut the heart of an ox into fine bits; put these into a beaker; cover with water and stir occasionally for four hours; then filter through a cloth, pressing the residue; stir the residue with more water in a beaker, and again filter through a cloth; slightly acidify these united extracts with acetic acid, boil and remove the coagulated albumen by filtration. Concentrate the

filtrate; add a solution of the normal acetate of lead, and remove the precipitated chlorides, phosphates, sulphates and carbonates by filtration. To this filtrate, freed from excess of inorganic acids, add some basic acetate of lead which throws down a precipitate containing impure inosite. Collect this precipitate, wash it with water, then suspend it in water and treat the mixture with a current of hydrosulphuric acid gas. Remove the precipitated sulphide of lead by filtration; concentrate the filtrate to a small volume; decant from any crystals that may form; add alcohol to the clear fluid, and set aside when inosite will crystallize.

Inosite may be obtained also by the method of Boedeker, which is as follows: To the syrup from which crystals of creatine have been obtained (see preparation of creatine), add from one to four times its volume of boiling alcohol. If a sticky, pasty precipitate forms, decant the supernatant clear fluid; but if a flocculent precipitate is formed, filter the solution through a warm filter. The clear fluid which has been decanted or the filtrate, after standing 24 hours, deposits crystals of inosite. The pasty precipitate, if such an one has formed, contains some inosite; consequently such a precipitate is dissolved in a little hot water, and this solution is treated with four times its volume of boiling alcohol and the supernatant fluid decanted from any residue and allowed to stand for 24 hours when the inosite will be deposited.

If the alcoholic solution fails to deposit inosite after standing 24 hours, add to it ether until a cloudiness appears and remains on agitating the fluid; then allow to stand for 24 hours longer, when inosite will be deposited in glistening scales.

Pure inosite forms in large rhombic plates and prisms, and contains two molecules of water of crystallization. It is soluble in water, insoluble in cold alcohol and ether. Its aqueous solution has a sweet taste, dissolves but does not reduce cupric oxide, does not undergo any kind of fermentation with yeast, and has no effect upon polarized light. By long exposure to the air at ordinary temperature or more rapidly at 100° , the crystals lose their water of crystallization and become opaque. When heated, inosite melts at 210° , and after cooling forms in fine needle-shaped crystals.

Inosite boiled with Fehling's solution does not reduce the copper, but changes the color of the solution from blue to green. It does not produce a brown coloration when boiled with potassic hydrate, or, in other words, fails to give Moore's test for sugar. It will be seen that inosite resembles grape sugar in its chemical composition, but the failure of the former to respond to the ordinary tests for the latter affords an easy method of distinguishing between the two.

If inosite be dissolved in water containing albumen and the solution be set aside in a warm place, as the albumen decomposes the inosite will be broken up, forming lactic and butyric acids. If an aqueous solution of inosite be boiled with basic acetate of lead, a jelly-like mass is precipitated.

Inosite is not changed by being boiled with dilute hydrochloric or sulphuric acids. If inosite be dried at 100° , then pulverized and dissolved with stirring in cold strong nitric acid and strong sulphuric acid be added to this solution, a white precipitate is thrown down. This precipitate which is represented by the formula, $C_6H_6O_6(NO_2)_6$, is *hexanitroinosite* and may be dissolved in boiling alcohol from which it crystallizes on cooling in rhombic tables and prisms. After the above compound has been deposited, the supernatant clear alcohol contains another substance which it deposits in groups of needles on concentration. This is *trinitroinosite*, and has the formula, $C_6H_6O_6(NO_2)_3$. Both of these compounds are explosive.

GLYCOGEN, — $C_6H_{10}O_5$.

Glycogen exists in the muscle, white corpuscles, and in all developing cells of the animal. The muscular tissue of the fœtus is especially rich in this constituent. It has been found in the placenta in large quantities; it exists in the embryo of the chick, and is abundant in the *ostrea edulis* and *cardium edule*. During foetal life the liver contains but little glycogen, while in the adult this organ seems to be the great manufactory and store-house of this substance. Only in structural disease of the organ, is the liver of any vertebrate animal free from glycogen.

Preparation.—Kill a large rabbit, in full digestion, by decapitation, quickly open the abdomen, remove the liver, cut into fine

pieces and place these in a dish of boiling water. Let the pieces cook until they harden ; then decant the fluid into a beaker ; rub the pieces of liver up in a mortar ; return the pulp to the dish ; add distilled water and boil for half an hour ; filter and cool the filtrate by surrounding the vessel with snow or by placing it in ice water. To the cooled filtrate add hydrochloric acid and potassio-mercuric iodide (prepared by dissolving mercuric iodide in a boiling solution of potassic iodide to saturation) alternately as long as a precipitate forms. Agitate well, allow to stand for five minutes, and remove the albuminous matters, which have been precipitated by the hydrochloric acid and potassio-mercuric iodide, by filtration. To the filtrate add alcohol, constantly stirring, until an abundant precipitate of glycogen begins to fall. An excess of alcohol is to be avoided, for after the complete precipitation of the glycogen the continued addition of alcohol will throw down other substances. Allow the precipitated glycogen to subside ; then collect it upon a small filter and wash with alcohol of 60 per cent. until the filtrate is no longer rendered turbid by the addition of a dilute solution of potassic hydrate containing a little ammonia ; then wash with alcohol of 95 per cent. ; then with ether, and finally with more alcohol. Dry in a dessicator over sulphuric acid. The repeated washing of the glycogen with alcohol, leaves it as a fine powder which can be easily shaken from the filter.

Glycogen is a white, amorphous, tasteless, odorless powder, which is freely soluble in water, insoluble in alcohol and ether. If it be dried without having been previously washed with strong alcohol, it forms a pasty mass. The aqueous solution of glycogen is opalescent, but becomes clear on the addition of potassic or sodic hydrate. The aqueous solution is dextrorotatory, turning the light three times as far as a similar solution of grape sugar. On concentrating an aqueous solution of glycogen, a pellicle forms on the surface of the liquid. Filtration through animal charcoal removes the whole or the greater part of the glycogen from its solution.

If freshly prepared glycogen be treated with a solution of iodine (sufficient metallic iodine dissolved in a solution of potassic iodide to impart a wine-red color to the solution), the glycogen is

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stained red ; if dried glycogen be treated in the same manner, a brown color is produced. If glycogen be boiled with dilute hydrochloric acid, the former is converted into grape sugar ; the same change is produced by the action of the saliva, pancreatic juice or blood. It dissolves, but does not reduce cupric oxide. In an ammoniacal solution of the sulphate of copper, glycogen dissolves, forming a deep blue solution from which it is precipitated on the addition of nitric acid. By the action of cold strong nitric acid, it is converted into xyloidin ; on being boiled with dilute nitric acid, oxalic acid is produced. By prolonged boiling with strong alkalis, glycogen is decomposed. The addition of acetate of lead to an aqueous solution of glycogen, simply produces a turbidity, and, if this solution be treated with a current of hydric sulphide, the sulphide of lead remains suspended until an alkali is added.

If to an aqueous solution of glycogen a few drops of blood be added and the mixture be kept on the water-bath for some time at a temperature of 40° , then freed from albumen and tested with Fehling's solution, sugar will be found to be present. The blood acts as a ferment converting the glycogen into sugar : this conversion consisting in the assumption of a molecule of water. A similar test should be made with a mixture of saliva and an aqueous solution of glycogen.

It will be seen both from the formula and from its various reactions that glycogen is a starch. It is especially abundant in the liver of animals which have been fed upon starchy or saccharine food. In some animals, the rabbit, for instance, after prolonged fasting the glycogen entirely disappears from the liver. Food consisting principally of fat does not increase the amount of this substance. What becomes of the glycogen of the liver is a question not positively decided. It is supposed to be gradually converted into sugar which is oxidized in the blood and assists in the production of muscular activity ; but how the blood oxidizes the sugar is not known.

PARALACTIC ACID,— $C_3H_6O_3$.

This substance is always present in the muscle and has been

found in the bile, in the urine after poisoning with phosphorus and in the bones in cases of osteomalacia.

Preparation.—This acid, which was first obtained by Wislicenus, is prepared as follows: To Liebig's extract of meat add four times its volume of tepid water; to this add, constantly stirring, about 8 parts of alcohol which throws down a precipitate; allow this precipitate to subside and decant the supernatant fluid. The greater part of the paralactic acid is contained in the fluid which has been decanted, but traces remain in the precipitate. In order to remove these traces, stir up the precipitated matters with warm water, add alcohol, allow to stand and again decant. Unite and concentrate the alcoholic solutions to a syrup on the water-bath; extract with alcohol; again evaporate the alcoholic solution to a syrup on the water-bath; render this syrup acid by the addition of a small quantity of sulphuric acid; then shake well with ether; remove the ether and agitate repeatedly with this agent. Evaporate the united ethereal extracts, when impure paralactic acid remains; dissolve this residue in a little water, add some carbonate of lead, boil and filter; treat the filtrate with a current of hydrosulphuric acid gas and again filter. Boil this filtrate until all the odor of hydric sulphide disappears and to this solution while yet hot, add carbonate of zinc to neutralization. The paralactate of zinc is formed and remains in solution. Concentrate the solution until on cooling crystals begin to form; then add five volumes of alcohol of 90 per cent. After standing a while, the mixture becomes turbid and is then filled with minute crystals. These may be collected upon a filter and washed with alcohol; they may be purified by repeated solution in water and precipitation with alcohol.

The crystals, as prepared above, are composed of the paralactate of zinc and the free acid may be obtained by treating a cold saturated aqueous solution of the crystals with a current of hydric sulphide gas, removing the precipitated sulphide of zinc by filtration, concentrating the filtrate to a syrup, extracting this syrup with pure ether, filtering the ethereal solution and allowing to stand until the ether evaporates spontaneously, when paralactic acid will remain as a syrup.

Paralactic acid is, at ordinary temperature, a liquid of a syrupy consistency and miscible with water in all proportions. It combines with many bases, acting as a monobasic acid and forming characteristic compounds. Of these, one of the most important is the paralactate of zinc, which by the spontaneous concentration of its aqueous solution forms in fine prisms often arranged in bundles. The paralactate of lime is formed when calcic hydrate is boiled with paralactic acid, the excess of lime removed by precipitation with carbonic acid gas and filtration and the filtrate concentrated.

NERVOUS TISSUE.

A complete analysis of the brain or nerves has never yet been made. The substances composing this tissue are of a very complex organization, are separated from one another with great difficulty and at best but imperfectly, and some of them are probably more or less changed during the process of extraction. A long list of chemical substances obtained from the brain has recently been given, but such a list must be accepted with caution; for a great many of the ultimate analyses from which the formulæ of these substances are computed have most likely been made upon mixtures rather than pure chemical compounds. Consequently a full history of all the substances which some claim to have discovered in the brain will not be given here: only a few of those best known and most thoroughly studied will be noticed.

CEREBRIN.

The formula of this substance is probably $C_{17}H_{33}NO_3$. It was first prepared by Müller who made many analyses of it and deduced the formula given above. Otto discovered a substance resembling Müller's cerebrin but containing no nitrogen.

Preparation.—Free a brain from its membranes and blood-vessels as completely as possible; wash with cold water; rub the brain up in a mortar; cover the pulp with cold dilute alcohol and allow to stand for three days with frequent stirring; then decant the alcohol. The alcoholic extract contains lecithin and neurin and may be used for the preparation of these, but it contains no cerebrin. The residue of brain insoluble in alcohol is

now repeatedly extracted with ether as long as this reagent dissolves any thing, ascertained by allowing a few drops of the ethereal extract to evaporate spontaneously and observing whether any residue be left or not. The ether dissolves cholesterine and lecithin but not the cerebrin. The residue which has proven to be insoluble in cold alcohol and ether is now boiled with alcohol with frequent stirring and the mixture while yet hot is filtered. The residue upon the filter is repeatedly washed with boiling alcohol. The united filtrate and washings are allowed to cool, when cerebrin mixed with lecithin is deposited. The cold supernatant alcohol is removed by either filtration or decantation: the residue consisting of impure cerebrin is repeatedly washed with cold ether in order to remove the lecithin, then boiled for an hour with baric hydrate. This mixture is then treated with a current of carbonic acid gas which precipitates baric carbonate and with it the cerebrin; filter and wash the precipitate first with cold water then with cold alcohol; suspend the precipitate in alcohol, boil and filter while hot. The boiling alcohol has extracted the cerebrin from the carbonate of barium and as the alcoholic filtrate cools, cerebrin is deposited. For further purification, the cerebrin is redissolved in boiling alcohol, from which it is deposited on cooling then finally washed well with ether and dried over sulphuric acid.

Prepared as above, cerebrin forms a white, odorless, tasteless, hygroscopic powder which consists of microscopic granules. It is insoluble in cold water, alcohol and ether, soluble in boiling alcohol or ether. In boiling water, it forms a pasty mass and dissolves to a slight extent; it is insoluble in boiling alkalis. When boiled with dilute mineral acids, cerebrin is quickly decomposed forming a sugar-like substance with laevorotatory power, but incapable of undergoing alcoholic fermentation and another substance whose properties have not yet been studied. Cerebrin is decomposed only after prolonged boiling with an alcoholic solution of potassic hydrate. With concentrated sulphuric acid, cerebrin is converted into an oily mass which at first is of a beautiful purple color, then gradually becomes brown and finally black.

Moist cerebrin, especially when mixed with lecithin, appears

under the microscope as granules or more frequently as fibres more or less twisted. Solutions of cerebrin in hot alcohol are without action upon litmus paper. If some cerebrin be placed upon platinum foil and gradually heated, it becomes brown at 80° , then melts and finally burns with a reddish flame.

LECITHIN, $-C_{42}H_{84}NPO_9$.

Lecithin is found in both the vegetable and animal, as a constituent of the fluids of the cell in the former and in all the principal fluids of the latter. It is a constituent of spermatic fluid, of the fluids and yolk of the egg, of the blood, bile, transudates, nerves and brain. It may be prepared from any of the above mentioned substances but is generally obtained from either the brain or the yolk of the egg, since these are rich in lecithin.

From Egg-Yolk.—Hoppe-Seyler prepares lecithin from the yolk of eggs as follows: The yolks freed from the whites are shaken with successive portions of ether, as long as any decidedly yellow tint is imparted to the ether. The removed ethereal extracts are discarded and the residue remaining insoluble in ether, is treated with a large excess of water, filtered, pressed and then extracted with alcohol on the water-bath at a temperature from 50° to 60° . The alcoholic extract is concentrated to a syrup as quickly as possible at the above temperature. This syrup is dissolved in a little absolute alcohol, and the filtered solution is kept in a covered glass vessel for from 12 to 24 hours at a temperature of from -5° to -20° . At the expiration of this time, a deposit which generally consists of granules, though sometimes of crystalline plates, forms. This precipitate is collected in the cold, pressed and dried in vacuo over sulphuric acid. By this method, its author claims that lecithin quite pure is obtained; but the loss is very great.

Strecker has introduced the following method of obtaining lecithin from the yolks of eggs: Extract the yolks with a mixture of alcohol and ether; heat the extract gently until the greater part of the ether is given off, then to the remainder after cooling add a solution of chloride of platinum acidified with hydrochloric acid. This precipitate is a double salt of lecithin and platinum, is soluble in ether and is precipitated from its ethereal solution

on the addition of alcohol; consequently, it is purified by being repeatedly dissolved in ether and precipitated with alcohol. Finally the ethereal solution is treated with a current of H_2S gas and the precipitated sulphide of platinum removed by filtration. The filtrate containing the lecithin is evaporated at a gentle heat. According to Hoppe-Seyler, lecithin prepared by this method is by no means pure. Evidently the lecithin thus obtained contains chlorine which may be removed by boiling an ethereal solution of the impure lecithin with the oxide of silver, removing the precipitated chloride of silver by filtration and the excess of silver from the filtrate with H_2S gas and a second filtration.

Instead of the chloride of platinum the same salt of cadmium may be used to precipitate the lecithin. In this case the double salt of lecithin and cadmium may be washed with ether, in which it is but sparingly soluble, and be dissolved in alcohol acidified with hydrochloric acid. The use of the chloride of cadmium has the advantage that the precipitate may be freed from fat by ether.

From the Brain.—A brain freed from its membranes and blood-vessels is rubbed up with a little water; the pulp kept at 0° is repeatedly extracted with ether; the residue is freed from any water or ether by pressure; the cake is digested with alcohol at a temperature of 40° ; the mixture is filtered while warm; the filtrate is kept at or below 0° for some time, when impure lecithin containing cholesterine is deposited; this is collected upon a filter and washed with cold absolute alcohol and ether. The mass is again dissolved in alcohol at 40° and the solution is surrounded by a freezing mixture, when lecithin is in part deposited while another part remains in the solution and is obtained by evaporation.

Lecithin prepared by the method of Hoppe-Seyler is a brittle, colorless substance which is soluble in alcohol, very freely soluble in hot alcohol, less but yet quite soluble in ether, also soluble in benzol, chloroform and bisulphide of carbon. In hot water, it swells and forms a pasty mass but does not dissolve.

If lecithin be boiled with baric hydrate, it is soon decomposed with the formation of cholin or neurin, glycerinphosphoric acid

If stearic acid enters into the above combination instead of oleic acid and palmitic acid, stearin-lecithin would be formed and from what has already been given, the student will be able to write its rational formula.

GLYCERINPHOSPHORIC ACID,— $C_3H_5PO_6$.

This acid is found in the body only as it results from the decomposition of lecithin; it is found in the brain in cases of softening of that organ, in the blood and urine in leucocythæmia and in various transudates. It can be prepared from the yolks of eggs, from brain or from any substance containing lecithin. It may also be prepared by the direct action of glacial phosphoric acid upon glycerin. It is a syrupy fluid which at ordinary temperature slowly breaks up into glycerin and phosphoric acid. It is a dibasic acid forming salts with various bases; of these, the baric and calcic compounds are insoluble in absolute alcohol, soluble in water. The calcic salt is less soluble in hot than in cold water and it crystallizes from its solution in the latter on being raised to the boiling point.

Preparation.—Mix pulverized glacial phosphoric acid and glycerin kept at low temperature; solution accompanied by considerable increase of temperature takes place and glycerinphosphoric acid is formed. Dilute the solution with water and neutralize with carbonate of barium in order to remove any excess of phosphoric acid. Remove the precipitated phosphate of barium by filtration and add to the filtrate a few drops of dilute sulphuric acid in order to precipitate any barium and again filter; concentrate this filtrate in vacuo over sulphuric acid; it will be impossible to obtain the glycerinphosphoric acid perfectly free from water for if the temperature be raised sufficiently to drive off the water, the acid will be decomposed.

For the detection and estimation of glycerinphosphoric acid in animal fluids or in the brain, the following process may be used: Rub the brain up in a mortar with an excess of baric hydrate; or render the fluid, as the blood or urine, alkaline by the addition of baric hydrate; heat gently in order to coagulate albuminous matters; filter; remove the excess of barium from the filtrate by treatment with carbonic acid and filtration; con-

concentrate this filtrate to a small volume on the water-bath ; allow to stand for some hours ; pour off the fluid from any crystals of creatine which may have been deposited ; concentrate this fluid in vacuo over sulphuric acid ; extract with absolute alcohol which removes urea and other substances soluble in this menstruum ; dissolve the residue, which has proven insoluble in absolute alcohol, in a little water ; filter ; evaporate the filtrate to dryness ; rub this residue up with some powdered carbonate of sodium and nitrate of potassium and keep the mixture at a red heat in a porcelain or platinum crucible until all the organic matter is destroyed ; dissolve the cooled ash in a little water ; to this solution kept at about 40° , add a nitric acid solution of the molybdate of ammonia ; allow to stand for 24 hours, then collect upon a filter the yellowish-white precipitate of the phosphomolybdate of ammonia which has formed if glycerinphosphoric acid were originally present ; dissolve this precipitate in dilute ammoniac hydrate ; to the clear solution add chloride of ammonia, ammoniac hydrate and sulphate of magnesium. This throws down ammonio-magnesian phosphate which may be collected, dried, heated and weighed as the pyrophosphate of magnesium, $Mg_2P_2O_7$. (See p. 27). From this the amount of phosphorus, of glycerinphosphoric acid and of lecithin may be computed.

CHOLIN, $-C_5H_{15}NO_2$.

Cholin, also known as neurin, exists normally in the body as a constituent of lecithin and when free is due to decomposition of lecithin.

Preparation.—Shake the yolks of eggs freed from the whites, first with ether, then with warm alcohol ; remove the ether and alcohol from the united extracts by distillation ; boil the residue for an hour with baric hydrate in order to decompose the lecithin ; treat the mixture with a stream of carbonic acid gas which precipitates all the barium not combined with the glycerinphosphoric acid ; remove the precipitated carbonate of barium by filtration ; concentrate the filtrate at a gentle heat on the water-bath to a syrup ; extract the syrup with absolute alcohol which dissolves the cholin, but does not dissolve the baric salt of glycerinphosphoric acid ; to the filtered alcoholic extract, acidified with

hydrochloric acid, add a solution of the chloride of platinum. The double chloride of platinum and cholin, which is formed, is insoluble in absolute alcohol and falls as a bright-yellow precipitate. Collect this precipitate upon a filter; wash with absolute alcohol; dissolve in water; treat the aqueous solution with H_2S gas and remove the precipitated sulphide of platinum by filtration; concentrate the filtrate to a syrup on the water-bath and dry in vacuo over sulphuric acid. In this way the chloride of cholin is formed and may be freed from chlorine by dissolving in water, shaking with recently precipitated oxide of silver, and filtering.

Cholin is a colorless syrup of a decidedly alkaline reaction, soluble in water and alcohol and unites with acids forming salts which are easily decomposed. The most characteristic of its salts are its double chlorides with platinum and gold. The former is soluble in water, insoluble in alcohol and ether and is deposited from its concentrated aqueous solution, after standing over sulphuric acid, in large orange colored rhombic prisms or six-sided plates, having the composition represented by the formula, $(C_5H_{15}NOCl) Pt Cl_4$. The double chloride of cholin and gold forms in fine yellow needles, which are also ~~is~~ soluble in alcohol and ether and which become brown on being heated. The chloride of cholin forms in colorless prisms, needles or plates, the latter often resembling the corresponding crystals of cholesterine. This salt is soluble in alcohol, but insoluble in ether.

THE URINE.

COLOR.

The normal urine of man is of a golden yellow color; while from various causes, some transient and unimportant, others more permanent and serious, this excretion may so vary in appearance as to present almost every shade of color. It must be remembered that what will be here given concerning the color of the urine applies only to the fluid and not to any deposits; consequently should any deposit be present, the same should be removed by filtration and the color of the clear filtrate determined. The color of the urine may be regarded as depending upon these two conditions, (1) variations in the proportion

of normal coloring matters present, (2) the introduction of abnormal coloring matters.

Pale urine is the result of an excess of water in this excretion and may be colorless. It is generally alkaline or neutral, sometimes feebly acid and is the normal urine of infancy and of extreme old age; while in others it may be due to the consumption of a large quantity of water either as such or as contained in food, especially vegetables and fruits, or to a pathological condition of the system as in diabetes, chlorosis and anaemia. Pale urine is generally of low specific gravity, the urine of diabetes mellitus being an exception to this rule. In all pale urines, the normal coloring matters are deficient in proportion to the water and the color of such urine is heightened by concentration.

On the other hand, if the normal coloring matters be in excess in proportion to the water, the urine will be more or less highly colored. This is the case when but little water is taken or when the water leaves the body through other avenues than the kidneys; thus, the urine excreted when the perspiration is greatly augmented is small in quantity, strongly acid and highly colored. Concentration has taken place in the body producing the same result as if the normal quantity of urine had been passed and then concentrated by the application of heat. Again the urine will be highly colored when it contains an excess of nitrogenous constituents. This may result from the consumption of much nitrogenous food or from the rapid disintegration of tissue as the result of disease; from the former cause the urine of the carnivora and of man, when living principally upon nitrogenous food, is highly colored, while from the latter cause result the reddish urines of febrile diseases. One of the sources of the normal coloring principles of the urine is in the process of the retrograde metamorphosis of muscular tissue and in this respect the same result follows whether it be from the disintegration of the muscle of the ox taken into our bodies as food or by similar changes going on in our own muscular system as the result of disease.

The abnormal coloring matters of the urine may be divided into two classes: (1) those which result from food or medicines; (2) those which are due to pathological conditions of the body.

In some persons the coloring principle of coffee is soon excreted by the kidneys and gives to the urine a brownish tint. Rhubarb, senna, santonine, hæmatoxylon, carbolic acid, creosote, tar and many other medicinal agents influence the color of the urine. Rhubarb colors the urine a greenish-brown and often leads one to suspect the presence of bile-pigments. If a dose of santonine be taken and the urine for the next 24 hours be collected it will appear normal in color if it be acid, but upon the addition of an alkali the urine will become crimson. It must be remembered that the addition of santonine and an alkali to normal urine will not produce this color; it is due to the action of the alkali upon the substance into which the santonine is changed during its passage through the body. Either the internal or external use of carbolic acid or creosote will often cause the urine to be more or less dark, sometimes quite black; an inunction of tar will produce the same result.

We have now to consider those colors of the urine produced by pathological conditions. Greenish-brown or reddish-brown urine may result from the presence of bile-pigments. Blood may produce a variety of shades; thus if the bleeding be from the bladder or urethra and especially if it be profuse the coloring matter yet existing as hæmoglobin, the color will be red; while if the blood has passed through the kidney, the corpuscles will often be disintegrated and the coloring matter so changed as to give to the urine a smoky or dark tint and indeed it may be black. In some rare cases, the urine after standing for some time becomes blue or more frequently a blue pellicle forms upon the surface or blue granules are deposited. This has been observed in various forms of albuminuria and in diabetes mellitus and is supposed to be due to the decomposition of indigo-forming substances.

The fact that a specimen of urine is of a normal color is not proof sufficient that it is normal in other respects. The pale urines indicate either a temporary excess of water or some chronic disease, never an acute form; while the highly colored, the red, brown and dark varieties are indicative of acute forms of disease, unless they be produced by the food.

THE AMOUNT OF URINE.

Formerly it was thought that it was only necessary to estimate the per cent. of urea and other constituents of the urine. Consequently, in many of the older works, we find long lists of figures given showing the number of parts per thousand of chlorides, phosphates, etc. A moment's thought will convince us that the great majority of these analyses are of no value. Suppose that you eat much solid food and drink but little water and other liquids; while your friend eats but little solid food and consumes large quantities of some drink; is it reasonable to suppose that the number of grammes of urea in a litre of the urine of each will, by any means, be the same? The old method has passed away and we now estimate the amount of urine and its various constituents passed in a given time. The most suitable period to take as the basis of our estimations is twenty-four hours; because, during this time man passes through a cycle of changes, which with greater or less variations are repeated every subsequent day.

Having decided upon the time for which the urine should be collected, the next question is how should it be done. It is necessary that the vessel should be perfectly clean, and we use this word, clean, in a scientific sense and not according to the ordinary acceptation of the term. Patients, who should know better, when requested to collect their urine will often bring it to the physician in a bottle from which they had poured some oil, rinsed it with a little water and called it clean. In order to cleanse a bottle for this purpose, it is best to wash it out first with water, then with a solution of caustic potash, again with water, then with dilute sulphuric acid, and then rinse it with distilled water until the rinsings cease to give an acid reaction when tested with litmus. The patient is then instructed to completely empty his bladder at a certain time, throwing this discharge away, and to collect in the prepared vessel all the urine excreted until the same hour of the next day. Caution must be taken to prevent loss of urine when at stool. After it has been collected, the urine should be measured in clean glass jars or cylinders graduated according to either the French or English system.

When we remember that in health, the kidney is one of the

channels through which the excess of water passes from the system, we shall appreciate the fact that in a healthy condition, the amount of urine will vary (1), with the amount of water ingested and (2), with the quantity that leaves the body by other avenues. To these, we must add a third physiological condition, which is constantly influencing the quantity of the renal secretion, i. e., the quality and quantity of solid food.

As a rule, the quantity of urine is from one-tenth to one-half more than the amount of water drank; but it must be borne in mind that this proportion may be reversed by excessive perspiration or by watery stools. I found, in experimenting upon this subject that when the average mid-day temperature was 72° F., in the shade, for every 1000 c.c. of water drank, I excreted 1220 c.c. of urine. In this case, I took but little exercise. The excess of water in the renal excretion over that ingested comes partly from the water contained, as such, in the solid food and partly from the oxidation of the hydrogen of the food. Moreover, when the atmosphere is very damp, more water may be absorbed through the lungs than is exhaled. It will be seen from this that by increasing or decreasing the quantity of water drank, we can, as a rule, correspondingly increase or decrease the amount of urine excreted in a given time. Can we make any use of this fact in treatment? We can in case the daily excretion of urine is too small, but if, on the other hand it is too large, I doubt the propriety of restricting the patient in the gratification of his thirst. In all such cases as the latter, the cause of the trouble lies deeper than the mere consumption of an excess of water, and this cause must be sought for, and the treatment directed to it; because the abnormal thirst is but an effect and follows the cause just as necessarily as darkness follows the withdrawal of light. I knew of a case of diabetes insipidus, which a man who wrote M. D. after his name (we suppose that in this case, these letters signify *disgrace to medicine*,) treated by locking his patient in a room and allowing her but a small quantity of water. The thirst still existed and its gratification was a necessity; consequently, the urine, as soon as it passed, was swallowed by the patient in vain endeavor to relieve her unbearable suffering. These diseases in

which there is an excess of urine passed, will be discussed in subsequent lectures. Fortunately for the physician the majority of his cases in practice will belong to the former class, i. e., when there is a deficiency of the urinary excretion. I want to impress the importance of attending to this subject; because it has been overlooked by too many. A great many persons drink too little water. The merchant goes behind his counter and in order to avoid frequent visits to the water-closet, drinks but little water; consequently, his urine is small in amount, of high specific gravity, strongly acid, and often deposits urates, uric acid and oxalate of lime in the urinary passages. The result is irritation of the bladder with cystitis, or a stone is formed. If the physician sees him in time to avoid these disastrous consequences and advises him to drink more, the reply often is, "Give me some medicine for it, I do not want to drink much water or I will have to go out every hour." As soon as his bladder becomes irritated micturition will necessarily be more frequent and his own actions compel him to traverse a rougher road than the one which he endeavored to shun. It must not be supposed, by my specifying the merchant that this class only commit this error. The same mistake is made by ladies who are out in society much; by the student who does not wish to be interrupted in his studies by the calls of nature; and even by the physician, who is so constantly attending to the wants of others, that he forgets his own. From the foregoing, I think that we are justified in deducing the following rule: If your patient complains of some irritation of the urinary tract and upon examination you find the amount of urine 1000 c.c. or less, the specific gravity 1028 or higher, the reaction strongly acid, no sugar or albumen, have him measure the amount of water that he drinks during twenty-fours and see if it is not correspondingly small. If this be the case, it is well to give some mild diuretic dissolved in much water. For this purpose, citrate or acetate of potash will often be found very suitable; because, during their passage through the body, these salts are converted into carbonates, which will decrease the acidity of the urine. It must be remembered that in no case should these remedies be used in quantities sufficient to render the urine alkaline.

We have next to consider the effect produced upon the daily amount of urine by the quantity of water excreted through other channels. One day when the mercury went up to 100° F. in the shade, I walked eleven miles at the rate of three miles an hour and spent the remainder of the twenty-four hours in my room, comparatively inactive; during this time I drank 2000 c. c. of water and excreted 562 c. c. of urine. It is owing to the diminished cutaneous exhalation, that more urine is excreted in winter than in summer. Whether normal sweat contains any urea or not is a question still under discussion. Funke and others claim to have found it present in large quantities; but it is evident that either they mistook something else for urea, or the sweat which they examined was not normal. My own opinion, founded upon experiments, is that in a perfectly healthy condition urea is not a constituent of perspiration. Be this as it may, it is well known that when the kidneys are so changed in structure as to fail in the performance of their function, not only urinary water, but the solids, both organic and inorganic may pass off through the skin. Consequently, in these diseases, the intelligent physician often causes, by means of the hot air-bath, a profuse flow of perspiration and in this way removes from the blood, urea, uric acid and other poisonous substances. In such cases, urea or the product of its decomposition, carbonate of ammonia, is also excreted by the lungs.

The third physiological factor, upon which the daily excretion of urine depends, is the solid food—its quality and quantity. It was long ago observed that man passed more urine when living upon animal food, than when he subsisted upon vegetables. Lehmann found that when his daily rations consisted of 39.79 oz. of animal food (eggs), he excreted 1202.5 c.c. of urine; while when he ate the same amount of vegetable food, he passed 909 c. c. of urine. In a series of carefully conducted experiments, I found that when I consumed in my food daily 225 grains of nitrogen, the average amount of urine was 960 c. c.; and when my food contained 155.9 grains of nitrogen, the urine excreted amounted to only 769 c. c. In these experiments all the food was weighed and the drink measured and the only change which was made

and which reduced the quantity of urine was the withdrawal of solid nitrogenous food. The effect of the kind of food has been observed in the lower animals. A cat, fed exclusively upon animal food, excretes seven and a half times as much urine for every pound of its body weight, as the horse, fed upon corn and hay, excretes. (Harley.) Many other experiments might be cited to show that the quantity of urine depends upon the quality of the food—whether it be animal or vegetable or mixed—and upon the quantity of nitrogen which it contains. The explanation for these facts is that nitrogenous food is a true stimulant and increases the rapidity of certain chemical changes going on in the body. Nitrogenous food hastens the oxidation and the consequent excretion of not only the non-nitrogenous substances that are taken in at the same time with the food, but also of the fat that may be stored up in the body. It will be remembered that a Mr. Banting proposed to reduce corpulent persons to any desired extent by feeding them exclusively upon animal food. His theory depends upon this fact, that the nitrogenous substances by acting upon the nerves increase the oxidation of the fat which has been stored up. The formula for stearine is $C_{57}H_{110}O_6$. It contains much hydrogen and when it is oxidized a corresponding amount of water will be formed as seen from the following equation :



We must free our minds of the old belief that the sole or even the principal office of nitrogenous food is to repair the waste of the muscular system ; for we have no evidence that such waste exists to any considerable extent ; but it is evident that this kind of food is a true nerve stimulant. I hope, though, that none of you will employ Mr. Banting's plan of reducing corpulency. Consider the extra amount of work that is thrown upon the kidney in eliminating the great quantities of urea, to say nothing of the water. Moreover, there is a safer and more reasonable way of removing any superfluous fat, as has been pointed out when discussing foods (in unpublished lectures)

Many other conditions have been mentioned by authors as influencing the amount of urine. As a rule women pass less

urine than men ; this is not due to any mysterious influence that sex has over this excretion ; but depends upon the fact that women eat less and are not so constantly engaged in physical exercise. It is equally evident why children pass more and old people less urine in proportion to the body weight than those in the prime of life. There are certain articles of food and drink which have a diuretic effect. This is true of onions, tea, coffee and wine or beer.

It will be seen from the preceding considerations, that it would be impossible to give exactly the number of cubic centimetres that constitute the normal daily excretion. An amount, which under certain conditions would indicate a serious disorder, would under other circumstances be a result of healthy action. In the examination of urine for either physiological or diagnostic purposes, the physician must be, as he should be in all of his professional work, both broad and deep in his observations. Every day, I see something which impresses upon me the belief that the most thorough analyses of the egesta are of but little value, as aids to treatment, without a corresponding knowledge of the ingesta and of the conditions surrounding the patient. There are these three important factors, (1) the quality and quantity of the ingesta, (2) the atmosphere in which the patient lives, and (3) the quality and quantity of the egesta, that should always be inquired into by the physician. There is now a tendency among medical men to depend too much upon the detection of abnormal constituents of the excretions and to neglect other investigations. For instance, a patient complains of nervousness, indigestion, and probably of some slight irritation of the urinary passages, the urine is examined and found to contain large quantities of oxalate of lime and uric acid, the physician inquires no farther, and prescribes nitro-muriatic acid. The prescription is all right, but the patient may be eating, all the while, such large quantities of starchy food, that the most heroic doses of nitro-muriatic acid will not suffice to oxidize it all ; or he may be sleeping every night in a room so poorly ventilated that the amount of oxygen inhaled is only sufficient to convert the carbonaceous part of the food into oxalic acid and not enough to produce carbonic acid ; or he may be

drinking so much wine that uric acid is necessarily produced in excess. The study of the excretions has richly repaid its investigators and it promises to yield to those who will continue to labor in its mines, gems brighter than any that have yet been brought to light. But we must remember that golden images can not be cast from molten lead; nor can Alpine plants grow in the burning sands of Sahara; neither can the excretions be normal so long as the food is abnormal; nor can man enjoy health so long as he violates the laws of hygiene.

From what has been said, we will be able to appreciate the fact that very different figures are given by different authors to represent the average daily excretion of urine. Valentin gives his average amount at 1447 c. c.; Lehmann, his at 1057 c. c.; Thudicum gives 1950 c. c. as an average for seventy-six days for a man aged 28 yrs., weight 70 kilos. My average, age being 26 yrs., and weight 65 kilos., for 100 days is 960 c. c.

A study of the hourly variations in the amount of urine excreted, presents some very interesting points and enables us to understand more fully the daily cycle of changes through which man passes. I will give three tables representing the hourly excretion for three consecutive days. The day, as here understood, begins at 12 M. At the expiration of each hour, with the exception of the time during which I slept, the urine was passed into a graduated glass and the amount noted. The figures represent so many c. c.:

TABLE NUMBER ONE.

Dinner at 12; Supper at 6; Breakfast at 9.45; Sleep from 11 P. M. to 7 A. M.

	P. M.											A. M.						
Hour.....	12	1	2	3	4	5	6	7	8	9	10	11	7	8	9	10	11	12
Amount.....	50	62	65	50	35	28	27	34	39	15	19	14	189	44	52½	60	52½	52½

TABLE NUMBER TWO.

Dinner at 4.30; No supper; Breakfast at 8.30; Sleep from 11 P. M. to 7 A. M.

	P. M.											A. M.						
Hour.....	12	1	2	3	4	5	6	7	8	9	10	11	7	8	9	10	11	12
Amount....	52½	52	63½	47	37	33½	24½	22½	19½	25	24	15	131	33½	36½	43	18½	45

TABLE NUMBER THREE.

Dinner at 1; no supper; no breakfast; drank 8 ozs. of water at 6 P. M.;
sleep from 11 P. M. to 7 A. M.

	P. M.											A. M.						
Hour.....	12	1	2	3	4	5	6	7	8	9	10	11	7	8	9	10	11	12
Amount.....	45	40	24	27	16	16	12	16	17	11½	11	10	68½	25	35	39	22½	45

In no case, with the exception indicated in table No. 3, was any food or drink taken between meals. It is very evident from the tables that from about 2 P. M. there is a gradual decrease in the amount until the hour of retiring; while on the other hand, from about 8 A. M. there is a gradual increase until mid-day. This decrease during the afternoon and increase during the forenoon is quite independent of the food and drink. Thus, in table No. 3, although dinner was taken at 1 P. M. and 8 ozs. of water consumed at 6 P. M. and neither food nor drink taken during the morning, still the forenoon increase and the afternoon decrease appear. In both tables 2 and 3, it will be noticed that the amount passed at 11 A. M. is small. This seems to be an exception to the forenoon increase; but the decrease in the amount passed at this hour is due to the fact that on each of these days, the preceding hour (from 9 A. M. to 10 A. M.) was devoted to physical exercise (walking) which caused the perspiration to flow freely. In noting this hourly variation, we have only written another line in that great volume of facts which demonstrate the plant-like life of man. Only under the influence of sunlight is the carbonic acid decomposed and the carbon transformed into plant tissue; likewise, the light of day is essential to the full activity of the organs of digestion, absorption and excretion.

We will now briefly consider the effects of remedies upon the amount of urine. In the selection of a diuretic, the physician should first ascertain the cause of the small excretion and then treat accordingly. It would be very unwise to administer, in every case of diminished excretion of urine, acetate of potash simply because that article is classed with the diuretics in the *Materia Medica*. Remember that rational men believe that every diseased state has its cause and in the condition now under con-

sideration it is the cause of the diminished flow that we must endeavor to remove. The amount of urine varies directly with the arterial pressure; consequently, if there be a want of vascular fullness, water is the best diuretic that can be given. In these cases, drinking large quantities of water increases the amount of urine, diminishes its specific gravity, lessens the acidity and, consequently, soothes any irritated part of the urinary tract. In fevers, water and sweet spirits of nitre serve the double purpose of cooling the body and increasing the amount of the renal secretion, of gratifying the desires of the patient and accomplishing the objects of the physician. If there be slight congestion of the kidney, as shown by the diminished excretion and by a dull pain in the loins, sweet spirits of nitre is again useful. If the urine be small in amount, strongly acid, containing free uric acid and producing irritation, acetate and citrate of potash, as has already been shown, are beneficial. But if there be general venous stasis from diseased action of the heart, digitalis should be combined with the salts of potash. The digitalis acts upon the heart, produces free circulation, increases arterial pressure, removes the stagnating blood loaded with carbonic acid and other poisons from the kidney, and prevents those changes in the renal structure which would necessarily follow from malnutrition. The salts of potash dissolve and probably oxidize the uric acid and thus prevent the formation of gravel and calculi. Both the digitalis and potash increase the amount of urine in these cases. Brunton has shown (On Digitalis, page 43,) that in health, this drug increases the amount of urinary water, and I have seen the daily amount of urine rise from 880 c. c. to 1100 c. c. within three days from the administration of five drops of the tincture of digitalis three times per day in a case of "irritable heart." If there be any inflammation of the urinary tract, as pyelitis, cystitis or urethritis, or if the condition known as "irritable bladder" (when the urine is concentrated and is strongly acid, and when there is a constant desire to urinate with but little relief from micturition) exists diuresis is best produced by the combination of either buchu, pareira brava, or uva ursi with a vegetable salt of potash (H. C. Wood, *Materia Medica*, page 475). In paren-

chymatous inflammation of the kidney, all irritant diuretics must be either avoided altogether, or given with the greatest care.

When the flow of urine is excessive from debility and consequent relaxation, it is best to build up the system by the use of tonics. For this purpose, iron, strychnia, and quinia have proven very efficient. The treatment of diabetes insipidus and other diseases in which there is an excessive excretion of urine, will be discussed in subsequent lectures.

THE REACTION.

The reaction of urine is best ascertained by its action upon blue and red litmus paper. If it be acid, it will color blue litmus paper red; while, if it be alkaline, it will color red litmus paper blue; and if it be neutral, it will produce no change upon either kind of the test papers. If the urine be found alkaline, it is important to decide whether this reaction is due to a volatile or to a fixed alkali. If it be due to a volatile alkali, ammonia, the blue color imparted to the test paper will disappear upon drying, but if due to a fixed alkali, the color is permanent.

The reaction of the mixed twenty-four hours' urine, if normal, is always decidedly acid when collected. This reaction is due to the presence of acid phosphate of soda, acid urates, kryptophanic acid, probably lactic, and perhaps other organic acids. If kept in a clean vessel and in a cool place, the acidity is increased, or the urine undergoes the acid fermentation within a few days. During this process, an organic acid—probably lactic from the sugar which Pavy has shown to be present in small quantities in normal urine—is developed and unites with the bases setting free uric acid; while the latter is converted by the oxygen of the atmosphere into oxalic acid, which immediately unites with the lime present, and the oxalate of lime thus formed is deposited in octohedral crystals. If the urine contains an excess of mucus, or if it be kept in a warm place, the acid fermentation either goes on so rapidly that it is not observed, or it does not occur at all. Be this as it may, the urine will sooner or later become alkaline. This depends upon the fact that the urea takes up two parts of water and is converted into carbonate of ammonia, as represented by the following equation :



That this decomposition is hastened by the presence of mucus may be proven by pouring into one beaker any amount of normal urine *without* filtration, and into another beaker an equal amount of *filtered* urine from the same specimen; setting the two beakers away and testing the reaction of each from day to day. It will be found that the specimen which has been deprived of its mucus by filtration retains its acid reaction much longer than the other. The same fact can be proven in another way. Divide a specimen of normal urine into two equal parts; to one of these add a quantity of mucus; set the two portions aside, and test as before. It will be found that the one containing an excess of mucus is first to become alkaline. This decomposition of urea into carbonate of ammonia may take place in the urinary passages, and from the experiments given above, it will be seen that this is especially liable to occur when the bladder pours out pus or an excess of mucus as is the case in cystitis. Why mucus hastens the decomposition of urea and the nature of the changes, if any, that occur in the mucus itself, are subjects which are not yet understood and which deserve careful investigation. Pasteur held that the change was due to atmospheric germs which found a nidus in the mucus, and consequently the more mucus a specimen of urine contained, the more suitable was it for the development of these germs. That this theory is entirely untenable must be admitted, when we remember that the decomposition goes on in the bladder, to which air has no access. *! see Nitro p. 82.*

While the mixed urine for twenty four hours is invariably acid when normal, the urine passed at different hours of the day, varies in reaction, and that passed at certain hours may be neutral or even alkaline, and still be normal. Dr. Bence Jones first observed that after a meal the acidity of the urine gradually decreased for a while until often it became neutral or alkaline. Dr. Roberts repeated the experiment of Dr. Jones, and found that after breakfast the acidity was sensibly decreased, within forty minutes, and continued to decrease until the expiration of the second or third hour; when the urine gradually regained its acidity. After dinner there was no perceptible change until the second

hour, and the greatest alkalinity was attained during the fourth and fifth hours. Dr. Jones thought that the alkalinity of the urine during digestion was due to the withdrawal of the acid from the blood to form gastric juice, and that the greater the alkalinity of the urine, the greater the acidity of the gastric juice, and *vice versa*. Dr. Roberts admits the probability of the theory advanced by Dr. Jones, but thinks it more likely that the decrease of acid in the urine after meals is due to the excess of alkalis in the food. (Roberts on Urinary and Renal Diseases, pages 38-40.)

I have made a great number of experiments upon the reaction of the urine passed at different times of the day, and while I think that Dr. Roberts is right in deciding that foods influence the reaction, I am compelled to believe that he has omitted many important circumstances upon which the reaction depends. The more I experimented, and the greater variations I made, the more fully was I convinced that the reaction of the hourly excretions of urine depends upon various and complicated factors. First as to the influence of the food. The degree to which the reaction is affected by food, depends upon the time of day at which the food is taken, as well as upon the kind and amount of food. The following tables, taken from a great number representing similar experiments, will illustrate my meaning. The positive sign signifies that the urine, passed at the hour indicated, was acid; while the negative sign represents an alkaline, and the cipher a neutral condition:

TABLE NUMBER ONE.

August 24. No food or drink taken until dinner; dinner at 1; supper at 6:15. Sleep from 11 P. M. to 7 A. M.

A. M.						P. M.											
Time when passed,	7	8	9	10	11	12	1	2	3.	4	5	6	7	8	9	10	11
Reaction.....	+	+	0	-	-	+	+	0	-	-	+	+	+	+	+	+	+

TABLE NUMBER TWO.

August 25. Breakfast at 9.45; dinner at 4.30; no food or drink after dinner. Sleep from 11 P. M. to 6 A. M.

A. M.						P. M.												
Time when passed,	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11
Reaction.....	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+

TABLE NUMBER THREE.

August 26. Breakfast at 8.30; dinner at 1; no more food nor drink taken until 1 P. M. of the next day. Sleep from 11 P. M. to 5 A. M.

	A. M.												P. M.											
Time.....	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11					
Reaction.....	+	+	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+	+					

On August 23, I took supper at 6 P. M., and as indicated in table No. 1, no food or drink was taken on the 24th, until 1 P. M.; nevertheless, the urine was neutral at 9 A. M., and alkaline at 10 and 11. What caused this alkalinity? Could it have been due to the supper of the preceding evening? It is well to remark here that during this forenoon I took no exercise: in fact did not leave my room. On the afternoon of the 24th, the urine was neutral at 2, and alkaline at 3 and 4. This was probably due to the food taken at 1 P. M. But supper was taken at 6 P. M., and the acidity of the urine passed at the expiration of each hour until 11 P. M. was estimated and not only did the secretion remain acid, but its acidity was increased. The food taken at dinner and supper of this day was weighed and, for the two meals, was identical both in quantity and quality. Here we have a certain amount of food taken at 1 P. M. causing the urine to become alkaline within two hours, while the same amount of the same kind of food taken at 6 P. M. does not lessen the acidity of the urine within five hours. On the morning of the 25th the urine was alkaline at 7, although breakfast was not taken until 9.45. Again, as shown in table No. 2, dinner was taken at 4.30, the urine being acid and had increased in its acidity at 11 P. M.; while on the subsequent morning, as shown in table No. 3, the urine was alkaline at 7, although breakfast was not taken until 8.30. It is evident, on an inspection of the tables, that food taken towards the close of day does not influence the reaction so quickly as that taken in the forenoon. It will be observed in table No. 3, that the urine was acid at 11 A. M. and alkaline during the preceding and subsequent hours. It may possibly be that the effects of the supper of the preceding day had ceased to be manifest while the breakfast had not yet produced its effects: but I think that the acidity possessed by the excretion of this hour

was due to the fact that during the hour from 9 to 10 of that morning, I walked constantly and rapidly. The conclusions which I have drawn from these experiments are (1). food, taken during the latter part of the day, undergoes very slowly those changes which are necessary before it can be excreted by the kidneys and (2), exercise increases the acids of the body and consequently the acidity of the urine. The first conclusion is but a corroboration of the facts ascertained with regard to the hourly excretion of urine and will be again emphasized when we study the variations in the amounts of urea excreted at different times of the day. The second conclusion would not be justifiable did it rest on the experiments given above, only; but other and, to my mind, sufficient evidence is at hand. During the Fall of 1877, I found thirty students sufficiently interested in this subject to estimate the acidity of the daily excretion of urine for from two to three weeks. During six days of the week, they attended lectures and clinics and performed laboratory work; while on the seventh day of these weeks of experimentation they took long walks through the country. The urine of the seventh day invariably contained more acid than that of any other day. The hours from 8 A. M. to 12 M. of each of the six days were passed in a poorly ventilated room listening to lectures and in physical inactivity. With but one exception out of the thirty, the urine passed upon leaving the lecture room at noon was alkaline and turbid from the precipitation of earthy phosphates. In the afternoon, the students were engaged in the chemical laboratory and consequently took more exercise. During this time the urine regained its acidity and maintained it until the following morning. At length, the time for examination drawing near, the laboratory work was discontinued, and the afternoons were devoted to close study, and the urine was constantly alkaline and turbid with phosphates. When the urine is alkaline either from food or insufficient exercise, the reaction is always due to a fixed alkali. From a long list of experiments, (Chem. Centr. 1878) R. Maly has also reached the conclusion that the acids of the body are increased by exercise. He finds that the acid phosphates of soda are especially augmented and mentions monosodic

phosphate (Na H, PO_4) as one of the constituents of the blood, resulting from muscular activity. The physiological evidence here given to prove that exercise in the open air increases the acids of the body is supported by clinical experience. What is the general condition of those patients, who are troubled with the deposition of phosphates in the bladder from urine alkaline with a fixed alkali? They are, so far as my experience goes, invariably those who are debilitated by age, by disease, by poverty, by either muscular inactivity or by want of pure air, or by both. It is true, as Roberts remarks, that an excess of fixed alkali in the urine is not so injurious as a volatile alkali. The urine with a fixed alkali is bland and the amorphous phosphates seldom form a stone, but are washed out with the urine; still they often cause some irritation, especially in old men with enlarged prostate. Moreover, urine alkaline from a fixed alkali always denotes a low state of vitality and should not be disregarded by the physician. In the treatment of these cases, two objects may be kept in view. These are (1), to relieve as speedily as possible, any irritation of the bladder and (2), to increase the vitality of the patient, and in this way to remove the cause of alkalinity. The latter is accomplished by the judicious use of tonics, by exercise and pure air. The former object is best attained by the administration of the weaker mineral acids (as carbonic and phosphoric) or of vegetable acids. In quite a number of cases of old men with urine alkaline from a fixed alkali and with irritation of the bladder causing frequent micturition, I have observed that drinking old cider rendered the urine acid and relieved the irritation very promptly. Carbonic and phosphoric acids act by combining with the excess of bases, (soda, potash and lime) in the blood forming acid salts which are excreted by the kidney and influence the reaction of the urine. Benzoic acid acts in a similar way, being converted during its passage through the body into hippuric acid, which combines with the bases forming hippurates. However, benzoic acid is not so useful in these conditions as it is when the urine is alkaline with ammonia. The administration of the strong mineral acids, as nitric and hydrochloric, in order to render alkaline

urine acid is in accordance with neither physiological nor chemical facts.

If the reaction of urine be due to ammonia, one of the following may be the cause; (1) the urine has been unduly retained in the urinary passages; (2) the bladder is not completely emptied at each micturition and some stale urine is left to decompose the normal as fast as it falls from the ureters; (3) there is some undue irritation of the urinary passages causing them to pour out pus, or an excess of mucus. The first one of these causes will be discussed fully in the lecture on retention. Suffice it here to say that the urine may be retained in the pelvis of the kidney, or in the bladder, and that the retention may be due to calculi, stricture, paralysis, enlarged prostate, morbid growths, and foreign bodies. The treatment consists in removing the cause. With regard to the second cause given above, it is well known that if normal urine be allowed to drop, at the rate which urine passes into the bladder, into a vessel containing putrid urine and the whole be kept at the temperature of the body, the urea of the normal urine decomposes very rapidly. It frequently happens that from enlarged prostate or other partial obstruction, the bladder is not completely emptied during micturition, consequently the remaining urine becomes putrid and decomposes the normal urine as it enters the bladder. In these cases, complete evacuation of the bladder should be secured, either by the removal of the obstruction, by drawing off the urine with a catheter, or, when these are impossible, by washing out the bladder frequently. To Sir Henry Thompson belongs the credit of calling attention to the fact that "*you can not completely empty every bladder with the catheter.* When the prostate is irregular in shape and throws out protuberances into the bladder, there are sinuses or spaces between them, which retain one, two or even more drachms of urine. Again there are not unfrequently numerous small saculi in the coats of the bladder which act in the same way." (Diseases of the urinary organs, page 194, third edition.)

We will now consider how irritation of the urinary passages may lead to the production of ammoniacal urine. This irritation may be due (1) to an abnormal condition of the urine when it

reaches the bladder, and (2) to the presence of some foreign body. Sometimes the urine, as excreted by the kidney, is unduly acid and irritates the mucous membrane of the passages. This causes the production of an excess of mucus, and we have already seen that urine containing much mucus becomes alkaline quicker than normal urine; consequently the urea is decomposed into carbonate of ammonia while the urine is yet in the bladder. Moreover, ammoniacal urine is very irritating and this change in the reaction from undue acidity to alkalinity only increases the inflammation and consequently the amount of mucus. Thus the mucus and the ammoniacal urine react upon each other, the latter increasing the irritation of the bladder and the former hastening the decomposition of urea. This condition may continue for years, and render the life of the person miserable. Foreign bodies set up a similar irritation and produce the same results. The treatment of these conditions will be discussed under cystitis.

The effects of the absorption of ammoniacal urine into the blood have been studied by MM. Gosselin and Robin (*Archives Generales de Medicine*, May and June, 1874). These experimenters first ascertained the effects on animals of subcutaneous injections of an aqueous solution of carbonate of ammonia. When large amounts of this were used, there were restlessness, cries, convulsive movements, slow pulse, a fall in temperature, albuminuria and diminution of the number of blood corpuscles. When but small quantities of the aqueous solution of ammoniac carbonate were used, the symptoms were slight, or none were observed.

The same investigators found that small and repeated injections of normal urine caused but slight local irritation, with a limited increase of temperature; that large quantities of normal urine were necessary to produce death, and that the only change observable at post mortem was slight renal congestion.

A mixture of carbonate of ammonia and normal urine was next used. After the injection of this mixture, severe local effects were soon manifest, the temperature rapidly increased and death quickly followed. Although large quantities of normal urine and carbonate of ammonia had been required, when used

separately, to cause death; still, but a small quantity of the mixture proved fatal. When putrid urine obtained from patients with cystitis was substituted for the mixture, the symptoms were much more severe and death followed more rapidly.

The conclusions of MM. Gosselin and Robin are as follows: (1.) "Urine spontaneously ammoniacal acts with greater intensity than a more concentrated mixture of carbonate of ammonia and normal urine." (2.) "Ammoniacal urine is very poisonous when injected subcutaneously and the intensity of its action varies with the amount of ammonia." (3.) "Local lesions and fever (similar to the conditions observed in the extravasation of urine) are manifest." (4.) "The pathological conditions correspond with those observed after death from urinary fever." (5.) "The poisonous effects are greatly increased when air is admitted." (6.) "The rapidity with which normal urine decomposes, when mixed with pus and blood and in contact with air, explains how febrile accidents occur after operations on the urinary organs, even when the urine was acid before the operation."

I have called your attention to these valuable experiments in order to have you fully appreciate the dangers that may follow upon the absorption of ammoniacal urine, especially after operations upon, or injuries to the urinary organs. In some subsequent investigations, MM. Gosselin and Robin (*Archives Generales de Medecine*, Nov., 1874) found that benzoic acid best prevented the absorption of ammoniacal urine. As has been already stated, the benzoic acid is converted into hippuric acid, which unites with the ammonia and other bases forming hippurates.

The acid may be taken in doses of from one to six grammes and is best given in syrup with some aromatic. The neutralization of the urine is generally accomplished within seven or eight days. (See *British and Foreign Medico-Chirurgical Review*, April, 1875.)

It now remains for us to consider undue acidity of the urine. The acid may be so much in excess as to cause a burning pain during micturition and the patient says that his urine "scalds." In these cases, the daily excretion is small. The undue acidity may be relieved with certainty by the administration of alkalis.

In the selection of medicines for this purpose, those should be selected which least disturb the stomach and bowels. In this respect individual peculiarities are at times very marked. Thus, although the acetates and citrates are generally entirely unobjectionable, in some persons they invariably produce nausea, while the carbonates cause no derangement of the stomach. The administration of tartrates is generally followed by more or less purging. Water used in these cases in large quantities is an antacid, since it dilutes the urine and in this way prevents the irritation. Indeed, in many instances the insufficient amount of water drunk is the sole cause of irritation of the urinary passages.

When the highly acid urine contains free uric acid or oxalate of lime or both, the administration of nitro-muriatic acid will, in many instances, prove very beneficial. This acid acts upon the stomach and liver, improving digestion and consequently rendering the changes in the food more complete, and converting the excess of uric acid and oxalic acid into urea and carbonic acid. The effects of the nitro-muriatic acid are due to its oxidizing and not to its acid properties.

SPECIFIC GRAVITY.

Since the urine consists of water holding in solution certain solids, its weight can never be as light as that of an equal volume of pure water; also since the difference between the weights ~~of~~ ~~equal weights~~ of equal volumes of urine and pure water will depend upon the proportion of solids contained in the former, the more concentrated a specimen of urine is, the higher will its specific gravity be. The most accurate method of determining the specific gravity of the urine consists in weighing a certain volume of the specimen at a certain temperature and dividing this weight by that of an equal volume of water at the same temperature. For this purpose, the urine and water may be measured and weighed in any small, clean flask or bottle; but it is more convenient to use a specific gravity bottle or pycnometer, which is a small bottle having a long stopper perforated with a capillary tube. This bottle is so made that when the stopper is accurately fitted it holds a certain number of c. c. (generally either 20 or 25) of water; since 1 c. c. of water weighs 1 gramme, it con-

tains the same number of grammes. The picknometer is filled to overflowing with the urine, the stopper is adjusted, the outside of the bottle wiped perfectly dry and the weight of the contained urine ascertained. Suppose that the picknometer contains 25 grammes of water and 25.5 grammes of the urine under examination, then if we consider the specific gravity of water as 1000, the specific gravity of the urine will be found from the following :

$$\frac{25.5 \times 1000}{25} = 1020.$$

This method is, as has been stated, the most reliable and whenever scientific accuracy is desired, it should be used ; but for the purposes of the physician, a more convenient method is desirable and it is furnished in the urinometer. This consists of a blown glass float with a bulb containing mercury for a weight and a shaft graduated so as to indicate the depth to which the instrument sinks in the fluid. The greater the proportion of solids contained in the urine, the less will the instrument sink and the more will its shaft project above the surface. The specific gravity of the urine is seldom above 1040, consequently the stem of the urinometer is graduated from 1000 to 1040. If the instrument be of convenient length and if only one be used, the lines on the stem indicating the depth to which the instrument sinks will be so close together as to render it difficult to decide within less than one degree as to which line coincides with the surface of the fluid ; consequently the best form consists of two separate urinometers, the stem of one being graduated from 1000 to 1020, and that of the other, from 1020 to 1040. It is only necessary in the use of the urinometer to place the instrument in the urine and read off the specific gravity.

After having found the specific gravity of a specimen, the weight of a given volume and the amount of solids contained in a given volume may be calculated. Suppose that during 24 hours, 1500 c. c. of urine are excreted and that the specific gravity of the specimen is 1020 ; now each c. c. of water weighs one gramme, but this urine is 1.02 times as heavy as water and each c. c. of this urine weighs 1.02 grammes and the weight of the 1500 c. c. will be found from the following :

N

$$1.02 \text{ grammes} \times 1500 = 1530 \text{ grammes.}$$

To ascertain exactly the total amount of solids contained in a specimen of urine is quite a difficult task and requires the use of complicated apparatus and much time. If a portion of urine be evaporated, even at the temperature of the water-bath, much of the urea is decomposed and passes off as ammonia; consequently the weight of the residue would fall short of that of the total solids originally contained in the fluid. Again the residue which is obtained by evaporation of urine is very hygroscopic, rapidly absorbs water from the atmosphere and this introduces another source of error. It is thus seen that a simple, even though it may not be perfectly exact, method of ascertaining the total amount of solids in the urine is desired. It has been ascertained as the result of numerous experiments made with the greatest care that if the specific gravity of a specimen of urine be less than 1018, the total amount of solids in 1000 c. c. of that urine will be represented by the product obtained by doubling the last two figures of the specific gravity considered as a whole number.

Suppose amount of urine for 24 hours = 1500 c. c.

Suppose the specific gravity = 1015.

Then total solids in 1000 c. c. = $15 \times 2 = 30$ grammes.

The total residue in 1500 c. c. is found by the following proportion :

1000 c. c. : 1500 c. c. :: 30 grammes : X, or 45 grammes.

It has also been found that if the specific gravity be above 1018, the total amount of solids in 1000 c. c. of the urine will be found by multiplying the last two figures of the specific gravity by 2.33.

Suppose amount of urine for 24 hours = 1200 c. c.

Suppose the specific gravity = 1020.

Then total solids in 1000 c. c. = $20 \times 2.33 = 46.60$ grammes.

The total residue in 1200 c. c. is found from the following proportion :

1000 c. c. : 1200 c. c. :: 46.60 grammes : X, or 55.92 grammes.

It now remains to consider the average specific gravity of normal urine and the variations that may occur in the same and

those which result from disease. From what has already been given with regard to the amount of urine excreted within 24 hours, it will be seen that it is both difficult and unwise to set up any absolute standard for the specific gravity of normal urine; for as a rule the amount and specific gravity vary inversely. The urine passed after drinking much water is known as *urina potus*, is pale and of low specific gravity sometimes as low as 1003. That excreted during sleep is called *urina sanguinis*, is of a brighter color, more acid and of a higher specific gravity, generally from 1012 to 1025; while the urine excreted after much solid food has been taken is known as *urina cibi*, is generally not so bright nor so acid as the *urina sanguinis*, but of a higher specific gravity, generally from 1015 to 1030. From this, the necessity of collecting all the urine excreted during the 24 hours is evident.

The specific gravity of the mixed 24 hours urine may vary in health from 1015 to 1030 and these limits may be passed temporarily without indicating any serious disorder; but it will be safe to say that if the specific gravity of the 24 hours urine continues for several days or weeks to be above 1030 or below 1015, some pathological condition of the body is indicated. A possible exception to this rule is furnished by the urine of pregnancy; for during the latter months of gestation the urine generally becomes more dense and may constantly have a specific gravity above 1030. If the urine be highly colored and of a high specific gravity, there is generally an excess of urea and some febrile affection is indicated. Pale urine of high specific gravity occurs in diabetes mellitus and in this disease the density may be as great as 1060, such an increase being an indication of the progress of the disease.

Albuminous urine is generally of low specific gravity and in parenchymatous inflammation the less dense the urine, the more serious the indication, since it is evidence of the retention of a large amount of urea. In amyloid degeneration of the kidney the urine often becomes more dense as the disease progresses and in the last stages the specific gravity may be 1040 or higher. This is due to the diminished amount of urinary water, the excretion for the 24 hours sometimes not measuring 100 c. c. In renal

cirrhosis, the specific gravity is less than 1020. In diabetes insipidus and in hysteria, the urine is of low specific gravity, the urinometer in some cases registering only 1002.

OTHER PHYSICAL PROPERTIES.

The odor.—The urines of different animals have characteristic odors which are due to volatile oils and in general resemble the odor of the fat of the animal. When recently passed, the odor is most perceptible because the temperature of the specimen is higher than it subsequently becomes unless heat be applied and because there is more of the volatile oil than there is after the urine has been passed for some time. The odor is often a valuable aid to one in determining whether a specimen be urine or not and if so, the urine of what animal. In making this determination the fluid should be heated and if necessary evaporated to dryness and the residue burned when, if the fluid be, or contains urine, the odor will be recognized.

Heating urine with nitric acid increases but also modifies the odor. Many articles of food and medicinal substances impart characteristic odors to the renal excretion. The internal use or even the inhalation of oil of turpentine produces in the urine the odor of violets. Asparagus imparts to the urine a peculiar and very disagreeable odor. Again the urine may have an abnormal odor as the result of pathological conditions, as the ammoniacal odor of cystitis and the peculiar fishy smell of some forms of albuminuria.

The Taste.—The urine of man has a bitter and a salty taste, the former being due to an organic principle, urochrome, and the latter to the chloride of sodium. In diabetes the taste is sweet and it was by the application of this test that sugar was first discovered in the urine; while in icterus the taste is bitter from the presence of bile. Drinking the urine which has been resorted to in cases of necessity has been found to increase the thirst.

The Temperature.—Patients sometimes complain and say that their urine scalds; now the urine receives its heat from the body and consequently, when in the bladder or when passing along the urethra, can not be of a higher temperature than the surrounding tissues. The irritation caused is not due to the high

temperature of the fluid, but may be caused by an inflamed or raw condition of the tissues or by the excessive acidity of the urine, or by both; for the latter not unfrequently produces the former. If the urine be excessively acid, the administration of an alkali and an increased consumption of water will soon relieve the difficulty; while if the tissues be inflamed, injections suitable to the case may be used.

Deposits.—The only deposit occurring in normal urine within 24 hours after its emission is a faint cloud of mucus which may be recognized by the ease with which it is distributed on agitating the fluid and by its insolubility in acids. After a greater or less length of time after emission, any urine will become alkaline and deposit phosphates, or before this period has been reached, it may undergo the acid fermentation and deposit oxalate of lime and uric acid. But any deposit, other than mucus, occurring in urine within 24 hours after emission must be regarded as pathological. Moreover it is not necessary that such a deposit should be visible to the unaided eye; thus oxalate of lime may be deposited in large quantity and the urine appear perfectly normal. The discussion of the various deposits will be given under the various substances forming such deposits.

UREA,— $\text{CH}_4\text{N}_2\text{O}$.

Urea is the principal organic constituent of normal urine and exists in the blood and in various transudates. It has been found in the amniotic fluid, the aqueous humor, lymph and chyle. Urea may be prepared synthetically or obtained from the urine.

To some urine (from 200 c.c. to 500 c.c.) add the baryta mixture (made by mixing two parts of a saturated solution of baric hydrate with one part of a saturated solution of baric nitrate) as long as the precipitate increases. Remove this precipitate, which consists of the phosphate and sulphate of barium, by filtration; concentrate the clear filtrate to a syrup on the water-bath; extract this syrup with alcohol; filter and evaporate the alcoholic extract to dryness on the water-bath; extract this residue with absolute alcohol; again filter and evaporate at 100° ; on cooling, this residue will be found to consist of a mass of crystals of urea. By this method quite pure urea is obtained; but the process is

attended with considerable loss, the urea being decomposed during evaporation.

Urea may also be prepared from human urine by the following process: Concentrate from 100 c.c. to 500 c.c. of urine of high specific gravity and of an acid reaction to a small volume on the water-bath; to this syrup kept at 0° , add an equal volume of strong nitric acid. After a short time a mass of crystals of nitrate of urea forms. Collect these upon a filter and dry by pressing them between folds of blotting paper; then dissolve them in water and add carbonate of barium with stirring as long as gas is liberated. The nitrate of barium is formed and the urea is set free. Evaporate the mixture to dryness at 100° and extract the residue with absolute alcohol; filter and concentrate the filtrate and allow to stand in a cold place when urea crystallizes.

Feed a dog for several days upon all the lean meat that it will eat. Collect the urine excreted by the animal during this time; concentrate it to a syrup on the water-bath; extract with alcohol; filter and again concentrate; extract this residue with absolute alcohol; filter, concentrate and allow to stand in a cold place when urea crystallizes.

Urea is the cyanate of ammonia and may be prepared artificially in various ways. Mix two parts of dry ferro-cyanide of potassium with one part of the black oxide of manganese on a thin iron plate; apply heat until the mixture burns thoroughly; extract the cooled residue with water; to the filtered aqueous extract add one and one-half parts of sulphate of ammonia; evaporate the mixture to dryness and extract the residue with absolute alcohol which dissolves the cyanate of ammonia or urea. On concentrating the alcoholic extract, urea forms in crystals.

Fuse the cyanide of potassium mixed with the oxide of lead; extract the residue with water, add sulphate of ammonia and proceed as above.

Urea forms in fine, long, four-sided prisms which are terminated at each extremity by short pyramids. The crystals are not hygroscopic, are freely soluble in water and alcohol, insoluble in anhydrous ether. The dry crystals may be heated to 110° with-

out decomposition, but in solution especially in the urine and more rapidly if the urine be alkaline, urea is decomposed with the formation of carbonate of ammonia.

Urea is a base uniting with acids to form characteristic salts. If urine or water containing as much as 10 per cent. of urea be treated with an equal volume of strong nitric acid and the mixture be kept in a cold place, nitrate of urea will be precipitated. This compound results from the simple combination of the acid with the base and is represented by the formula, $\text{CH}_2\text{N}_2\text{OHNO}_3$. It crystallizes in rhombic plates or prisms and is sparingly soluble in cold, more freely in hot water, insoluble in strong nitric acid. The study of this salt is important, since the detection of urea in many fluids depends upon the formation and recognition of these crystals.

If a solution containing as much as 20 per cent. of urea be treated with oxalic acid, the oxalate of urea is formed and also crystallizes in rhombic tables. These crystals contain water and are represented by the formula, $\text{CH}_4\text{N}_2\text{OH}_2\text{C}_2\text{O}_4 + 2\text{H}_2\text{O}$. This salt is sparingly soluble in cold water and consequently is sometimes recognized by its crystalline form in the microscopical examination of concentrated urine.

Urea combines not only with acids but also with some bases; thus, if a solution of urea be treated with one of mercuric nitrate a white precipitate consisting of the oxide of mercury, urea and nitric acid is thrown down. The exact proportion of these constituents contained in the precipitate depends upon the concentration of the solutions and the amount of free acid present. According to Liebig, there are three different compounds formed with mercuric nitrate and urea; the first contains four equivalents of mercuric oxide, the second three, and the third one, to one equivalent of urea. If any of these compounds be suspended in water, the mixture treated with H_2S gas and the sulphide of mercury removed by filtration, nitrate of urea will exist in the filtrate and may be recognized on concentration by its microscopical appearance. Upon the fact that urea is precipitated by mercuric nitrate depends the ordinary method of estimating the quantity of the former. ✓

Urea is decomposed in the presence of putrid animal matter, the decomposition consisting in the assumption of a molecule of water and the formation of carbonate of ammonia. This change has already been referred to and represented by an equation as the cause of the ammoniacal odor of putrid urine. If a solution of urea be treated with one of the hypobromite of soda, the former is rapidly decomposed with the formation of water, carbonic acid gas and nitrogen. If there be an excess of the alkali present, the carbonic acid unites with the base forming a carbonate, while the nitrogen passes off as a gas and may be collected, measured and the amount of urea calculated. Upon this fact depends the hypobromite method of estimating urea. Similar decompositions are caused by the action of nitrous acid and hypochlorite of soda on urea.

Physiology.—Urea is the principal product of the retrograde metamorphosis of nitrogenous material. It was for a long time supposed to be a production of "vital force," but in 1828 Wöhler succeeded in preparing urea artificially and to-day the chemist manufactures as good an article in his retort as is formed in his body. It is the great vehicle for carrying off the waste nitrogenous matter of the food and is probably formed in various parts of the body. Indeed some urea is found in the small intestines and its presence there is a result of the processes of digestion; so that even before the food has been absorbed, a part has been so changed as to be of no farther value either in the liberation of force by farther changes in itself or in building up tissue. It is quite evident that in the liver more urea is formed; the nitrogenous parts of the food being broken up into urea, uric acid and other less highly oxidized nitrogenous compounds on the one hand and on the other, into non-nitrogenous substances, such as glycogen.

A true knowledge of the physiology of urea can be obtained only by a study of the value of nitrogenous foods; because, with the exception of insignificant traces, the excreted nitrogen of the system is found in the urine as urea and uric acid, principally as the former. The old theory, advanced by Liebig, and which stood unquestioned for a long time, divided foods into two principal classes, viz., the heat producing and the tissue forming.

According to this theory, the principal or sole use of nitrogenous food was to build up muscular tissue; consequently, it was held that muscular exertion was rendered possible by the liberation of force resulting from the disintegration of muscular tissue itself and that the amount of urea excreted within a given time depended upon the amount of muscular exertion put forth and was independent of the kind and amount of food. In order that the theory of Liebig may be fairly represented and understood, the following quotation will be given: "Boiled and roasted flesh is converted at once into blood; while the uric acid and urea are derived from the metamorphosed tissues. The quantity of these products increases with the rapidity of the transformation in a given time, but bears no proportion to the amount of food taken in the same period. In a starving man, who is in any way compelled to undergo severe and continued exertion, more urea is secreted than in the most highly-fed individual if in a state of rest."

After some years, thoughtful men began to question these assertions, which seem to have been made without any substantial basis of experimentation. Lehmann soon found that the quantity of urea and uric acid excreted did depend to some extent upon the food. Fick and Wislicenus proved by direct experimentation that work could be done upon a non-nitrogenous diet and without increased disintegration of the substance of the muscle. Drs. Parkes, Pavy and Haughton have experimented upon this subject and reached the same general conclusions. In order to show the effect of the kind and amount of food upon the excretion of nitrogen, I will give some experiments made upon myself and relating to this subject. For 15 days I accurately measured and weighed all my food, taking the same both in quality and quantity each day. During the last five of these days I collected the urine and estimated the amounts of urea and uric acid and calculated from these the quantity of nitrogen excreted. The relation between the nitrogen of the food and that of the urea and uric acid is shown by the following:

Nitrogen of the daily food=225.107 grains.
Nitrogen daily excreted =230.205 grains.

It will be seen from this that after the continued consumption of the same amount of nitrogenous food for 10 days, the nitrogen of the food and that of the excretions are practically the same; the difference of about five grains being slight. At the expiration of the first period of 15 days, the daily amount of nitrogenous food was diminished and this smaller quantity was taken for a second period of 15 days. During the last five of these days, the urine was again collected and the amounts of urea and uric acid estimated; the relation between the nitrogen of the food and that of the urea and uric acid is represented by the following:

Nitrogen of the daily food=155.899 grains.

Nitrogen daily excreted =155.394 grains.

It should be stated that during both of these periods, my food consisted of inorganic, starchy, fatty and nitrogenous articles and that the change from the first to the second period simply consisted in omitting a part of the nitrogenous food. During both of these periods but little muscular exercise was taken. The waking hours of the day were spent in reading and conversation. I walked to the library and back to my room each forenoon and to the laboratory and back each afternoon, in all not one mile per day. The little physical exercise with the reading kept me from feeling dull. I retired each night promptly at 10 o'clock and arose at 7 o'clock.

These experiments are certainly sufficient to convince one that the amount of urea excreted within a given time does depend largely upon the food. Indeed this is the universal conclusion of those who have experimented upon this subject.

Now during a third period which embraced only 5 days, I took the same amounts of inorganic, starchy and nitrogenous foods as in the second period, but increased my fatty food by one ounce of butter. During each of these five days, in addition to the little physical exercise taken during the other periods, I walked 11 miles on a rough railroad bed at the rate of 3 miles per hour, passing the remainder of the time as during the two preceding periods. The object of this period of experimentation was to ascertain whether the additional exercise would increase the excretion of urea. The urea and uric acid for each day were

estimated as before. The relation between the nitrogen of the food and that of the urea and uric acid is represented by the following :

Nitrogen of the daily food=155.899 grains.

Nitrogen daily excreted =149.129 grains.

From this, it is seen that the amount of nitrogen excreted as urea and uric acid was not increased by muscular exertion. (For further details in these experiments, see the Physician and Surgeon, Jan., 1879.) The experiments of Prof. Haughton and others prove the same. Prof. Haughton found that under ordinary conditions and when his physical exercise never equaled a walk of five miles per day, his average excretion of urea was 501.28 grains ; while the average amount of urea excreted during five days, in which the average amount of physical exercise was equal to a walk of 20.74 miles, was 501.16 grains. (See Medicine in Modern Times, p. 125 et seq.)

From the many experiments made upon this subject and the great similarity of the results obtained, we are justified in asserting that in a healthy man, the amount of urea is not increased by any reasonable amount of muscular exertion. Of course, every one knows that in certain diseased conditions, the muscular tissue of the body is disintegrated and increases the amount of urea ; but we are now studying physiological and not pathological conditions. For this reason, as Dr. Pavy has shown, the experiments of Prof. Flint on Weston are of but little value ; for, to quote the words of Prof. Flint, " At 10.30 P. M. of this day (the fourth of experimentation) Weston broke down completely. He could not see the track, and was taken staggering to his room, having reached, apparently, the limit of his endurance." We should not feel warranted in deducing any physiological laws from experiments upon a man who " could not see, and who was taken staggering to his room."

Dr. Edward Smith has shown (see Cyclical Changes in the Human System) by a long series of experiments, which are an honor to the profession, that muscular exertion does increase the amount of carbonic acid exhaled, and physiologists are now generally united on this point. We are to measure the amount of

physical exercise by the amount of carbonic acid exhaled and not by the amount of the urea and uric acid excreted ; moreover, there is not proof sufficient to justify the old idea that every part of the human body is replaced every few years by new material.

Hundreds and probably thousands of experiments have been made in order to determine the effects of medicinal agents upon the elimination of urea. Let us see how some or the majority of these experiments have been made and then we can judge of their value. In one medical journal, I find that Dr. ~~Jun~~ estimated the amounts of urea passed by three healthy persons and three others who had renal disease in 24 hours without medication, and then estimated the amounts of urea passed by the same persons in 24 hours beginning with or succeeding a jaborandi sweat, and found that in five of these persons the amounts of urea were increased, while in the other, the amount was slightly decreased. The conclusion drawn is that jaborandi increases the amount of urea excreted within a given time. This drug may have the above effect, but the conclusion is certainly not warranted by the experiments. It is not stated whether these persons ate much, little or nothing during these days. All experiments upon the elimination of urea, which do not consider the amount of nitrogenous food, are of but little value. Another way of making these experiments is to estimate the amount of urea passed during a number of consecutive days without medication, then for the same number of days with medication, then again for the same number of days after medication ; take the average for each period and compare results. Even this method is far superior to the first one given ; for in the first, only one day without medication, and with or after medication was taken into consideration and consequently errors, which may arise in this method, stand a chance of being corrected by the extension of the time. But even in the second method, the experimenter generally *presumes* that he eats *about* the same amount of food each day. Now presumptions do very well when no more accurate knowledge is possible, but it is hardly wise for the *scientific* physician of this day to build upon the sands of presumption when the rocks of certainty not only lie near by, but with regard

to foods (thanks to Payen, Letheby and others who have given us the per cent. of nitrogen and other constituents of the most common foods) they are already carved and fitted.

It will be well to consider some of the circumstances which affect the excretion of urea. The effects of variations in the kind and amount of food have already been sufficiently referred to. Any agent which interferes with or improves digestion may correspondingly diminish or increase the amount of urea excreted in a given time. In cases of indigestion an abnormally large proportion of material may pass from the body as faeces; consequently, so much nitrogen escapes conversion into urea and the amount of the latter is necessarily lessened. Again if the food is only partially or poorly digested and is absorbed in this condition, the process of oxidation is retarded to a corresponding extent and that which should form urea will pass from the body as uric acid or as the yet less highly oxidized substances, xanthine and hypoxanthine. For a similar reason, the excretion of urea is influenced by any thing which helps or hinders the processes of oxidation which normally occur in the blood; thus in diseased conditions of the lungs, the amount of urea will often be found sensibly diminished. This constituent of the urine may also be increased by an increased consumption of water; in this case the blood is more nearly deprived of its waste material as it passes through the kidneys, the water extracting the soluble substances. The heat of fever consumes the muscular tissue of the body and consequently augments the quantity of urea. Again the urea may be retained in the body after it has been formed; this is the case in the last stages of structural diseases of the kidneys. In diseased states, it may also pass from the body through other avenues than the kidneys; thus cholera stools and the perspiration of those who suffer from renal disease may contain urea.

The formation of urea in the body is not only a normal but a necessary process and if the material for its formation is not furnished as food, then the muscular tissue of the system will be disintegrated and supply the place of the food. The products of the retrograde metamorphosis of nitrogenous material are necessary to the maintainance of the functions of life; but these, like

other stimulants, must not be allowed to accumulate in excessive quantity in the blood; for in large quantities they are true poisons, producing serious and often fatal disturbances of the nervous system as seen in the convulsions of uraemia. If an animal be starved, the urea for a while rapidly diminishes and then remains stationary until death. The production of this substance falls to its minimum and as soon as this fails, death results.

The relation between urea and uric acid has been worked out by experiments, both in the test tube and in the body. If uric acid be given an animal, it increases the amount of urea; thus, Neubauer found, that by giving rabbits from 2 to 3 grammes of uric acid, the amount of urea was increased from 2.1 to 4.2 grammes. (Harley). This same experiment has been verified by myself upon many animals.

The amount of urea excreted by a healthy man living upon ordinary mixed diet will vary from 18 to 36 grammes for the 24 hours. An amount, which at one time may be abnormal, may at another time be perfectly normal.

Pathology.—From what has been said concerning its physiology, it will not be difficult to understand why urea is in excess in some diseases and deficient in others. By the expression, “an excess of urea,” one of two things might be understood. These are, (1) an excess in proportion to the water and (2) an absolute excess for the 24 hours. A specimen of urine may contain an excess of urea as understood in the first sense and a deficiency in the second. We decide as to whether urea is in excess in proportion to the water as follows: Place a few drops of the urine under examination on a glass slide, add an equal volume of strong nitric acid and set aside in a cool place for five minutes, at the expiration of this time, if urea were in excess in proportion to the water, a mass of crystals of nitrate of urea will have formed and be plainly visible to the unaided eye; while if there be but a normal or deficient proportion of urea, no crystals will be seen. In order to test for a deficiency, evaporate some of the urine to one-half its volume, place a few drops of this on a glass slide, add nitric acid and allow to stand as before. If at the expiration of this time no crystals are seen, the urea is deficient in proportion to

the water. In all cases in which there is an excessive proportion of urea, the specific gravity is necessarily high. Of course if the proportion of urea be excessive and the amount of urinary water also abnormally large, the absolute quantity of urea for the 24 hours will be excessive. In those cases where there is only a relative deficiency of urinary water, the treatment has already been discussed in considering the amount of urine, and further remarks made here will apply to those cases in which there is an excessive excretion of urea within a given time. The exact amount can be ascertained only by quantitative estimation.

As a rule, the higher the temperature of the body, the greater the quantity of urea that is formed. This is true in febrile diseases; for instance in typhoid fever the daily excretion of urea may reach 75 grammes or even higher and this too, when but little food is taken and consequently the enormous excretion of urea represents disintegration of muscular tissue. In typhus fever, the temperature of the interior organs of the body rises to 105° and higher, and this excessive heat is the result of the combustion of the body itself. If we could know the cause of the increased temperature or if we could understand why it is that the tissue is so rapidly disintegrated then the removal of the cause would be the rational treatment; but we do not know these things, consequently the physician either does nothing at all or supplies other fuel for the fires of the fever. As yet we know no way of quenching these fires, consequently we furnish fuel as wine and beef-tea in order to preserve the body itself from combustion.

The amount of urea is increased in intermittent fever and in inflammatory diseases as in pneumonia, also in the exacerbations of some chronic diseases and in the paroxysms of epilepsy. The increase of urea in diabetes mellitus affords an interesting study. In health much of the force necessary to the maintainance of animal heat and the exercise of the body is furnished by the combustion of starchy food which is transformed or burned into carbonic acid and water. In diabetes mellitus the starchy food escapes combustion and the nitrogenous material must be consumed in its stead. For this reason, it is that the diabetic patient has a voracious appetite and for the same reason he gradually fails.

Says Prof. Haughton, "The diabetic patient resembles a racing steamboat on the Mississippi, whose supply of coals is exhausted and whose cargo furnishes nothing better than lean pork hams, to throw into the furnace to maintain the race. It can not be wondered at that our poor patient, under such disadvantageous conditions, fails to keep in the front."

In Asiatic cholera, the conditions are the reverse of those of fever. In this disease the temperature is diminished, the heat is not sufficient to maintain life, and the circulation is impeded; consequently there is a deficient formation of urea. It is useless to supply material for combustion because it can not circulate through the system. It is desirable to restore the due amount of heat to the body and thus render oxidation possible.

Deficiency of urea may be due either to decreased formation or to diminished elimination; while the former is undoubtedly the cause of the small amount of urea found in the urine of cholera, the latter condition prevails in structural diseases of the kidney. Retained urea acts as a poison and if the retention be marked and be not speedily relieved, death soon follows. The symptoms produced by retained urea are known as those of *uraemia*. The nervous system is affected and convulsions often repeated may follow. It is questioned by some whether these symptoms are produced wholly by retained urea or in part by other causes. In the breath of some persons suffering with these convulsions, carbonate of ammonia has been obtained and this has led some to believe that the nervous disturbance was caused by carbonate of ammonia and the term, *ammonaemia* has been suggested to designate this condition. The truth no doubt is that in some instances the symptoms are due to retained urea; in others, to the reabsorption of carbonate of ammonia from the urinary passages; and in still others, to the retention or undue formation of other nitrogenous substances; or the symptoms in a particular case might be due to all these causes combined. Of course, the carbonate of ammonia arises from the decomposition of urea. We must recognize the fact that the nitrogenous constituents of our food, during their passage through our body, undergo certain changes whereby a series of new substances is produced; each of these

is one degree nearer the confines of inorganic nature than its predecessor and all of these in moderate quantities are valuable as nerve stimulants, but are dangerous when allowed to accumulate in the system. Our ancestors ate much more nitrogenous food than we do, and consequently the physician of the past resorted to bleeding more frequently than the physician of the present would be justified in doing. Bleeding was then found beneficial because by this means the excess of nitrogenous material was quickly and surely removed.

The uraemic symptoms of pregnant women are due in some cases to non-elimination of urea and in others to the fact that the bladder is not thoroughly and frequently emptied and the urea is decomposed and absorbed as carbonate of ammonia. In the great majority of these cases, no examination of the urine is made and the true condition of the patient is not suspected until her frame is convulsed by the poison. All of this might have been foreseen and prevented in the great majority of cases by a timely examination of the urine. The urine of every pregnant woman should be repeatedly examined and the physician of the present day, who has the care of such a patient and fails to make such examinations, is guilty of criminal negligence.

URIC ACID, $-C_5H_4N_4O_3$.

Uric acid is the principal constituent of the urine of reptiles and birds. In the urine of carnivora and of man, it is found in small quantities; while in the urine of herbivora, it is replaced by hippuric acid. Uric acid in small quantities is a normal constituent of the liver, spleen, lungs, muscle, brain and blood. In the lower animals, an accumulation of uric acid in the blood can be produced by ligating the ureters or by extirpation of the kidneys. Uric acid is not unfrequently found in various transudates; while it is found in man in largest quantities in gravel and calculi of either the free acid or urates.

Preparation.—Uric acid is obtained with ease and in a state of purity from the urine of serpents as follows: Dissolve the powdered excrement in a hot dilute solution of sodic hydrate (one part of caustic soda dissolved in ten parts of water); boil

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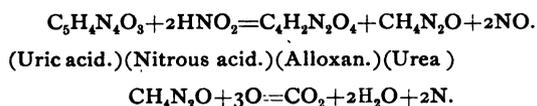
this solution as long as the odor of ammonia is given off; filter while hot; treat the filtrate with a current of carbonic acid gas until a granular precipitate falls; allow to stand for 24 hours; collect the precipitated acid urate of soda upon a filter; wash it with a little cold water, then redissolve in a hot dilute solution of sodic hydrate and add hot dilute hydrochloric acid sufficient to make the mixture strongly acid. Allow this to stand for 24 hours, when pure, white uric acid will be deposited in rhombic tablets. The supernatant fluid may be decanted, the crystals washed with water then with alcohol and dried. If guano be treated as above, uric acid will be obtained in larger quantity than from the urine of serpents; but it will not be so pure.

Uric acid is obtained from the urine of man by the following method: To some (not less than 200 c. c.) clear urine of specific gravity not less than 1025, add 5 per cent. of hydrochloric acid and allow to stand for 24 hours in a warm place. The hydrochloric acid combines with the bases and sets uric acid free. At the expiration of the 24 hours, the specimen will be found to contain a brick-dust deposit and reddish crystals will be observed floating upon the surface of the fluid and adhering to the sides of the beaker. By means of a glass rod with a small piece of rubber tubing drawn over one end, gently agitate the fluid and rub the crystals from the sides of the vessel. All the crystals will then subside. Decant the supernatant fluid and add to the sediment a little water; agitate gently and again decant; then wash by decantation with alcohol; collect the crystals on either a filter or watch-crystal and dry at 110° . As thrown down from the urine, the crystals of uric acid are more or less colored. If it be desired to obtain uric acid from urine of low specific gravity, such urine should be concentrated and then treated as above. If albumen be present it should be removed by precipitation with a little acetic acid, heat and filtration, and the filtrate concentrated and treated as above.

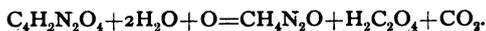
Pure, dry uric acid is a white, odorless and tasteless powder. The pure acid does not crystallize so readily nor take such a variety of shapes as that which contains more or less coloring matter. It has already been stated that when normal urine under-

goes the acid fermentation, uric acid is liberated from its combination with the bases; in these cases, the acid is deposited in beautiful crystals of a reddish or brown color. The most perfect crystals of uric acid are long rhombic plates; but in the majority of instances, these are greatly modified. Frequently in the urine, the crystals will be found arranged in bundles or rosettes which, not unfrequently, are visible to the unaided eye. These crystals contain no water and are insoluble in alcohol and cold dilute acids and practically insoluble in water, 1 part of the acid requiring 1800 parts of boiling, and 14000 parts of cold water for solution.

To a few drops of nitrous acid in a small dish or watch-crystal, add some pure uric acid. A brisk effervescence immediately occurs and after standing for a few moments, a white substance will remain suspended in the acid. Microscopical examination will show that this substance, which is *alloxan*, $C_4H_2N_2O_4$, is composed of beautiful colorless cubes or octohedrons. The nitrous acid has oxidized the uric acid forming alloxan, urea and nitrous oxide; while the urea is immediately decomposed into carbonic acid, nitrogen and water. These changes are represented by the following equations:

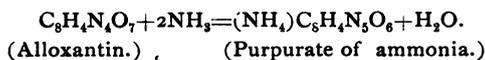
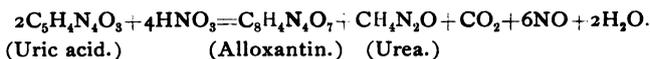


The alloxan, after standing in the acid for some time, is gradually oxidized into oxalic acid, carbonic acid and urea, and since nitrous acid consists of nitric acid containing the oxides of nitrogen, as soon as these oxides are decomposed, the urea combines with the nitric acid forming the nitrate of urea. The crystals of nitrate of urea will be recognized on microscopical examination. The change by which alloxan is converted into urea, oxalic acid and carbonic acid is represented in the following equation:



To some uric acid in a small evaporating dish add nitric acid; place the dish on the water-bath and heat to dryness. If this experiment be made in a laboratory where there is much ammonia in the atmosphere, the residue left on the sides of the dish as

the nitric acid evaporates will take a beautiful crimson tint; otherwise a white residue remains and becomes crimson on the addition of a little ammonia or on being placed under a bell jar filled with the vapor of ammonia. The uric acid has been changed by the action of the nitric acid into alloxantin, $C_8H_4N_4O_7$, and the alloxantin unites with ammonia forming the purpurate of ammonia. These changes are represented thus:



Murexide test.—The purpurate of ammonia is also known as murexide and consequently the above reaction is known as the murexide test. It forms a delicate means of detecting the presence of uric acid and is consequently of great value in the analysis of urine. Certain precautions are necessary in its application. In the first place, other organic matters must be removed as far as possible; because some will give a similar color when carried through the process and others will interfere with the development of the color when uric acid is present. In applying this test to urinary deposits the supernatant fluid should *never be evaporated* but should be removed either by decantation or filtration. The sediment should then be washed by decantation or on the filter with a little cold water and then with alcohol. Neither of these will dissolve either free uric acid or acid urates and in these conditions only, is uric acid deposited in the urine. The washed sediment is then placed in a clean porcelain dish, nitric acid added and the mixture heated to dryness on the water-bath. To the residue, kept warm on the water-bath, add a little ammonia, when if uric acid were originally present either as free acid or as a urate, the crimson color will be developed. The addition of an excess of ammonia must be avoided and if the quantity of material under examination be small, it is better to simply blow a spray of ammonia over the dish.

To some uric acid add water sufficient to form a semi-fluid mass. To this, add permanganate of potash, allow to stand for a short time at ordinary temperature (heat destroys the test) and

clears up on the application of heat. Its ready solubility on being warmed affords an easy means of recognition; for the acid urate of potash does not dissolve until the temperature is raised nearly to the boiling point; while a deposit consisting of the acid urate of ammonia does not dissolve until the mixture is brought to the boiling point. The acid urate of soda may be produced artificially by treating a solution of uric acid in caustic soda with a current of carbonic acid gas, until a precipitate forms. Prepared in this way, it appears under the microscope in granules. It may also be obtained by mixing a hot solution of uric acid in sodic hydrate with a solution of the bicarbonate of soda, or by boiling uric acid in a solution of the phosphate of soda. By either of these methods it forms in fine needles which often are arranged in balls and bundles. The salt is soluble in 1200 parts of cold, and 125 parts of hot water.

The acid urate of potash, $\text{KC}_5\text{H}_3\text{N}_4\text{O}_3$, resembles the corresponding salt of soda and may be prepared in a like manner. It occurs as a urinary deposit and requires 800 parts of cold, and 80 parts of boiling water for solution. Besides these two salts, quadriurates of potash and soda occur in the urine and may be prepared by adding acetic acid to solutions of uric acid in sodic and potassic hydrates until a feebly acid reaction is obtained. Both sodic and potassic quadriurates are amorphous. The potash salt has the formula, $\text{KC}_5\text{H}_3\text{N}_4\text{O}_3 + \text{C}_5\text{H}_4\text{N}_4\text{O}_3$.

The acid urate of ammonia, $\text{NH}_4\text{C}_4\text{H}_3\text{N}_4\text{O}_3$, occurs as a urinary deposit and often in dumb-bells or in balls of radiating needles, especially in ammoniacal urine. It may be obtained by adding ammonia to uric acid suspended in boiling water, when it appears in fine needles.

Acid urate of lime is rarely met with in urinary deposits, when it appears in fine needles which may be mistaken for tyrosine. It may be obtained by adding chloride of lime to a solution of uric acid in sodic or potassic hydrate, when the acid urate of lime is precipitated in an amorphous form.

Physiology.—Like urea, the uric acid, which is excreted in health, results from the food; while in disease, it may be due to disintegration of the muscular tissue of the body. It is therefore

ES H₂ N₄ O₃

evident that an increased consumption of nitrogenous food will increase the amount of uric acid formed; consequently in many instances, it is found that the urea and uric acid vary in the same ratio. But there are other circumstances which may cause the amounts of these substance to vary inversely and to these will we now direct our attention. It has already been seen that uric acid outside of the body is converted by means of oxidizing agents into urea. The former is the result of less perfect oxidation. That deficient oxidation increases the amount of uric acid and correspondingly decreases that of urea is proven by every fact which we know concerning the variations of the amounts of these substances. Wine drinking increases the quantity of uric acid, because the alcohol is more readily acted upon by the oxygen of the oxyhaemoglobine and the nitrogenous constituents of the food escape combustion. Again, constant drinking of wine leads to disease of the liver and this organ plays an important part in splitting the albumen of our food into carbohydrates, urea and uric acid. Again in diseases of the lungs, when but an insufficient supply of oxygen reaches the blood, the quantity of uric acid is increased and that of urea correspondingly decreased. In venous stasis, the same result follows because the blood is not oxidized sufficiently. In indigestion the processes of retrograde metamorphosis are retarded and the nitrogen leaves the body just so much farther removed from urea. Those living in poorly ventilated houses excrete an excess of uric acid and oxalate of lime; the same is true of those who take but little physical exercise. U.

It is said that uric acid can not result from imperfect oxidation, because birds take in large quantities of oxygen in proportion to their body weight and yet their urine consists principally of uric acid. We should remember that the products of chemical changes depend upon the conditions under which they take place. Oxygen and uric acid are not the only essentials for the production of urea; the conditions must be favorable. A diamond may be exposed to the oxygen of the atmosphere for centuries and yet remain unchanged, yet is this proof that the product of the oxidation of the diamond is not carbonic acid? Large quantities

of arsenic may be given to a pig without causing death ; would it be wise for a physician to decide from this, that the same amount of arsenic would not injure his patient? The surrounding conditions of the nitrogenous food in the body of birds are very different from those attending the changes of the same food in man and as long as these differences exist, it is very unwise to expect that the products of the changes in the two will be identical.

The average daily excretion of uric acid by a healthy man is about .4 of a gramme. Free uric acid is not a constituent of normal urine ; consequently when we speak of the amount of uric acid in normal urine, we refer to that existing in a combined state. It is combined with sodium, potassium, ammonia and lime. Since free uric acid does not occur in normal urine and the urates, when normal, are in solution, any deposit of either shows that there is some abnormality.

✓ *Pathology.*—Free uric acid may be deposited from any of the following causes : (1) the urine may be unduly acid, the stronger acids taking up the bases and setting the uric acid free ; (2) there may be an absolute excess of uric acid formed, so that the normal amount of bases is not sufficient to take up all the acid ; (3) the proportion of alkaline bases may be abnormally decreased.

Not unfrequently, either while the urine is in the passages or after it has been passed, by a species of acid fermentation, already referred to, other acids are developed which decompose the urates and deposits crystals of uric acid. If this takes place after the passage of the urine, of course, it can be of no pathological import ; but if such changes go on while the urine is still in the passages, the most serious results may follow from the deposition of these crystals, and the formation of gravel and calculi. In such a case as this, the chemical, rational treatment would consist in the administration of alkalis ; thus preventing any farther acid fermentation, and at the same time dissolving any of the crystals that may have been deposited. In using these remedies, ammonia salts are to be avoided ; because the urate of

ammonia is but little more soluble than free uric acid: salts of potash and soda should be used.

If the free uric acid be due to excessive formation, this should be prevented by the administration of oxidizing remedies, which would convert the excess of uric acid into urea. In such cases as this, oxalate of lime will generally be found deposited with the uric acid, and the treatment should consist of fresh air, exercise of both body and mind, and of acid tonics. Now there is one class of diseases, in which there is excessive formation of uric acid, that cannot be reached by oxidizing agents alone. I refer to those diseases of the heart and lungs which interfere with the circulation, producing venous stasis. In these cases, attention must be given to the organ diseased, and in the mean while the deposition of uric acid or urates prevented by the use of alkalis; for no amount of acid tonics could prevent the excessive formation of uric acid, so long as the blood, loaded with carbonic acid and nitrogenous poisons, stagnates in the veins; but as soon as the circulation is normal, the proportions of uric acid and urea become natural.

Urates are the most common constituents of urinary deposits. They vary in color from white to crimson; the higher the color the more serious are the indications. The nature of the deposit may be determined by the fact that it is cleared up by heat by the separation of the deposit by decantation or filtration, and the application of the murexide test, or by the addition of nitric acid, and the formation of crystals of free uric acid. The deposition of urates after passage of the urine may be due (1) to deficiency of water; not being enough to hold the urates in solution when the temperature of the body ceases to aid; (2) to excess of urates. Any sudden change of life may cause a temporary deposit of urates; for instance, a person of sedentary habits takes violent exercise and perspires freely; more than the ordinary amount of water passes off through the skin, and leaves a deficiency to be excreted by the kidney; consequently, as soon as the urine cools, urates are deposited in a large quantity, attracting the attention of the person, and if he be ignorant on this subject, often frightening him greatly. It is true that the urine should

contain no deposit, and this is an abnormal condition, but one that nature herself will right in a short time. Urates were formerly called critical discharges and there is one class of affections in which they may be so regarded. I refer to severe febrile affections of an inflammatory nature; in these cases, the sudden appearance of a deposit of urates is indicative of a change for the better; because they show that so much of the poison has been eliminated. But if there be a frequent deposit of urates, it should not be overlooked nor passed lightly by, but should be studied and it will be found that everything that I have said with reference to free uric acid applies equally to such a deposit of urates. Since the causes, effects and treatment are the same as given under uric acid, a repetition will be unnecessary.

Hitherto, we have only considered the injurious effects following upon an excess or upon a deposit of urates, but the non-elimination of this substance, and a consequent deficiency in the urine, demands our attention. It is this which leads to gout; the urates are deposited in parts of the body, especially in the joints. A deficient excretion of uric acid can be determined with accuracy only by a quantitative estimation. The elimination of the retained uric acid may be secured by the use of phosphate of soda, bicarbonate of potash, liquor potassæ, etc., which dissolve and eliminate the uric acid and acid urates. After this, the excessive formation in the body may be prevented by colchicum, quinia, digitalis, etc.

HIPPURIC ACID,— $C_9H_9NO_3$.

This acid is a constant and important constituent of the urine of the horse, ox and other herbivorous mammals. It is also found, though in small quantity, in human urine, in which it is increased by the consumption of certain fruits, as plums, and by the administration of benzoic acid, the balsam of Peru and some other medicinal agents. Hippuric acid has been detected in the scales which form on the skin in ichthyosis.

Preparation.—Hippuric acid is best obtained from the urine of the horse or ox as follows: Concentrate from 600 c. c. to 1000 c. c. of the *fresh* urine to a syrup; extract this with alcohol;

filter the alcoholic extract and concentrate on the water-bath until all the alcohol is driven off; to the cold residue add cold hydrochloric acid and allow to stand when hippuric acid crystallizes. These crystals may be collected upon a filter and dried by pressure between folds of blotting paper. They may be purified by solution in boiling water, agitation with animal charcoal, filtration and concentration.

The traces of hippuric acid may be obtained from the urine of man by the following process: Concentrate 1000 c. c. of the urine to a syrup on the water-bath; to this syrup, add alcohol acidified with hydrochloric acid; agitate well and filter. Wash the residue with alcohol, neutralize the united filtrate and washings and heat on the water-bath until all the alcohol is driven off. To the remaining portion, add oxalic acid, then shake with pure ether to which ten per cent. of alcohol has been added. Allow to stand until the ether separates. Shake repeatedly with ether and remove the ether from the united ethereal extract by evaporation. To the residue, add milk of lime to a feebly alkaline reaction, warm and filter. Wash the residue on the filter with water which dissolves the hippurate of lime. Concentrate the filtrate and add hydrochloric acid sufficient to produce an acid reaction. Allow to stand when the hippuric acid crystallizes; pour off the supernatant fluid and test the crystals by microscopical examination. Place some of the crystals in a test tube, add a few drops of strong nitric acid and heat to dryness when the peculiar odor of nitrobenzole will be given off if the crystals are hippuric acid.

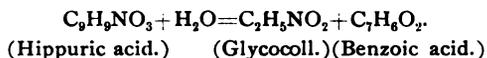
Hippuric acid forms in large four-sided prisms which are terminated at each extremity in short pyramids. These crystals are sparingly soluble in cold water and ether, freely soluble in alcohol. Its solubility in alcohol affords an easy means of separating hippuric acid from uric acid. If a urinary deposit is suspected to be composed of, or to contain both uric and hippuric acids, collect it on a filter and wash it with alcohol acidified with hydrochloric acid, when the hippuric acid will be dissolved and may be obtained in the crystalline form on concentrating the filtrate.

The solution of hippuric acid in hot water has a decidedly acid reaction. This is a monobasic acid and unites with many

bases forming characteristic crystalline salts. The hippurate of silver, $\text{AgC}_9\text{H}_9\text{NO}_3 \cdot \text{H}_2\text{O}$, is soluble in hot water and is deposited on cooling in fine, glistening needles. If to an aqueous solution of some hippurate, a few drops of a neutral solution of ferric chloride be added, a brown, amorphous precipitate of the hippurate of iron falls. This precipitate is insoluble in water, soluble in dilute alcohol and in the urine.

In the presence of decomposing matter, hippuric acid is soon changed into benzoic acid; for this reason, all tests for this acid should be made with fresh urine. A disregard of this fact has caused some experimenters to report that hippuric acid is not a constant constituent of the urine of the herbivorous mammals.

If some crystals of dry hippuric acid be heated in a test tube, it will be decomposed; benzoic acid will be given off and deposited upon the upper part of the test tube. Besides benzoic acid, an oily, red fluid with an aromatic odor is obtained by the dry distillation of hippuric acid. If hippuric acid be boiled with nitric acid for some time and the solution then allowed to cool, benzoic acid crystallizes from the solution. The hippuric acid has been decomposed into benzoic acid and glycocholl, the latter remaining combined with the nitric acid after the former has crystallized. The same change is produced by boiling hippuric acid with hydrochloric or sulphuric acid and is represented by the following equation:



If benzoic acid be administered internally, it appears in the urine as hippuric acid. If 15 grains of benzoic acid be taken at bed-time, and the urine passed on rising the next morning be concentrated and acidified with hydrochloric acid, crystals of hippuric acid will soon form and may be recognized by microscopical examination and by their solubility in alcohol.

Physiology.—Evidently, hippuric acid is formed in the body by the combination of benzoic acid and glycocholl; but the question arises here, what is the source of the benzoic acid necessary to the production of hippuric acid? Do we obtain sufficient benzoic acid as such in our ordinary food to account for the hippuric

acid excreted? This question must be answered in the negative, for it has been shown by Weissmann that when living upon albuminous food only, man continues to excrete hippuric acid. It is true that some fruits contain considerable benzoic acid; thus Ducheck found that after eating greengages, his urine contained a large excess of hippuric acid, and subsequent investigation showed him that this fruit contained benzoic acid in considerable quantity; this has been confirmed by Thudicum. But it has been found that our ordinary foods contain no benzoic acid, and the same was ascertained to be true of the fodder which constituted the food of a cow whose urine contained much hippuric acid. This question as to the source of benzoic acid seems now to be settled by the discovery of Ure, that benzoic acid is produced by the oxidation of albumen. Proust has also obtained oil of bitter almonds and benzoic acid by the action of sulphuric acid and bichromate of potash upon albuminous substances. From this, it is evident that the sources of benzoic acid in the human body are at least two: one constant and furnishing but a small quantity of the acid; the other variable, sometimes furnishing large quantities and at other times being absent. The oxidation of the albuminous constituents of the food, and when food is wanting, of the nitrogenous tissues of the body furnishes the small but constant supply of benzoic acid from which results some of the hippuric acid daily excreted. From this source, is derived the hippuric acid which has been found to be present in the urine of man after prolonged fasting.

When foods or medicines containing benzoic acid are taken, the hippuric acid is greatly increased. Besides greengages, which have already been referred to, cranberries and blackberries have been found to increase the amount of hippuric acid. Reason as well as experiment would show that there is a limit to the amount of hippuric acid which may be obtained from this source. This limit depends upon the amount of glycocholl available for this purpose in the body; thus Ducheck found that after the administration of 1 gramme of benzoic acid, 0.714 of a gramme of hippuric acid could be obtained from the urine, which contained no benzoic acid; consequently all of the benzoic acid

administered in this case was probably excreted as hippuric acid. However, the same experimenter found that after the administration of benzoic acid in quantities of 2 grammes and over, the amount of hippuric acid remained at about 1.8 grammes and did not increase with increased consumption of the benzoic acid; while in these cases, the unchanged benzoic acid could be detected and estimated in the urine. It is but just to state that the experiments of Ducheck have been questioned and need confirmation.

It is a question as to where the benzoic acid combines with the glycoll. Kühne found that benzoic acid administered to jaundiced patients was not converted into hippuric acid, but reappeared in the urine unchanged; from this he supposed the liver to be the seat of transformation. On the other hand, it was found that benzoic acid, administered to a dog whose bile was completely removed through a fistula, was changed and appeared in the urine as hippuric acid. This subject also needs further investigation and furnishes a rich field for experimentation.

From what has been said, it will be seen how unwise it is for one to study the excretion of hippuric acid without any regard to the kind and amount of food. What has already been said with regard to the importance of taking into consideration the nature of the ingesta when studying the amount of urine, the reaction, the quantity of urea and uric acid, will apply here and in fact to the study of every constituent of the egesta. The average amount of hippuric acid excreted during 24 hours by a healthy man, living upon ordinary mixed food, is about 8 grains.

Quite a number of experiments have been made by Erdmann and others to show that the amount of hippuric acid excreted by horses varied as the animal was at rest or at work. It was thought that when the animal was at work a part of the carbon of the hippuric acid was converted into carbonic acid and excreted by the lungs; while the remainder of the carbon appeared in the urine as benzoic acid. In confirmation of this theory it was found that the urine of some horses used for ploughing contained benzoic acid, but no hippuric acid; while that of other horses kept at rest contained much hippuric acid and no benzoic

acid. However, subsequent investigation has shown that the presence of benzoic acid found in the urine of the horses used in ploughing was due to the decomposition of the hippuric acid after emission, and if the fresh urine of any horse be examined it will be found to contain hippuric acid. Most observers are now united in the opinion that the daily excretion of hippuric acid, like that of urea and uric acid, is not modified by muscular exertion.

Pathology.—On account of its solubility in water and the small amount generally present, hippuric acid seldom forms a deposit; though such deposits are occasionally observed. It is found in long, needle-shaped crystals, often arranged in groups. They are to be distinguished from uric acid by the fact that the hippuric acid crystals contain but little or no coloring matter, are semi-transparent and are longer than uric acid crystals. But in all cases the deposit should be collected and washed with boiling alcohol. From the alcoholic solution, hippuric acid, if present, recrystallizes on cooling. Hippuric acid may possibly be mistaken for phosphate of lime, but the former occurs only in urine that is decidedly acid; while the latter is generally found in urine that is feebly acid, neutral or alkaline. But in cases of doubt, a drop of hydrochloric acid should be added to the crystals when, if they are phosphates, they will quickly disappear, while any hippuric acid will remain undissolved. Crystals of the acid phosphate of lime occur in urine that is strongly acid, but the solubility of the phosphate in hydrochloric acid distinguishes it from hippuric acid.

PHOSPHATES.

Phosphorus is widely distributed in nature, existing in both the inorganic and organic worlds: but no where is it found naturally in the free state. Its attraction for oxygen is so great that when separated from its combinations by artificial processes, phosphorus must be kept excluded from the air. Phosphates of the alkalis and alkaline-earths are essential constituents of the soil and assist in the formation of the earth's rocky crust. The mineral apatite is a phosphate of lime and is used by the farmer as a fertilizer. In the growing plant, this element enters into new combinations

and in this condition is transferred to the bodies of animals. In the animal world, phosphorus is found alike in the most highly organized and in that structureless microscopic speck which seems to be but little removed from inorganic nature. In the higher animals, phosphorus is present as an essential constituent of bone, blood, muscle and brain, and is a necessary attendant of all physical and mental activity. The singular compactness of the bones of birds is due to the comparatively large amount of phosphate of lime entering into their composition. The white corpuscle, that mysterious amœba of the blood, contains phosphorus as an essential ingredient. Muscular and mental activity increase the consumption of this element. Indeed its relations to the quality and quantity of brain-work have been so marked that some one has asserted that, ("A man with too little phosphorus in his brain is an imbecile, and one with too much, a maniac.")

In cases of administration of a poisonous dose of free phosphorus, the various tissues of the body undergo fatty degeneration. The liver, spleen, kidneys and even the minute arterioles undergo chemical changes whereby the albuminous parts are destroyed and fat remains. Those processes of decay which normally occupy three score years and ten, are accomplished within a few days. The urine is generally albuminous, contains bile-acids, bile-pigments and pieces of epithelium tinged with bile and breaking up into oil globules. Leucine and tyrosine are often found, showing serious derangement of the liver.

J. In the urine, phosphorus exists as phosphoric acid combined with the bases calcium, magnesium, sodium and potassium. Accordingly, there are alkaline and earthy phosphates; calcic and magnesian phosphates belonging to the latter class, while sodic and potassic phosphates constitute the former. In normal urine, the excretion of alkaline phosphates is much greater than that of the earthy; for the simple reason that alkaline bases are more abundant in our food and blood than earthy bases. However, the exact proportion between the quantities of these two kinds daily excreted varies greatly with our food.

Of the earthy phosphates, the magnesian is the more abundant in the urine of growing children, because a greater part of the

lime is taken up in the growth of the bones and teeth ; while the urine of old people contains more calcic than magnesian phosphate. (Harley.) If normal urine be rendered alkaline by the addition of ammoniac hydrate, the earthy phosphates are precipitated. The magnesian phosphate combines with the ammonia and is deposited as ammonio-magnesian or triple phosphate, in pennate or stellate crystals ; while the calcic phosphate is simply thrown out of solution and forms a granular deposit. If instead of adding ammonia directly to the specimen, the urine be allowed to stand until the urea gradually decomposes, with the slow formation of ammoniac carbonate, the ammonio-magnesian phosphate crystals will be prismatic and not stellate or pennate. Thus, if the urine has undergone decomposition in the bladder, microscopical examination will reveal prismatic forms of the triple phosphate. Ammonio magnesian phosphate is never deposited in urine which is not ammoniacal ; consequently, if the specimen under examination be acid, or be alkaline from a fixed alkali, or if the odor of ammonia be not perceptible, the presence of these crystals need not be suspected. Of these forms, the prismatic only is to be regarded as of pathological import ; for the presence of the stellate or pennate crystals indicates the accidental or intentional addition of ammonia to the urine after its passage, and consequently does not in any way show an abnormal state of the patient. Whether the prismatic form be indicative of any condition affecting the health of the individual depends upon the time elapsing between the passage and examination of the specimen. If these crystals are formed in the bladder, the urine will be ammoniacal when voided and will immediately deposit a sediment which will be found on microscopical examination to contain the prismatic triple phosphates. This is the case when there is excessive irritation of any part of the urinary tract and generally arises from retention ; in such a case the retained urine becomes ammoniacal and increases the irritation, while the mucus which is poured out hastens the decomposition of the urea.

On the other hand, if perfectly normal urine be set aside after its emission, it will sooner or later become alkaline and deposit triple phosphates ; consequently it is necessary that the analyst

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know whether decomposition took place within the body or after emission, before he is ready to announce the pathological indications of his examination.

All forms of triple phosphates are beautiful microscopic objects. The stellate and pennate crystals are sometimes composed of large interlacing rods; while at other times minute and delicate fringing borders the larger branches. The prismatic crystals are not unfrequently large enough to be visible to the unaided eye, and form beautiful polariscopic objects. They consist of various modified prisms with many truncations. Not unfrequently the careless student finds these crystals of a deep blue color; a drop of acid touches some part of the microscope and dissolves a little of the copper and this solution stains the crystals. If the prisms be very short, they may be mistaken for octohedrons of oxalate of lime; consequently, the microscopic examination should be confirmed by the chemical test. If the crystal be a triple phosphate, it will disappear upon the addition of acetic acid; while if it be an oxalate, it will be insoluble in acetic, soluble in hydrochloric acid. Again creatinine may be mistaken for prismatic phosphates; but creatinine is generally found in acid urine.

Phosphates of Lime.—In the urine there are at least two phosphates of lime and it is a matter of no little importance as to which forms a deposit. The neutral phosphate of lime, $\text{Ca}_3(\text{PO}_4)_2$, may occur as a sediment in urine that is either feebly acid, neutral or alkaline. It never occurs in a crystalline form, but always as a granular mass. When urine containing a sediment of this salt is agitated, the deposit is easily distributed and floats through the fluid as a cloud of mucus, from which the phosphate is distinguished by its ready solubility in acids. When it is deposited in feebly acid urine, this phosphate may be mixed with crystals of uric acid and consequently may be mistaken for urates, but the phosphate does not dissolve on the application of heat and is thus distinguished from urates. In neutral urine and in that alkaline from a fixed alkali, the neutral phosphate of lime is mixed with magnesian phosphate. Whether a deposit of amorphous phosphates contains this salt of lime or consists wholly of mag-

nesic phosphate may be decided by the following process: Collect the deposit upon a filter, wash it with a little water and dissolve in hot acetic acid. To this solution add oxalate of ammonia when any lime, which may be present, will be precipitated as an oxalate. After the removal of the precipitated oxalate of lime by filtration, magnesium may be precipitated from the filtrate on the addition of ammonia. In ammoniacal urine, this substance is mixed with crystals of ammonio-magnesian phosphate.

The neutral phosphate of lime is less soluble in hot urine than it is in cold urine; for this reason, it often happens that the application of heat to a specimen of urine which is clear, yet but feebly acid, causes a turbidity. The precipitate thus formed is distinguished from albumen by its solubility in nitric acid. It is this phosphate which gives the cloudy appearance to urine which is passed soon after eating. Mixed with magnesian phosphate, it may be thrown down from normal urine on the addition of sodic or potassic hydrate.

Acid Phosphate of Lime, CaHPO₄.—This substance is occasionally found as a urinary deposit. It occurs only in urine that is decidedly acid and it invariably appears in a crystalline form. These crystals may be needle-shaped, prismatic, or rhombic tablets. The needle-shaped variety is distinguished from uric acid by the absence of coloring matter and from both uric and hippuric acids by the ready solubility of the phosphate in dilute hydrochloric acid. The prismatic form can not be distinguished by optical examination from the corresponding form of the triple phosphates; but the triple phosphate occurs as a deposit only in ammoniacal urine, while this form of calcic phosphate is deposited only in acid urine.

Calcic phosphate may be obtained artificially in a crystalline state by dissolving some of the amorphous deposit to saturation in acetic acid and allowing the acid solution to stand for some days, when crystals will be deposited. Again, if a solution of phosphate of sodium be treated with one of chloride of calcium, an amorphous precipitate of the phosphate of lime is thrown down. After this has stood for some days, microscopical examination will reveal minute, thin, rhombic plates.

Alkaline Phosphates.—On account of their free solubility in both acid and alkaline urines, these phosphates never form a spontaneous deposit. They may be separated from the earthy phosphates by rendering the urine alkaline and then filtering. The earthy phosphates, being insoluble in alkaline urine, are thus removed. From the filtrate the phosphoric acid, which is combined with sodium and potassium, may be precipitated as ammonio-magnesian phosphate on the addition of ammonia and sulphate of magnesium.

Sodic Phosphate, Na₂HPO₄.—This salt may be obtained from the urine by the following method: Concentrate some urine to a syrup on the water-bath; allow to stand for some time when many of the urinary salts will be deposited in a crystalline mass. Pour off the supernatant syrupy fluid into a clean, small beaker and add to it alcohol in excess. The phosphate of sodium is precipitated by the alcohol and after standing for some time may be obtained for microscopical examination. It appears in colorless, transparent, rhombic prisms which are freely soluble in water. This salt is identical with the ordinary sodic phosphate of the pharmacopœia.

Acid Phosphate of Sodium, NaH₂PO₄.—This salt may be prepared artificially by boiling uric acid with a solution of the ordinary phosphate of sodium when the uric acid takes up one equivalent of the sodium and is converted into a urate. The acid phosphate exists in the syrupy fluid from which the ordinary phosphate is obtained; but it crystallizes more slowly after the addition of the alcohol and may be found in the sediment after the mixture has stood for three or four days; or its deposition may be hastened by the addition of ether to the mixture of alcohol and the syrupy fluid. It forms in a variety of shapes, the most common of which is the rhombic prism. The acid phosphate of potassium, KH₂PO₄, is found in the urine in very small quantity and resembles the corresponding salt of sodium.

Physiology of Phosphoric Acid.—The food of man contains large quantities of phosphorus, as phosphoric acid combined with various bases, and smaller quantities existing in complex organic compounds, as albumen. One pound of beef contains on

an average about 60 grains of phosphates; while an equal weight of bread contains as much as 65 grains. Moreover, potatoes and other articles of food are rich in phosphates. Albumen contains phosphorus and this, during its passage through the body, is oxidized, and appears in the urine as phosphoric acid combined with bases. In the growing child, much of the phosphorus of the food is used in the construction of various tissues; as bone, muscle and brain contain this element. But in the adult, the amount of phosphorus needed for the repair of tissue is probably very small and the amount excreted varies with the food. If no food is taken, then the tissues of the body are consumed in the production of force necessary to the maintenance of life and consequently phosphates continue to appear in the urine. But from what has been said, it is evident that when no food is taken, the excretion of phosphorus falls to a minimum, unless there be some disease which causes undue disintegration or oxidation of tissue.

The exact physiological office of the phosphorus, which is present in the food as inorganic matter, is not known. Evidently it can not be a direct source of force from changes within itself; for it has already reached the limit of oxidation and must return to the plant in order to receive a new supply of force, or to be deoxidized. No doubt our ordinary food contains more of this inorganic material than is absolutely necessary; but that some of it is essential to the healthy activity of the body is a fact proven beyond dispute. It is probable that the inorganic salts of phosphorus are of value and are even necessary in the food of the adult principally on account of their influence over other substances; thus, in the blood, the acid phosphates hold certain substances in solution and prevent the formation of others. That the urine contains some phosphorus in organic combination may be proven by estimating the amount of phosphoric acid contained in a given volume of urine, and then evaporating another equal portion of the same urine to dryness, burning the residue until all the organic matter is destroyed, dissolving the ash in water acidified with acetic acid, and estimating the amount of phosphoric acid in this solution. It will be found that the quantity has been increased

by burning. The phosphorus of the organic matter has been converted into phosphoric acid. The exact nature of the organic compound, which contains phosphorus and is constantly present in the urine, is not known. Both lecithin and glycerinphosphoric acid have been found in the urine, but these observations have not been sufficiently numerous to enable us to decide whether one or both of these are constant constituents of the urine or not.

The quantity of phosphoric acid contained in the 24 hours urine is very variable, even in a state of perfect health. These variations depend upon the kind and amount of food and probably to some extent upon the time of day at which the meals are taken. From a few experiments made upon this point, it seems that when late dinners are taken, less of the phosphates and indeed of all the constituents of the food is absorbed; while the fæces are so much richer in this constituent and increased in total amount. A healthy man living upon ordinary mixed food will excrete from 2 to 5 grammes of phosphoric acid (estimated as P_2O_5) during 24 hours. Drinking much water increases the excretion of phosphoric acid. This is probably due to, at least, two causes: (1) a greater proportion of phosphates are held in solution in the intestines and consequently a correspondingly great quantity is absorbed; (2) the water increases arterial pressure and consequently augments the quantity of urine and urinary salts.

Pathology.—The study of the pathology of phosphates divides itself into two distinct parts. These are, (1) deposition of phosphates in the urinary passages; (2) an excessive excretion of phosphoric acid. Both of these conditions may exist at the same time, but they are not necessarily dependent upon each other, and indeed most frequently exist separately. Phosphates may be deposited even when they are excreted in abnormally small quantities; the deposition depends upon the reaction of the urine and not directly upon the amount of phosphates present. Again there may be three times as many phosphates present as there should be, and still the urine be perfectly clear and contain no deposit. The pathological conditions indicated by a deposition of phosphates and those indicated by an excessive excretion are

wholly different. The presence of phosphates in a deposit is to be ascertained by microscopical and chemical examination; while the presence of an excess of phosphoric acid can be ascertained only by a quantitative examination of the 24 hours urine.

(a) *Deposition of Phosphates.*—Only the earthy phosphates are ever deposited spontaneously in the urine, and when such a deposit occurs, it is important to decide as to the exact nature of the phosphates which form the deposit. The deposition of ammonio-magnesian phosphate in the urinary passages is always due to some local cause. This form of phosphates may be deposited either in the kidney or in the bladder and is a frequent constituent of both renal and vesical calculi. The treatment must be directed to the local cause; thus if there be retention of urine, this must be relieved; if there be undue irritation of any part of the urinary tract, from which an excess of mucus is poured out, the irritation must be relieved. (For details in regard to ammoniacal urine, see p. 169, et seq.)

The normal phosphate of lime is found deposited in the urine with the triple phosphates, in the cases mentioned above; but in other instances, the deposit will be found to consist wholly of the amorphous phosphates of lime and magnesium. This deposit is due to an excess of fixed alkali, or to an excess of the earthy phosphates themselves. If the urine be alkaline from a fixed alkali, the cause is to be sought, either in the food, or in an excess of alkalis in the blood, or in a low state of vitality (see p. 167). The acid phosphate of lime sometimes occurs as a urinary deposit and may form calculi. The treatment should consist of exercise in the open air and the administration of the weak mineral acids, as carbonic and phosphoric, and of organic acids, as acetic (in old cider) and benzoic.

(b) *An Excess or Deficiency of Phosphates.*—In diseases of the stomach, as in gastric catarrh, the excretion of phosphates in the urine is diminished; while the fæces contain an excess of this constituent. If lactic or butyric acid replace the hydrochloric acid of the gastric juice, less of the mineral ingredients of the food will be dissolved and absorbed; while if acids are wholly wanting in the secretion of the stomach, still smaller quantities

of the phosphates will reach the blood. In structural diseases of the kidneys, the amount of phosphoric acid in the urine is often very small and the quantity is not increased by diuretics in proportion to the increase in the water. In diseases which interfere with the free action of the respiratory organs, the excretion of phosphorus as phosphoric acid is sensibly diminished. This is due to the fact that the oxidation of the albuminous constituents of the food is imperfect and the phosphorus from this source is not converted into phosphoric acid. In these diseases, the amount of phosphorus excreted in organic combination is probably increased, but experimentation is needed on this point. In acute febrile diseases, the amount of phosphoric acid has been found to be much less than normal; but this is probably due to the low diet.

In inflammatory diseases of the nervous system, the patient, although living upon a low diet, excretes more phosphoric acid than in health. This probably arises from the disintegration of the nervous tissue and the oxidation of the phosphorus of the lecithin. Harley found that a man, who had received an injury in the neighborhood of the fourth ventricle (as diagnosed from the presence of sugar in the urine), eliminated 8.749 grammes of phosphoric acid; "and this, too, at a time when he was taking very little food, and that little poor in phosphates."

It has been shown (p. 122) that in rickets, osteomalacia and some other diseased conditions, the bones contain but a small proportion of inorganic matter. Now during the progress of these diseases, the urine contains an excess of phosphoric acid. This essential constituent of the osseous system is unduly removed and washed out of the body with the urine. It is probable that the solution of the mineral constituents of the bones is affected by the abnormal development of some acid. If we knew the nature of this acid and the cause of its development, the rational treatment of these diseases would probably be found; but as it is, the physician can do no better than to see that his patient is properly fed and clothed, has plenty of fresh air and good water, and to administer phosphates to supply the place of these removed, and iron, quinia and strychnia to tone up the system.

SULPHATES.

The greater part of the sulphuric acid contained in normal urine is combined with potassium, while traces of the sulphates of sodium and lime are occasionally detected. Of these, the lime salt is the only one that ever occurs in deposit and it is rarely met with. The sulphate of lime crystallizes in long, needle-shaped crystals which are much finer than those of hippuric acid and resemble tyrosine; from the latter, the lime salt is distinguished by the ready solubility of the tyrosine in ammonia. Crystals of calcic sulphate are not unfrequently observed in the urine of the horse; and they may be obtained in abundance by giving the horse sulphate of magnesium in his drink, collecting the urine passed by the animal afterwards, acidifying and allowing it to stand, when crystals of gypsum will form.

Since the salts of lime in normal urine are in solution, the appearance of the specimen affords no evidence as to whether this constituent is in excess or not. If any urine be acidified with hydrochloric acid and then treated with a solution of the chloride of barium, the sulphate of barium is precipitated and will be found insoluble in acids. } Meet.

Physiology.—If sulphur be taken into the body as soluble sulphates, free sulphur, or in organic combination, it is partially or wholly oxidized and increases the amount of sulphates in the urine. Our food contains alkaline sulphates and organic compounds, as albumen, which furnish some sulphur. The first of these passes through the body unchanged, with this exception, that the sulphate of soda is converted into the corresponding salt of potash; thus, if sulphate of soda and chloride of potash be taken into the body, a mutual exchange takes place and the sulphuric acid is excreted as a potash salt and the chlorine appears in the urine as chloride of sodium. The other source of the sulphuric acid of the urine is in the oxidation of the sulphur of certain organic constituents of the food and of the body. That all of this sulphur is not completely oxidized when excreted in the urine may be proven in the following way: To 500 c. c. of normal urine, add sufficient hydrochloric acid to render it strongly acid and then remove all the sulphates by precipitation with

chloride of barium and filtration. To be sure that all the sulphuric acid has been removed, add a little more baric chloride to the filtrate and if no precipitate forms, all the sulphates have been removed. Now treat the filtrate for several hours with a current of chlorine gas. Soon a white precipitate will be observed to fall. The chlorine has oxidized the sulphur which had existed in organic combination, and as fast as this is oxidized to sulphuric acid, it precipitates the excess of baric chloride in the solution and falls as baric sulphate. After all the organic matter has been destroyed by the chlorine, the precipitated sulphate may be collected upon a weighed filter, dried and weighed.

While the amount of sulphuric acid excreted in a given time depends largely upon the food, this constituent does not entirely disappear from the urine of one who abstains from food. The small quantity, which continues to be present, is due to the oxidation of the tissues of the body. Any thing, which improves oxidation, increases the excretion of sulphates; for this reason, fresh air and nitro-muriatic acid increase the quantity of this urinary constituent. The average amount of sulphuric acid excreted as such in the 24 hours urine is about 2.2 grammes; while the unoxidized sulphur furnishes about .2 grammes more. If a person is inactive and breaths impure air, much of the sulphur will be excreted in organic compounds; while on the other hand, if he exercises body and mind as he should and obtains sufficient pure air, the greater part of these organic compounds will be changed into inorganic matter. Finally, to condense and conclude, it may be said that the amount of sulphuric acid excreted in a given time depends, (1) upon the food, and (2) upon the conditions under which the food passes through the body.

Pathology.—Sulphuric acid bears the same relation to the unoxidized sulphur of the food and tissues as urea bears to the less highly oxidized nitrogen of the same food and tissues; consequently it is found that sulphur and urea are decreased or increased by the same causes. In cases of indigestion, the sulphuric acid of the urine is diminished; the same is true in cholera and for the reason given for the diminished formation

of urea in this disease. It is not simply the small amount of sulphuric acid that may be present in the urine that is to be regarded as of pathological import; but it is the corresponding increase in the quantity of sulphur that leaves the body in organic combination. All escape of unoxidized food from the body is so much lost in the production of force. When an excess of nitrogen leaves the body as uric acid, or an excess of carbon as oxalic acid, or an excess of sulphur as cystine and other organic substances, then so much latent force escapes conversion into manifest force. Of course, the body of man is not so perfect that it can obtain and utilize all the force, which exists in the latent condition in the food; but it is conducive to health to secure the normal degree of oxidation.

In skin diseases, the amounts of both urea and sulphuric acid are decreased; while uric acid and unoxidized sulphur are correspondingly increased. In the same diseases, oxalate of lime is generally found in large quantities in the urine. It is a question as to whether these products of an imperfect oxidation cause the diseased condition of the skin, by poisoning the blood, or whether the impairment of the function of the skin is the cause of the arrest in oxidation. It is probable that these conditions, after being inaugurated, mutually react upon each other and that treatment appropriate for each should be used at the same time.

When there is a deficiency of sulphuric acid and an excess of unoxidized sulphur in the urine, the latter should be converted into the former. This can be done by the use of nitro-muriatic acid and exercise in the fresh air. The acid acts here, as in cases of an excess of uric acid, by virtue of its oxidizing properties, especially in improving digestion, and not because it is an acid.

In fevers, the tissues of the body are consumed or burnt and sulphuric acid is formed, just as it would be by oxidizing sulphur, and is found in the urine in increased quantity. In diabetes, the sulphuric acid is increased in proportion with and for the same reason that urea is augmented in that disease.

As has been stated, sulphate of lime is sometimes found in deposit; but it is not known ever to be present in the urine of man in quantities sufficient to produce pathological results. In

the herbivora, this salt sometimes forms calculi. Beale observed crystals of gypsum in the uriniferous tubules.

CYSTINE,— $C_3H_7NSO_2$.

This is the only one of the well known organic constituents of the urine which contains sulphur. It is sometimes found as the sole or principal constituent of urinary calculi of men and of dogs. At other times, it may be detected in urinary deposits or in solution in the urine.

Cystine is purified by dissolving the stone in ammonia, filtering and allowing the filtrate to evaporate spontaneously, when the cystine forms in colorless, six-sided plates. These are distinguished from uric acid crystals of the same form by the absence of color in the cystine crystals and their ready solubility in ammonia. From acid solutions, cystine is precipitated by the addition of carbonate of ammonia; and from alkaline solutions, by acetic acid. Cystine is insoluble in water, alcohol and ether; soluble in ammoniac hydrate, fixed alkalis and carbonates of sodium and potassium, but insoluble in carbonate of ammonia. It is soluble in the mineral acids and in oxalic acid, but insoluble in tartaric and acetic acids. If a solution of cystine in sodic or potassic hydrate be boiled, the cystine is decomposed with the formation of an alkaline sulphide, ammonia, and an inflammable gas. With the mineral acids, cystine forms crystalline salts which are easily decomposed.

If some cystine be placed upon a piece of silver, then moistened with a drop of a solution of sodic hydrate and heated, the silver will be stained brown. An alkaline sulphide has been formed, and in turn, this acted upon the silver producing the sulphide of silver. Again if a solution of cystine in sodic hydrate be boiled in a test tube with acetate of lead, a black precipitate of the sulphide of lead will be formed.

Mueller dissolves cystine in potassic hydrate, dilutes the solution with water and then adds some nitroprusside of potassium, when, if cystine be present, a beautiful violet color is produced. He holds that this is a more delicate test than any other.

It must be remembered that cystine may be present in solu-

tion in the urine ; indeed I have found it frequently in the urine but never in deposit. The urine is frequently neutral or slightly alkaline and often contains traces of pus showing some irritation. To such urine as this, acetic acid should be added as long as a precipitate is formed. This precipitate, which is amorphous, should be collected on a filter, washed with a little water and then dissolved in ammoniac hydrate. The ammoniacal solution should be evaporated gently on the water-bath, when the characteristic crystals of cystine will be obtained. If the urine should be acid, carbonate of ammonia should be added and the precipitated cystine mixed with phosphates should be collected, washed and dissolved in ammoniac hydrate as before. The phosphates being insoluble in ammonia will remain upon the filter. Fresh urine containing cystine has a sweet-briar odor ; while after decomposition sets in, hydrosulphuric acid gas is given off and may be recognized by its odor and by blackening silver.

Physiology.—Cystine is probably an intermediate stage in the formation of sulphuric acid by the oxidation of the sulphur of the food and tissues. The liver is supposed to have some influence over the formation of cystine and it is probable that it results from the splitting up of the albuminous constituents of the food. In some diseased states, the liver on post mortem microscopical examination has been found to contain crystals of cystine. Cystine resembles taurine in the per cent. of sulphur which it contains and may result from failure to oxidize the sulphur of the taurine. At present only conjectures can be offered with regard to the physiology of this substance, as all positive knowledge on this point is wanting.

Pathology.—The condition, which is represented by the presence of cystine in the urine, is known as cystinuria. This is not so rare as is generally supposed. But few cases are reported for the reason that a person may excrete cystine in his urine for years, and suffer from no particular pains which would call attention to the urinary organs ; then again, comparatively few physicians in general practice ever suspect and test for cystine. Within the past three years, I have met with two cases of cystinuria. The first was a lady of 30 years of age, unmarried, and who com-

plained of dull headaches, probably due to indigestion, and also of slight irritation of the bladder. The daily excretion of urine was found to be normal in quantity, but was slightly ammoniacal, and after standing for some hours formed a dirty white deposit, which consisted of mucus, phosphates and pus. It was by a mere accident that I was led to suspect cystine. The urine had been examined frequently, and a small bottle full, closely corked, had been standing upon my table for several days awaiting examination. One day, I happened to observe the bottle, and took it up, thinking that I would throw it out and obtain a fresh specimen for examination. I removed the cork and observed immediately a strong odor of hydrosulphuric acid gas; while a silver watch placed over the mouth of the bottle was soon blackened. The addition of acetic acid threw down a slight flocculent precipitate, which was collected upon a filter and dissolved in ammoniac hydrate. The ammoniacal solution was gently evaporated in a watch-crystal on the water-bath, and the residue examined under the microscope, when beautiful six-sided plates of cystine were observed. This residue was then further tested by dissolving it in potassic hydrate and boiling this solution with some acetate of lead, when the sulphide of lead was formed. After this, several analyses of the 24 hours' urine were made; but the quantity of cystine was not estimated. The following expresses an average analysis:

Total quantity for the 24 hours=1440 c. c.
 Deposit, slight and of a yellow color.
 Color of the fluid, yellow.
 Odor, of sweet-briar.
 Reaction, alkaline from ammonia.
 Specific gravity, 1011.
 Crystals of ammonio-magnesian phosphate.
 Pus, present in small quantity.
 Phosphoric acid= 2.60 grammes.
 Urea =14.48 "
 Sulphuric acid = 1.38 "
 Chlorides = 7.20 "
 Albumen, a trace and due to pus.

It is seen from this that both the urea and sulphuric acid are present in small quantity. The uric acid was not estimated. The

cystine disappeared from the urine when the patient took nitromuriatic acid and abstained from food containing much sulphur, as beef and eggs. Also the pus disappeared from the urine after this treatment had been followed for some time. However, both the cystine and pus returned as soon as the patient began to eat meat. It is probable that in this case, the cystine was deposited in the bladder, causing some irritation, and the pus, which was poured out, caused decomposition of the urea.

The second case was that of a little boy of 8 years of age. He was anaemic, and had been troubled with sick-headache and dizziness. It was found on inquiry that he was very fond of eggs, and ate largely of them. Cystine was precipitated and detected as in the preceding case. He was requested to abstain from his favorite food, and he was given two drops of the strong nitromuriatic acid in a tumbler half full of water, after each meal. The medicine was continued for a month, and although a year has elapsed, the symptoms have not returned.

In neither of these cases, was there any evidence of the disease being hereditary. On the other hand, several cases have been reported in which different members of the same family were subject to deposits of cystine. (The greatest danger in cystinuria is of the formation of a stone.) It is true that as long as so much sulphur is leaving the body without undergoing the process of oxidation, the person can not be in the enjoyment of perfect health.

The predisposing causes to this disease are excessive use of foods containing sulphur, want of fresh air and proper exercise; to these, some would add a hereditary disposition; this no doubt has its influence in this as well as in other diseases, but it is more probable that different members of the same family often have this disease because they live in the same atmosphere; they partake of the same kind of food and breathe the same kind of air.

CHLORIDE OF SODIUM,—NaCl.

This compound is abundantly distributed in nature, being found in large deposits, and in the water and air. It has been proven experimentally that animals entirely deprived of this arti-

cle of food do not thrive so well as those which are supplied with it in due quantity. But however essential common salt may be to the healthy condition of man, the majority of people take in their food more of this constituent than is absolutely necessary; this is shown by the large quantity of sodic chloride that is daily excreted in the urine.

To some normal urine in a test tube, add nitric acid sufficient to produce a decidedly acid reaction, then add a few drops of nitrate of silver. A voluminous, white precipitate of the chloride of silver falls, and upon boiling the mixture, this precipitate forms a clot and soon subsides on cooling. Pour off the supernatant fluid, and boil the clot with nitric acid in which the precipitate will be found to be insoluble. Pour off the nitric acid and shake the clot with ammoniac hydrate, when solution takes place. The chloride of silver is soluble in ammoniac hydrate and insoluble in nitric acid. In making this test, it is quite necessary that the urine be poured off from the precipitated chloride of silver; for, if this is not done, on the addition of ammoniac hydrate, the chloride of silver will be dissolved; but at the same time the earthy phosphates will be thrown down, and the novice will think that the chloride does not dissolve.

If a solution of sodic chloride in pure water be concentrated, this salt forms in cubes; but in the presence of urea and some other organic substances, sodic chloride crystallizes in octohedrons; consequently from the urine it always appears in the latter form. These crystals should be obtained from the urine and examined with care; for not unfrequently the beginner places a drop of urine on a glass slide and begins his microscopical examination, soon the water evaporates and large colorless octohedrons form and are mistaken for oxalate of lime. These crystals may be obtained in quantity from the urine by the following process: Concentrate from 200 c. c. to 500 c. c. of normal urine to one-sixth its volume, filter and continue the concentration of the filtrate on the water-bath until a syrupy fluid is obtained; set this aside for 24 hours, when a mass of octohedral crystals of sodic chloride will be deposited. These crystals are mixed with urea and the phosphates of sodium and potassium; from these impurities, the sodic

chloride may be freed by the following method: Collect the crystalline mass and press between folds of blotting paper, then place in a crucible and ignite until all the organic matter is destroyed; dissolve the ash in water, boil the solution with animal charcoal and filter. Concentrate the filtrate to a small volume on the water-bath and allow to stand, when sodic chloride crystallizes; while the alkaline phosphates remain in solution in the supernatant fluid, and may be poured off.

Physiology.—Chloride of sodium plays an important part in the animal system. According to Liebig, it influences the development of cells and probably assists in their preservation. The amount of this constituent excreted daily in the urine is less than that taken in with the food. Some of it escapes with the fæces, and some in the perspiration; moreover, some of the chlorine is used in the production of the gastric juice, while a part of the sodium is taken to the liver, and there becomes the base of the glycocholates and taurocholates. In the blood, the presence of sodic chloride assists in holding the albumen in solution and influences the shape of the blood corpuscles. In muscle, bone, and brain, this salt is present not only as common salt, but in organic combination with other substances; thus, if some finely divided muscle be thrown upon a filter and washed until the filtrate no longer contains chlorides, and then the muscle be transferred to a crucible and burnt, the ash will be found to contain common salt.

If a large quantity of salt be injected into the veins of an animal, it is rapidly eliminated in the urine and perspiration, while it is also increased in the saliva. The rapidity with which this substance is excreted after it has been taken with the food, depends upon the time of day at which it is taken. Like urea and in fact all other constituents of the urine, more salt is excreted during the hours of the forenoon than during the same number of hours later in the day. If large quantities of salty water be drunk at night, there is no marked increase in the amount of salt excreted until the next morning. Evidently every experiment upon this subject goes to prove that the processes of life are carried on with greatest vigor during the hours when we are under the influence of sunlight.

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It is an established fact that in health, the increased consumption of salt increases for a while the excretion of urea. This is probably due to the stimulating action of salt upon the kidneys. An interesting example of this seems to be furnished in cases of diabetes insipidus when pneumonia supervenes, when during pulmonary hepatization and when chlorides are absent from the urine, the amount of the urine often becomes normal. In a case of diabetes insipidus under my observation for a long while, the daily excretion of urine fell, during the stage of pulmonary hepatization of an intercurrent attack of pneumonia, from 12000 c. c. to 1500 c. c. As soon as the chlorides began to reappear, the quantity of urine began to increase, and after the patient recovered, she again excreted daily about 12000 c. c. of urine. A similar case is mentioned by Senator (see Ziemssen's Cyclopædia, Vol. XVI, p. 1031). We would hardly be justified in saying that in these cases, the return of the urine to the normal quantity was due to the arrested excretion of chlorides; but it is an interesting fact that if typhus fever (Pribram), acute rheumatism (Dickinson), erysipelas (Senator), pneumonia (Senator and myself), supervene in diabetes insipidus, there is a diminution of the amount of urine excreted. Now in these same diseases, typhus fever (Parkes), acute rheumatism (Folwarczny), erysipelas (Parkes), pneumonia (first observed by Heller and Redtenbacher), common salt is diminished and frequently is not found at all in the urine. Another fact of interest in this connection is that in diabetes insipidus the amount of chlorides is excessive; thus Vogel found that in a case of this disease as much as 48 grammes of sodic chloride were excreted in the 24 hours urine.

It is evident that the quantity of chloride of sodium excreted in the 24 hours urine in health is very variable. According to my experiments, the daily excretion of salt may vary from 5 to 15 grammes, the average being about 6 grammes. It must be remembered that these figures refer to the quantity of chloride of sodium and not to that of chlorine. In cases of starvation, chlorides wholly disappear from the urine, the system refusing to yield that contained in the tissues until more is furnished.

Pathology.—In certain inflammatory diseases, as pneumonia,

the common salt in the urine is diminished and frequently this urinary constituent is absent. This decrease occurs even when the patient consumes much salt in his food. It was formerly supposed that the chlorides were retained in the inflamed lung; but the retention of chlorides is not a condition wholly peculiar to pneumonia, but exists in all acute febrile diseases. They have been found deficient and absent in phthisis, typhus and typhoid fevers, erysipelas, acute rheumatism and cholera. Beale found that when chlorides were absent from the urine in pneumonia they were abundant in the sputa; and the other excretions in the above mentioned diseases should be examined. In all these diseases, the diminished excretion of salt is an unfavorable symptom; while the subsequent increase or the reappearance, after having been absent, is an indication of improvement, and often this is the first evidence of a change for the better furnished the physician. An increased excretion of common salt is not known to be by itself indicative of any pathological condition, but to depend wholly upon the food. The excessive excretion of salt in diabetes insipidus has already been referred to; but this has not been sufficiently studied to enable us to draw any general conclusions. To conclude, we may say that so far as this constituent is concerned, the urine is abnormal when it contains in the 24 hours' excretion, less than one gramme of sodic chloride.

It must be remembered that since common salt is soluble in both acid and alkaline urine, it never forms a spontaneous deposit, but is to be tested for, in the solution, with nitrate of silver as already given.

OXALIC ACID, $-\text{H}_2\text{C}_2\text{O}_4$.

Oxalic acid may be produced by the imperfect oxidation of many organic substances; thus, if one part of sugar be boiled with six parts of nitric acid of specific gravity 1.3 as long as red vapors of the oxides of nitrogen are given off, and the solution then be evaporated on the water-bath, oxalic acid will remain in a crystalline form. In the body, it may result from the partial oxidation of many substances; while if the process of oxidation was completed, carbonic acid would be produced. Some of these changes are represented in the following equations:

INCOMPLETE OXIDATION.

- (1) $C_5H_4N_4O_8 + 3H_2O + 2O = H_2C_2O_4 + 2CH_4N_2O + CO_2$.
(Uric acid.) (Oxalic acid.) (Urea.)
- (2) $C_4H_2N_2O_4 + 2H_2O + O = H_2C_2O_4 + CH_4N_2O + CO_2$.
(Alloxan.)
- (3) $C_4H_6N_4O_3 + 2H_2O + O = H_2C_2O_4 + 2CH_4N_2O$.
(Allantoin.)
- (4) $2C_{57}H_{110}O_6 + 216O = 55H_2C_2O_4 + 4CO_2$.
(Stearine.)
- (5) $C_6H_{10}O_5 + 9O = 3H_2C_2O_4 + 2H_2O$.
(Glycogen.)

COMPLETE OXIDATION.

- (1) $C_5H_4N_4O_8 + 2H_2O + 3O = 2CH_4N_2O + CO_2$.
- (2) $C_4H_2N_2O_4 + H_2O + 2O = CH_4N_2O + 3CO_2$.
- (3) $C_4H_6N_4O_3 + 2O = 2CH_4N_2O + 2CO_2 + H_2O$.
- (4) $2C_{57}H_{110}O_6 + 326O = 114CO_2 + 110H_2O$.
- (5) $C_6H_{10}O_5 + 12O = 6CO_2 + 5H_2O$.

Free oxalic acid in solution may be detected by the addition of chloride of calcium, when calcic oxalate will be deposited. Fortunately, when oxalic acid is present in abnormal quantity in the urine, it is always combined with calcium and deposited in the crystalline form. Crystallized calcic oxalate contains water, and is represented by the formula, $CaC_2O_4 \cdot H_2O$. It generally forms in quadratic octohedrons with one axis shorter than the other. These crystals are colorless and have sharp angles. Besides this form, oxalate of lime may be found in diamond-shaped crystals, in dumb-bells, or in discs. This salt is insoluble in water, alcohol, ether, alkalis, alkaline carbonates and acetic acid; soluble in hydrochloric, nitric and sulphuric acids, also soluble to some extent in solutions of the acid phosphate and urate of soda.

Of the four crystalline forms mentioned above, the octohedral is the most common and the most characteristic. Even when other forms are present, some octohedrons will generally be found and indicate the nature of the deposit. The only other substance in the urine that crystallizes in octohedrons is chloride of sodium, and this may always be distinguished from crystals of oxalate of lime by the solubility of sodic chloride in water.

Diamond-shaped crystals may be either uric acid or oxalate of lime, consequently in all cases of doubt, the chemical test should be resorted to; a drop of hydrochloric acid should be added, when the crystals, if oxalate of lime, will be dissolved; if uric acid, they will remain undissolved; or a drop of potassic hydrate may be added, and would dissolve any uric acid, but be without immediate effect upon the oxalate of lime. The dumb-bells may be oxalates, urates or carbonates. A drop of acetic acid would dissolve the carbonates; while the oxalates would be insoluble in acetic acid, but soluble in hydrochloric acid. By the action of either the acetic or hydrochloric acid, the urates would be converted into free uric acid which would take a crystalline form and remain undissolved. Discs may be either carbonates or urates as well as oxalates, and the true nature of such a deposit is to be ascertained by the application of the chemical tests as already given.

The beginner should always prepare crystals of oxalate of lime, and study them closely before he ventures to analyze specimens for diagnostic purposes. These crystals may be prepared by adding a few drops of a dilute solution of oxalic acid to some normal urine (200 c. c. or more). After this has been standing for some hours, octohedral crystals of calcic oxalate will be deposited, and may be found on examination of a drop of the urine taken from the bottom of the beaker and placed under a microscope. Care must be taken to avoid adding an excess of oxalic acid to the urine; for if this is done the oxalate is thrown down in an amorphous condition, or the crystals will be imperfect.

Within a greater or less time after emission, normal urine will deposit oxalate of lime; consequently, the examination for this substance in the urine should be made within 48 hours after emission, in order to be of any value for diagnostic purposes. If it be desired to make a close examination of a specimen for oxalate of lime, the 24 hours' urine should be collected, immediately placed in a conical vessel, allowed to stand for 12 hours, and then a drop of the urine from the bottom of this vessel examined under a microscope which magnifies at least 300 diameters and whose

defining power is good. It should be remembered that a natural (one to which nothing has been added after emission) specimen of urine never (with the exception of those passed after the administration of large quantities of oxalic acid) contains sufficient oxalate of lime to form a visible deposit. Urine containing this substance is generally acid in reaction.

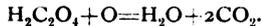
Physiology.—Many articles of food contain oxalic acid and other substances which may be converted into oxalic acid during their passage through the body. Oxalic acid taken in the food is partially or wholly oxidized to carbonic acid as it passes through the body. Buchheim and Piotrowsky have shown, by experiments upon themselves, that when from 1 gramme to 7 grammes of oxalic acid, as free acid or combined with an alkali forming a soluble oxalate, were taken into the stomach in divided doses within from 6 to 8 hours, from 8 to 15 per cent. of the acid could be recovered from the urine. In these cases the urine contained a visible deposit of oxalate of lime, which was in some instances amorphous, in others crystallized in dumb-bells. When calcic oxalate was taken into the stomach, in quantity sufficient to contain 7 grammes of oxalic acid, only from 1 to 2 per cent. of the acid could be recovered in the urine. It must be remembered that these quantities were taken in divided doses and that the administration of 3 or 4 grammes of oxalic acid in one dose would probably prove fatal.

It is well known that rhubarb contains oxalic acid in considerable quantity; now I found that when 5 young men, all in apparent good health, ate the same quantity of rhubarb, and the urine of the next 24 hours was collected and examined, that the excretion of two of the men contained a crystalline deposit of oxalate of lime; while in the urine of the others, no oxalic acid could be detected. I suppose that in the three, in whose urine no oxalic acid could be found, the oxalic acid of the food was oxidized during its passage through the body. The peculiarities of individuals upon the elimination of this acid unchanged, when taken with the food, or for purposes of experimentation, need further investigation.

It is often desirable, as in the experiments mentioned above,

to determine the amount of oxalic acid excreted during 24 hours. For this purpose, collect the urine and concentrate it to one-sixth its volume on the water-bath or steam-bath. Render the concentrated fluid strongly acid with acetic acid in order to hold the earthy phosphates in solution, then add chloride of lime which throws down any oxalic acid as the oxalate of lime. Allow the precipitate to stand for 48 hours, then collect it upon a filter, wash with a little water and then dissolve in hot hydrochloric acid. This solution, filtered, in order to remove any uric acid, is neutralized with ammonia, then acidified with acetic acid. The oxalate of lime, which is now precipitated, is collected upon a weighed filter, dried at 120° and weighed. As a confirmatory result, the oxalate of lime may be redissolved in hot hydrochloric acid and the lime precipitated from this solution, by the addition of dilute sulphuric acid and alcohol, as calcic sulphate, CaSO_4 . This may be collected, dried, ignited and weighed, and from this, the amount of calcium and its corresponding amount of oxalic acid may be calculated.

That oxalic acid in the system results from the imperfect oxidation of other substances is now considered as an established fact. It is one of the intermediate stages in the process of retrograde metamorphosis and may result from either the starchy, fatty, or albuminous food. It is probably present in small quantities in normal blood, but its physiological existence is of short duration and the matter of which it is composed normally passes on to the production of carbonic acid and water as represented by the following equation :



Pathology.—The continued presence of a deposit of oxalate of lime in the urine is indicative of a condition of the system designated by the term *oxaluria*. It must be remembered that an occasional deposit of oxalate of lime may occur in the urine of a healthy person, and it must be repeated here that *all examinations of the urine for the detection of this substance, in order to be of any value in diagnosis, must be made within 48 hours after the emission of the urine.* No doubt that many a patient has been treated for oxaluria, when the formation of oxalate of

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lime in his urine was due to changes going on in the urine after emission, and having no connection whatever with any condition of the patient.

From what has been given concerning the chemistry and physiology of oxalic acid, it will not be difficult to understand some of the circumstances which may lead to oxaluria. In the first place, indigestion is a frequent cause of the appearance of oxalates in the urine. The food is but partially fitted for absorption and the processes of oxidation are retarded just so much. In these cases, the indigestion must be treated. The cause of the indigestion must be sought. It may be that the patient's food is not of the right kind; it may contain so much starch that all of it can not be completely oxidized by the oxygen of the oxyhaemoglobine; or the patient may be breathing impure air and the oxygen of the blood may not be sufficient to oxidize a normal amount of food. Again the excessive use of alcohol is a frequent cause of oxaluria; this is true for two reasons, (1) the excessive use of alcohol deranges digestion, (2) the alcohol furnishes the oxygen of the blood a fuel more readily consumed than that furnished by the solid food; consequently, the latter is only half burned and that which should pass off as gas (carbonic acid) through the lungs, falls as cinder (oxalic acid) through the kidneys. The physician must investigate the conditions under which his patient lives. For the purpose of assisting in the oxidation of the food, and stimulating the action of the liver upon the food, I know of nothing better than nitro-muriatic acid. In cases of nervous prostration, this acid may be given with strychnia and other tonics. The nitro-muriatic acid is much more efficient when it is kept undiluted until it is used; then from 3 to 5 drops should be added to a tumbler of water, the mixture is then stirred and taken through a glass tube. Thus prepared, it forms a slightly acid, pleasant drink; but very few patients will have anything to do with the strong acid; they observe the color and odor of the chlorine that is given off when the stopper is removed, and then cry out in holy horror against the "terrible thing." In truth, this preparation needs to be handled with care, for a drop upon any article of clothing will soon destroy the texture. Con-

sequently, it is better that the physician should prescribe the less efficient acidum nitromuriaticum dilutum of the pharmacopœia to the majority of his patients. This should be given in doses of from 10 to 15 drops in water as recommended for the other form. It must be remembered that the dilute acid should be frequently renewed, as it soon loses its chlorine and oxides of nitrogen upon which its virtue depends. Both the dilute and stronger preparations should be kept in well stopped bottles and protected from the light.

In phthisis, emphysema of the lungs, and pneumonia there is frequently a deposit of oxalate of lime in the urine. This arises from a deficient supply of oxygen and the oxalates are frequently accompanied by crystals of free uric acid and deposits of acid urates. In cases of venous stasis arising from disease of the heart or lungs, oxalates, uric acid and urates are deposited. It is well known that this condition of venous stasis causes chronic hyperæmia of the kidneys and that albumen then appears in the urine ; but often long before the appearance of the albumen, the urine will contain oxalates, urates, and uric acid in deposit.

In skin diseases, oxalates and uric acid are almost invariably present in the urine either as an occasional or constant deposit. The frequent occurrence of uric acid and oxalate of lime in the urine in eczema and psoriasis has led some to believe that, in health, considerable quantities of soluble urates and oxalates are excreted by the skin. They find the amounts of these substances in the urine increased in these diseases, and conclude that this increase is due to the supposed fact that the uric and oxalic acid, which normally pass out through the skin, are now forbidden that avenue of escape and, consequently, are present in the urine. Any thing which interferes with the action of the skin, correspondingly retards oxidation and this, no doubt, is the true explanation of the increase of uric acid and oxalate of lime in eczema, etc. ; for in all these cases, the amount of urea is diminished. Only in suppression of urine, is it positively known that the skin excretes either urea, uric acid, or oxalic acid, and in suppression, any or all of the urinary constituents may be present either in the faeces, perspiration, vomited matters, or pulmonary exhalations.

The continued presence of an excess of oxalic acid in the body causes a greater or less disturbance of the nervous system. The patient often becomes very much alarmed and fancies that he will soon die. One day, he will complain of a severe headache and will imagine that his brain is diseased; probably within less than 24 hours, he will again summon his physician requesting that his heart be examined, thinking that *that* organ is diseased; but more frequently the patient's attention is called to the urinary organs. He becomes irritable, dejected and is unreasonable in his desires and demands. Upon examination of the urine of such a person, a few octohedral crystals of the oxalate of lime will generally be detected. Such cases demand the most serious attention of the physician; but I regard these peculiar symptoms as evidence of a diseased condition of the imagination rather than of any serious disorder of the body. The treatment should consist in out-door sports, or what is still better, active employment in the open air with some object in view. Endeavors should be made to relieve the anxiety of the patient by attracting his attention to other objects. About two years ago, a young man, of nervous temperament and a close student, came to me complaining of dizziness in the head, pain in the loins, and frequency of micturition. His urine was examined and found to contain a few crystals of calcic oxalate. I prescribed nitro-muriatic acid and advised the patient to take more out-door exercise. I saw him every day for about two weeks, during which time his complaints increased; he would tell me that he knew he could not live many days. Knowing that he was fond of hunting and fishing, I finally persuaded him to join a party of friends who were going to spend a week in fishing. A week spent upon the shores and waters of one of our beautiful lakes, together with the healthful air of early June, caused my patient to forget that there ever was known such a monster as disease.

In other cases, the extreme nervous excitement, instanced above, is not observed; and the first complaint is of want of energy, sick-headache, pain in the region of the kidneys and bladder with frequent desire to micturate. In a typical case of this kind, the urine will be strongly acid and, when passed, will

often be cloudy; on standing, quite a deposit forms and will be found to consist of finely divided pieces of epithelium. This deposit is without any form and is generally supposed to be mucus: but chemical examination will show that it contains no mucine. For the microscopical examination of this deposit, a good microscope with a magnifying power of 400 diameters and, what is more essential, with good defining power, is needed; moreover, a trained eye and a skillful hand are quite essential. With these requisites, such a deposit will be found to contain besides the amorphous pieces of epithelium, numerous minute octohedrons of calcic oxalate. I have mentioned a skillful hand as one of the requisites in this examination; the importance of this aid will be appreciated when we remember that the detection of these crystals often depends upon the skill with which the fine adjustment of the instrument is moved in order to catch the reflection from the sides of the octohedron. It is true that often large octohedral crystals will be found in these deposits, but I am of the opinion that these result from the growth of the smaller ones after emission; because, if the specimen be examined within an hour after it has been passed, only the minute crystals are present, while after several hours have elapsed, many large ones will be found.

These minute crystals with their sharp points pierce and irritate the walls of the bladder and the substance of the kidney. That these crystals penetrate the substance of the kidney can hardly be questioned; indeed they have been found in this situation by Crosse and Meckel; while they have been detected in the blood by Garrod. The pain caused by these crystals in the kidneys and bladder is constant and dull; but often so marked as to cause both patient and physician to believe that there is structural disease of the kidney. Several times when physicians have requested me to make examinations of specimens of urine for albumen and casts, saying that they knew their patients to have "Bright's disease" and only wanted to know the proportion of albumen and the nature of the casts, I have found neither albumen nor casts, but a great abundance of these minute crystals. If such a case be taken under care at this stage, relief may be

secured with certainty. Plenty of fresh air and good water, especially should this be free from lime, with proper food and nitro-muriatic acid will seldom fail to remove the oxalates from the urine and the pain from the kidneys and bladder. However, there is one word of caution that must be given here. In some of these cases, there is but little urine (from 400 c. c. to 800 c. c.) passed during the 24 hours, and this is strongly acid. Now if the irritation has existed for any length of time, all the nitro-muriatic acid, that can be given the patient, will not bring relief until the quantity of urine is increased. In such cases, the patient should be requested to drink much water, and I have also found the fluid extract of buchu, taken in quantities of from one-half to one pint per day, very beneficial. Again if the pain be severe, on account of the irritation produced by the excessively acid urine on the raw surface of the walls of the bladder, speedy relief may be obtained by the administration of carbonate of soda or some other alkali. Of course no one will give the alkali and acid at the same time, nor one immediately after the other.

If the formation and consequent irritation of these oxalates be allowed to continue, one or both of two very serious results may follow. These are (1) structural disease of the kidney, and (2) the formation of a stone. The continued irritation produced by these crystals is not an unfrequent cause of parenchymatous inflammation of the kidney. Year after year the irritation may continue, and finally the substance of the kidney begins to break down; this organ soon becomes incapable of performing its function, and death results.

Calculi composed exclusively of oxalate of lime are very rare. Many calculi contain this substance as a constituent, and may consist principally of it; but there is generally either uric acid or phosphates, or both, present. Uric acid and oxalate of lime coexist in the same stone so frequently, because both result from deficient oxidation, and may depend upon the same cause. Calculi of oxalate of lime are often coated with a layer of phosphates. The oxalic stone is very rough and presents many protruding points, indeed so marked is its irregular surface that the term *mulberry calculus* has been used to designate a stone com-

posed of oxalate of lime. Now such a stone can not exist for a long while in any part of the urinary tract without causing considerable irritation. If it be in the bladder, the walls of this organ closing down upon the stone, when the urine is forced through the urethra, are wounded by the rough surface of the calculus; consequently, cystitis often follows, the urine becomes ammoniacal, phosphates are thrown down and deposited upon the stone. If the calculus of oxalate of lime be formed in the pelvis of the kidney, pyelitis and often occlusion of the ureter result; the urine is retained either in part or altogether, and decomposition of urea with consequent deposition of phosphates follows. In this way a small calculus of oxalate of lime may receive layer after layer of phosphates upon its surface and become a large stone.

Suppose that a stone of oxalate of lime has formed, is there any medicinal agent by which it may be removed? In considering this question, we will suppose that the calculus is in the kidney; for if it be in the bladder, it would be very unwise in the physician and unjust to the patient to depend upon the slow and uncertain action of medicines given by the stomach or injected into the bladder, when the knife of the surgeon affords a speedy and certain removal. But cutting down upon, and thus removing a stone from the kidney has been attempted as yet but a few times, and has been attended with but partial success. Consequently, the physician must do the best he can, and the line of treatment which I have followed with some success is briefly as follows: In the first place, all lifting of heavy weights or any thing which may cause a strain upon the small of the back is positively forbidden. From three to five drops of the strong nitro-muriatic acid are given after each meal as already directed. This is done to prevent the further deposition of oxalate of lime. From one to two hours before each meal, from one to six drachms of the phosphate of sodium are given in a broth. This is done in order to dissolve the stone already formed. For the phosphate of sodium, the carbonate or citrate of this base or potash may be substituted. If there be only small pieces of gravel of oxalate of lime in the kidney, this treatment long continued will be found

beneficial; but if there be a large stone, one of a quarter of an inch or more in diameter, I know of no medicinal agent which will remove it.

Hassall and Beale think that the dumb-bell form of oxalate of lime forms renal calculi more frequently than the octohedral variety. I have found the dumb-bell form constantly in the urine in two cases of renal calculi; but have observed the octohedral constantly present in a large number of cases; while in one instance, sometimes one form and again the other, and at still other times, both would be present in the deposit.

XANTHINE,— $C_5H_4N_4O_2$.

Pure xanthine is a glistening, white, amorphous powder, which becomes wax-like on being rubbed. It is practically insoluble in water, one part of xanthine requiring as much as 14000 parts of cold, and 1400 parts of hot water for solution. It is also insoluble in alcohol and ether, but soluble in the caustic alkalis and the mineral acids. It has feeble, basic properties and forms salts with the strong acids. If a solution of xanthine be evaporated on the water-bath, the xanthine is deposited in crystalline scales. From a concentrated ammoniacal solution, xanthine is precipitated on the addition of argentic nitrate as $Ag_2OC_3H_4N_4O_2$. This precipitate is soluble in hot nitric acid, from which is deposited on cooling xanthine-silver nitrate, $C_3H_4N_4O_2AgNO_3$. The ammoniacal solution of xanthine is also precipitated by the acetate of lead, chloride of lime and chloride of zinc.

If a watch-crystal be partially filled with a solution of sodic hydrate, some chloride of lime be added, and the mixture be well stirred, then a little xanthine be added, a dark-green ring soon forms around the spot where the xanthine was dropped: this color soon changes to a brown and finally disappears.

If some xanthine be placed in a clean porcelain dish, covered with a few drops of nitric acid, and then heated to dryness, a yellow residue remains. If this residue, while yet warm, be treated with a drop of sodic or potassic hydrate solution, a deep purple color is developed.

Preparation.—Stædeler recommends the following method of

obtaining xanthine from muscular tissue or from the heart, liver, or spleen: Cut the organ or tissue into fine pieces; rub these up in a mortar with ground glass; add dilute alcohol to the pulp, stir, warm, and press through cloth; digest the residue for an hour with water at 50° and again filter through cloth; unite the alcoholic and aqueous extracts and remove the alcohol by distillation. Filter the remaining fluid in order to free it from coagulated albumen; concentrate the filtrate and add to it, first some acetate of lead, then basic acetate of lead, and after it has stood for some hours, add mercuric oxide. Suspend the precipitate formed by the mercury and lead, and treat with a current of hydrosulphuric acid gas; remove the precipitated sulphides by filtration, and evaporate the filtrate to dryness on the water-bath, when xanthine and hypoxanthine remain. If this residue be treated with cold, dilute hydrochloric acid, the hypoxanthine will be dissolved, and may be removed; while the xanthine remains insoluble.

Xanthine may be obtained from normal urine, but it is present in quantities so small, that large quantities of urine must be used in its preparation. Thudichum gives a method of obtaining xanthine from the urine without the evaporation of a large quantity of urine; but I have not found his method very satisfactory. The method proposed by Neubauer, for obtaining xanthine from normal urine is as follows: Concentrate from 100 to 200 pounds of urine on the water-bath; treat with the baryta mixture and filter in order to remove phosphates and sulphates. Concentrate the filtrate to a syrup and allow to stand for some time; decant the supernatant fluid from the salts which have separated by crystallization; dilute this fluid with a little water and add acetate of copper; boil this mixture for a short time, and collect the dirty brown precipitate, which has formed, on a filter; wash with cold water until the wash-water no longer contains chlorine (tested for with nitrate of silver); dissolve the precipitate with warm nitric acid and add to this solution some nitrate of silver, which reprecipitates the xanthine; dissolve this precipitate in hot, dilute nitric acid, and filter while hot. As the filtrate cools, xanthine-silver nitrate will be deposited. This compound, freed from nitric acid by being

digested with ammonia, is treated with hydrosulphuric acid, and filtered while hot. The filtrate, on cooling, deposits impure xanthine which may be purified by solution in hot nitric acid, and filtration through animal charcoal. This filtrate is neutralized with ammonia, evaporated to dryness and the residue is washed with water, which removes the ammonia salt, while the xanthine remains pure and insoluble.

Physiology.—Xanthine is found in the various tissues of the body, having been obtained from the liver, pancreas, spleen, muscles, and blood. It is an intermediate product of oxidation; although it has never, in the test tube, been directly oxidized to uric acid. Rheineck has reduced uric acid to xanthine with a very dilute solution of sodium amalgam; while on the other hand, xanthine is obtained by the action of nitrous acid on either guanine or hypoxanthine. Xanthine, in very small quantities, is a constituent of normal urine; the daily amount not being more than one grain. It exists in large proportion in the excrement of spiders.

Pathology.—In two instances, I have found xanthine deposited with uric acid in the urine of patients with enlarged spleen. In one of these cases, the daily excretion of uric acid was as much as 23.5 grains. The deposit, which was quite heavy, consisted of urates, uric acid and xanthine; these were separated by dissolving in strong sulphuric acid, and then diluting with water; when the uric acid was reprecipitated, and the xanthine remained in solution. Although xanthine, as prepared from muscle and normal urine, is granular and amorphous, when in great excess in the urine, it is deposited in small oval crystals. Prof. Langenbeck once extracted a calculus, the size of a hen's egg, which on analysis was found to consist entirely of xanthine: indeed calculi of xanthine contain no other constituent. Whether pieces of gravel contain xanthine or not may be ascertained by the tests, (1) with nitric acid and potassic or sodic hydrates, (2) with caustic soda and chloride of lime, and (3) by their ready solubility in ammoniac hydrate.

HYPOXANTHINE,— $C_5H_4N_4O$.

Hypoxanthine, known also as sarkine, has been found as a normal constituent of muscles, and of the substance of the liver,

spleen, lungs, and marrow of the bones. In the blood and urine of leucocythaemia, hypoxanthine is present in abnormal quantity. It resembles xanthine very much in its reactions and is a true animal alkaloid, uniting with acids to form salts. Hypoxanthine forms in fine, microscopical needles, which are soluble in 300 parts of cold, and 78 parts of hot water; insoluble in alcohol. It is freely soluble in the caustic alkalis and the mineral acids.

If an ammoniacal solution of hypoxanthine be treated with nitrate of silver, a double salt of silver and hypoxanthine is precipitated. This salt has the formula, $\text{Ag}_2\text{OC}_5\text{H}_4\text{N}_4\text{O}$, and forms a gelatinous mass. If an aqueous solution of hypoxanthine be treated with nitrate of silver, a precipitate having the composition represented by the formula, $\text{C}_5\text{H}_4\text{N}_4\text{O Ag NO}_3$, is thrown down, and will be found to be soluble in hot, strong nitric acid, from which it falls in crystalline scales on cooling. Hypoxanthine forms double salts with some other bases, among which are barium, copper, and platinum.

Of the mineral acids, hydrochloric is the best solvent for hypoxanthine. This solution consists of the formation of the chloride of hypoxanthine, and if it be evaporated to dryness on the water-bath, this salt remains in glistening tablets. From its solution in the alkalis, hypoxanthine is precipitated by a current of carbonic acid gas. By the action of oxidizing agents as nitrous acids, hypoxanthine takes another atom of oxygen and is converted into xanthine. The basic properties of this substance are quite marked, and its chloride, nitrate, sulphate, and other salts have been closely studied by Thudichum and others.

Physiology.—Hypoxanthine is formed by the oxidation of guanine, and we have here a physiological chain, the known links of which are guanine, hypoxanthine, xanthine, uric acid and urea. These are stages through which nitrogenous constituents of our food and tissues pass on their return to the inorganic world. Each of these contains C, H, N, and O: but there is a progressive increase in the oxygen until urea is reached, and then one step further carries this once highly organized matter back to inorganic nature. Under the influence of the heat of fever, the urea is sometimes converted into carbonate of ammonia within

the body. Thus, the tissues of the fever patient may really be burned to ashes. I look forward to the time when the physiologist will be able to trace matter from the inorganic world, through all its various changes in the plant and animal, until it returns to dust. If such knowledge be ever attained, the physician will endeavor to ascertain two things; (1) the means of preventing arrest in these progressive changes, and (2) the means of preventing the too rapid transformation of matter. Many diseases arise from each of these causes; thus in cholera, there is arrested transformation. Life depends upon the liberation of force resulting from the oxidation of the food. Stop this oxidation, or process of force liberation, and life for that individual ceases. But in the majority of cases, the processes of life are not suddenly arrested: but are retarded and gradually brought to a stop. The fire is not immediately extinguished, but the cinders and ashes are allowed to accumulate and shut out the air. On the other hand, in all acute febrile diseases, the transformations go on too rapidly; too much force is liberated, and the tissues of the body are consumed in this over production of force. Says Prof. Haughton, "An additional amount of work, equivalent to the body lifted through nearly one mile per day, is spent in maintaining its temperature at fever heat. If you could place your fever patient at the bottom of a mine, twice the depth of the deepest mine in the Duchy of Cornwall, and compel the wretched sufferer to climb its ladders into open air, you would subject him to less torture, from muscular exertion, than that which he undergoes at the hand of nature, as he lies before you, helpless, tossing and delirious, on his fever couch."

Pathology.—Hypoxanthine has been found deposited in the urine in severe diseased conditions of the liver, spleen, and kidney. The treatment, in all these cases, is to secure oxidation, and normal activity of the lungs and skin.

GUANINE,— $C_5H_5N_5O$.

It is not positively known that this substance ever occurs in the urine; but it is of value here on account of its relation to xanthine, hypoxanthine, and uric acid. Guanine is present in Peruvian guano, from which it may be easily obtained. It has

been found combined with lime in the scales of some fish, and has also been extracted from the muscle, liver, and pancreas of man. It has been detected in the muscles, tendons and joints of diseased pigs.

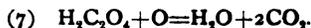
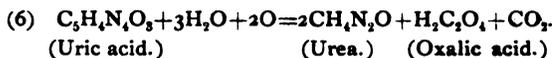
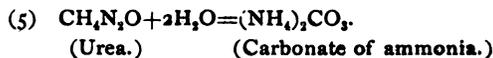
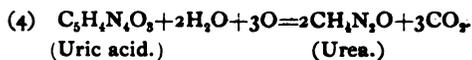
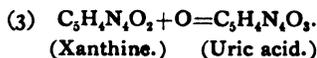
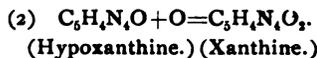
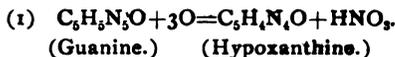
Preparation.—Boil Peruvian guano with water and milk of lime until some of the filtered solution is colorless; then filter through cloth. Urea and some other substances are contained in the solution; while uric acid and guanine remain undissolved. Now boil the residue with a solution of carbonate of sodium, repeatedly, until the filtered fluid ceases to give a precipitate on the addition of acetic acid. The united filtered extracts, made with the solution of carbonate of sodium, are treated with acetic acid, until a decidedly acid reaction is obtained. This precipitates the uric acid and guanine. The precipitate is allowed to stand for 24 hours, then the supernatant fluid is removed either by decantation or filtration. The residue is boiled with hydrochloric acid and filtered. The guanine being soluble in hydrochloric acid, passes through the filter, while the uric acid remains insoluble. From its solution in hydrochloric acid, the guanine is precipitated on the addition of ammoniac hydrate.

Guanine is a white, amorphous, odorless, tasteless powder, which is insoluble in water, alcohol, ether and ammonia. It is soluble in the mineral acids and in sodic and potassic hydrates. With the mineral acids, guanine forms crystalline salts, the best known of which is the chloride: this salt also forms double salts with several bases, among which are mercury, platinum, and zinc.

If some guanine be placed on platinum foil, a few drops of nitrous acid be added and then heated to dryness, a yellow residue remains and by caustic soda is colored red: this color being changed to a purple on the application of heat. By means of nitrous acid, guanine is converted into xanthine: while by chlorate of potash and hydrochloric acid, it is converted into xanthine, parabanic acid, and guanidine, CH_5N_3 .

Physiology.—If guanine be taken into the stomach, it is oxidized as it passes through the body, and increases the amount of urea. But there is a limit to this oxidation of guanine in the

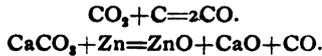
body, and if very large quantities be taken, all of it is not excreted as urea. The relations between the different compounds, which have been studied here, and which arise during the retrograde metamorphosis of nitrogenous food and tissue, are best represented by the following equations :



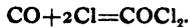
In the fourth equation, the normal degree of oxidation of uric acid is represented; while in the sixth equation, the imperfect oxidation of uric acid is represented. The reaction represented by the fifth equation should not take place in the body; but does occur in the bladder in cystitis, and in the blood in suppression of the urine, and in cases of extreme fever heat. From these studies, we see that the final principal products of the beef-steak, which we eat, and likewise of our own tissues are water, carbonic acid and ammonia; while the sulphur and phosphorus of the highly complex organic tissue are excreted as sulphuric and phosphoric acids, inorganic substances.

The water, ammonia and carbonic acid, which result from the oxidation of animal tissue, are returned to the plant. Here a series of chemical changes is inaugurated, whereby these substances are deoxidized, or furnished with a new supply of force. It is an interesting fact, which can be only mentioned but not discussed here, that this deoxidation can, in a great number of cases, be accomplished by artificial means. Thus, the chemist can build up urea from carbonic acid; first he takes the carbonic acid

and forms carbon monoxide. This may be done either by passing the carbonic acid over red-hot charcoal, or by heating chalk with zinc or iron :



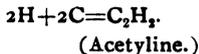
Now, equal volumes of carbon monoxide and chlorine are placed in glass balloons and exposed to the sunlight, when the chloride of carbonyl is formed :



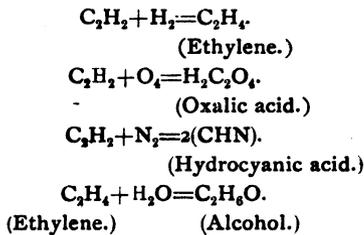
If carbonyl chloride be treated with dry ammonia, urea and chloride of ammonia are formed :



By means of a powerful galvanic battery, an electric arc is passed between carbon poles in an atmosphere of hydrogen, when these two elements unite and form acetylene :



From acetylene, a variety of organic substances may be built up, as represented by the following equations, taken from the Lehrbuch of Gorup-Besanez :



Chemical force, or chemism, is that which causes atoms to unite or to rearrange themselves to form molecules; and every molecule, simple or compound, inorganic or organic, formed in air, water, earth, plant, or animal, is produced by the *chemical* combination of its atoms. Every change in the atomic arrangement of any substance during absorption, assimilation, or excretion is due to chemical force. Every proximate principle formed in the plant or in the animal is a chemical formation,

Although the formation of every molecule is due to chemism and it is the province of chemistry to study these formations, the building up of these molecules into cells is an entirely different thing and does not fall within the domain of chemistry. Why certain molecules form liver cells, while others produce bone cells, and still others, nerve cells, is a subject which in no way concerns the chemist. It is a chemical fact that oxalic acid precipitates lime from its solution in normal urine, but why the molecules of oxalate of lime unite so as sometimes to produce octohedral, and at other, dumb-bell, at still other, diamond-shaped crystals is no part of chemistry to investigate. The formation of the crystal is to be studied in the laboratory of physics.

ALBUMEN.

Urine containing albumen may be either of high or low specific gravity, though, generally, if much albumen be present, the specific gravity will be low. If sugar be present with the albumen, the specific gravity may be high; again, in certain structural diseases of the kidneys and even in the later stages of these diseases, the urine may be very dense on account of the small amount of water present. In amyloid degeneration of the kidney, I have seen the specific gravity of the 24 hours' urine rise to 1040 just before death. In this case, the total urine for the 24 hours did not measure 200 c. c. From this, we see that a high or normal density is not proof sufficient of the absence of albumen.

The best test for this substance is heat and nitric acid; first applied separately and then combined. Heat coagulates albumen; but if we rely on this test alone, we will sometimes overlook it when present, and at other times, get a cloudiness when the solution contains no albumen. If the solution be neutral or alkaline, heat will often fail to coagulate the albumen until an acid has been added. In many cases, on heating a specimen of urine, a cloudiness, due to the precipitation of phosphates, appears; but redissolves on the addition of a drop of nitric acid. On the other hand, nitric acid alone may in some instances throw down a precipitate of either nitrate of urea or acid urates, which would be redissolved on the application of heat. Consequently, the best way is to apply heat to one part, nitric acid to a second,

and both heat and nitric acid to a third. Another chance of error here lies in the fact that a coagulum of albumen may be redissolved on the addition of nitric acid, if either too little or too much of the acid be added. If but little nitric acid be added and there be an excess of phosphates present, the nitric acid unites with the bases and forms free phosphoric acid: now albumen is soluble in free phosphoric acid, and more nitric acid must be added to reprecipitate the albumen from this solution. An excess of nitric acid added to a faint cloud of albumen may form an acid-albumen which passes into solution. In order to avoid these sources of error, it is well to add the nitric acid slowly, a drop at a time; and to about a drachm of urine, in a test tube, from 3 to 15 drops of the ordinary reagent, nitric acid, should be added.

It is necessary to remember that albumen, when present in the urine, is in solution and never in deposit. Albuminous substances, as mucus, may be deposited, but true albumen is in solution and the test must always be applied to the clear fluid. If any deposit be present, it must be removed either by filtration or decantation. Often the urine will be cloudy from suspended mucus which will not fall as a deposit; in such cases the urine must be filtered, and if necessary, must be passed through several filter papers, until it is clear. After a perfectly clear fluid has been obtained, apply the test with heat and nitric acid, and then if any coagulum or cloudiness appears and remains (when tested as recommended, with both heat and nitric acid), the presence of albumen is certain. Albumen is not always present in quantity sufficient to give a distinct coagulum; for even in structural diseases of the kidneys, as renal cirrhosis, the amount of albumen is often very small and only sufficient to produce a distinct opalescence, when the urine is treated with heat and nitric acid.

Physiology.—When we speak of albumen in the urine, we mean that kind, which is precipitated by heat and nitric acid; for there is a variety of albumen, precipitated by chloroform and absolute alcohol, which is a normal constituent of the urine. We must constantly bear in mind, that there are many kinds of albumen, and if certain of these get into the circulation, they are

unfit for use in the body and must be excreted; this accounts for the temporary albuminuria caused by indigestion. Sometimes after one has eaten a large meal, especially if it consisted of food not easily digested, and be taken late in the day, albumen temporarily appears in the urine. Also if excessive exercise be taken soon after a meal, or indeed at any time of the day, albumen may be present in the urine even in considerable quantities. Moreover, in cases of this kind, hyaline and epithelial casts have been known to appear in the urinary deposits. Albumen in the urine may be due to the presence of blood or pus, as in haematuria and cystitis, or directly from the serum of the blood as in structural diseases of the kidneys.

PATHOLOGY.

LITERATURE.—Bartels and Ebstein in Ziemssen's Cyclopaedia. Harley on the "Urine and its Derangements."

(a) HAEMATURIA.

Blood in the urine may be detected by the color, by microscopical examination, or by the spectroscope. Bloody urine is always albuminous. The color will vary from bright red to a smoky or even a black tint. The fluid is dichroistic, red by transmitted and green by reflected light, if much blood be present. The corpuscles may generally be detected by the microscope; but sometimes they are completely disintegrated, then the spectroscope may be used to advantage. The source of the blood can almost invariably be ascertained. If there be clots large enough to be visible to the unaided eye, the blood must have passed into the urine below the secreting structures. If from the bladder, the clots will often be quite large and may obstruct the passage through the urethra. If the coagulation has taken place in the ureter, the shape and size of the clots will so indicate. These have been mistaken for entozoa. When from the pelvis of the kidney, the coagula are much smaller than those from the bladder and may preserve the shape of the calices. If from the substance of the kidney only, the clots will be microscopic in size having been formed in the tubules, and the urine will generally have a smoky tint. Profuse bleeding from a wounded kidney or a highly vas-

cular cancer of that organ may cause large clots to be formed in the pelvis of the kidney, or in the bladder. When the urine has a bloody color and no corpuscles can be found, and it is not convenient to apply the spectroscope, Heller's test for blood-pigment may be used. This consists in boiling the urine with a solution of caustic soda, when the earthy phosphates will be precipitated and will entangle the blood-pigment forming a brick-dust or red deposit. But the spectroscopic examination is by far the best method of testing for blood-coloring matters.

(b) PUS AND EPITHELIUM.

Whenever there is sufficient pus in the urine to give a compact deposit, it could not have come from the kidney only, since in the severest inflammation of the kidney substance the amount of pus formed is small. If the pus be from the bladder, it will contain more mucus, be ropy, and the urine will generally be alkaline from a volatile alkali ; the urea having been decomposed, while in the bladder, into carbonate of ammonia. This decomposition takes place very rapidly, as the mucus, which is poured from the irritated walls of the bladder, acts as a ferment. In suppurative cystitis, the greater part of the pus and mucus is passed after the water, while in pyelitis the pus will be distributed through the urine, which will generally be of acid reaction. There is a chance of making a very serious mistake here : suppose that a specimen of urine be found to be acid and to contain traces of pus. Now this pus may have come from the kidney or from the urethra, and often it will be impossible to decide from an analysis of a specimen, collected in the usual way, whether there be a diseased condition of the kidney or simply an inflamed condition of the urethra. This question can be decided with certainty only by adopting the following procedure : when the patient goes to urinate, the first drachm or two passed should be collected in a small clean beaker or other vessel, and the remainder of the urine in a second vessel. Now if the pus be from the urethra, the urine first passed will wash it all out ; while the second portion of the urine will contain no, or very little pus. On the other hand, if the pus be from the kidney, it will be distributed in the bladder and the second portion of the urine will contain as much

as that passed first. To Sir Henry Thompson, I believe, belongs the credit of first calling attention to this serious source of error in the analysis of urine.

In all these cases, the epithelium should be closely studied. This may be from the uriniferous tubules, pelvis, ureter, bladder, urethra, or vagina. If the individual epithelial cells are normal in appearance, but unduly increased in amount, there is indicated only an excessive desquamation which may be due to simple hyperaemia and may be temporary or even physiological in its nature; thus, during pregnancy, the urine often contains a visible, dirty white deposit, which consists principally of vaginal epithelium. However, if the cells contain oil or are broken, there is some degeneration of the part from which they came. In fatty degeneration of the kidney, the renal epithelium will contain and may consist principally of oil globules. This condition follows poisoning by phosphorus as well as it results from a general diseased state of the system. In these cases, care is needed to distinguish between oil globules in the epithelium and those that may be floating freely through the fluid; the latter most frequently are due to some accidental cause, having been introduced into the urine after its emission. The student should make it a rule to study epithelium closely and decide upon its source in every instance, because these cells are overlooked by some, and mistaken for casts by others.

(c) CASTS.

It was formerly supposed that all casts were formed by the coagulation of albumen, from the blood-serum, in the uriniferous tubules. But it has been ascertained by chemical analysis that the different kinds of casts vary in their composition and that the majority of them are not composed of fibrine nor of any protein substance. As they vary in their origin, they likewise vary in their pathological importance. In examining for casts, the greatest care and patience is demanded. It is true that in many instances, they will be found in abundance on every slide examined; but in other cases, it will be necessary to pour the urine into a conical glass and leave undisturbed for from one to twelve hours and then examine the sediment most thoroughly.

Having found the casts, it will remain to determine their exact nature and pathological significance. Casts vary in diameter from .01 to .05 of a millimetre, and in the length with the place of formation.

Epithelial casts.—These are cylinders or pipes formed by the removal of the epithelia of the tubes in mass. The distinct cells can be recognized. These casts are caused by inflammation of the mucous membrane.

They may be produced by highly concentrated, acid urine containing urates or other irritating substances.

Granular casts consist of a mass of aborted epithelia, differing from the epithelial casts in the fact that the individual cells are not fully developed. In some cases, these granules are closely adherent; while in others, they seem to be on the point of breaking up. The cells are generally as large as a pus cell and somewhat darker.

Bloody casts consist of coagulated fibrine with blood corpuscles entangled and are formed in haematuria. Under the microscope these appear as dark granular masses.

Hyaline casts are smooth, structureless, and consequently often escape detection. They may be detected upon the addition of iodine in iodide of potassium, or of a dilute solution of carmine, when they will be stained yellow, or red, respectively. They are narrow, glass-like in appearance, sometimes containing a few fine granules or oil globules, and are formed by the coagulation of albumen in the uriniferous tubules and simply indicate albuminuria. These casts are often found in urine of persons suffering with severe febrile disease, when the post mortem reveals no pathological condition of the kidney whatever.

Waxy casts have the appearance presented by melting a piece of wax, dropping it on a slide and allowing it to cool. They are distinguished by their glistening appearance and by being of a faint yellow color; they are formed by an abnormal secretion from the mucous membrane of the kidney. Not unfrequently the largest casts found are of this class.

It will be seen from these short descriptions of the various kinds of casts, how important it is for the physician to know the

nature of any casts that may be present in his patient's urine. To make an analysis of a specimen of urine and report casts without indicating the kind of casts is about as senseless as it is for a physician to find albumen in his patient's urine and then diagnose "Bright's disease," and rest satisfied in his conscience, that he has made a correct diagnosis and must prescribe certain medicines given in his medical books as having been found beneficial in "Bright's disease." How many articles do we find in our medical journals giving the treatment found "wonderfully successful" by some physician in so many cases of "Bright's disease?" We read these articles through, and find that the physician knew his patient to have this disease because albumen, and sometimes casts are mentioned, was found in the patient's urine. Seldom is anything said concerning the nature of the casts and often they are not mentioned at all. The presence of the albumen may or may not have been due to structural disease of the kidney; and if structural disease of this organ did exist, it is quite important for the reader to know, before he can apply the recommended treatment, to his own patients, whether it was renal cirrhosis, or amyloid degeneration, or some other form. Is it not time that American physicians should follow the example of many foreign medical men and either restrict the term "Bright's disease" so as to have it mean something, or, if this can not be done, banish it entirely from the vocabulary of medicine?

It must be understood that in the following notes on renal diseases, it has been aimed to give only the condition of the urine as briefly as possible, and not to give a history of the diseases.

DIFFUSE DISEASES OF THE KIDNEY.

- (1.) Hyperæmia.
- (2.) Parenchymatous inflammation.
- (3.) Induration of the connective tissue.
- (4.) Amyloid degeneration.

Hyperæmia.—By this term is meant an engorgement of the blood vessels of the organ under consideration; an undue supply

of blood, with the various effects that may follow. This condition will be best discussed under two heads, according to the causes which produce it :

(1). From irritant poisons.

(2.) As caused by diseased conditions of other organs, as the lungs or heart, more especially the latter.

When due to the first cause, this condition of the kidney is frequently known as one of *active* hyperæmia, in contradistinction to the word *passive*, by which the other is known ; or the first is called acute and the second chronic. The poisons, which most commonly produce the acute form, are cantharides, turpentine, mustard, nitrate of potash, and cardol (oleaginous liquid from the *anacardium occidentale*.)

All slow poisons, all the effete products of the body, as oxalate of lime, which soon disease the kidney, and all those which act directly upon the tissues of the kidney, as arsenic, phosphorus, and the mineral acids, are excluded from this list ; because they produce other changes than that of simple hyperæmia.

The *anatomical* condition of the kidneys, produced by cantharides, turpentine, etc., is not sufficiently understood at the present day to be described in detail. Both kidneys will be affected alike, and they will be found presenting a well marked catarrhal aspect.

In poisoning by cantharides, cystitis is often the most apparent lesion, often causing the pathologist to overlook any affection of the kidney.

The symptoms are distinctly marked, since in the majority of instances, the irritation is not confined to the urinary tract, but the digestive suffers as well ; so that nausea and vomiting will frequently attract attention before the patient's notice has been called to the urinary tract. Here the first manifestation is a desire to pass water at short intervals of time ; but the amount of water is not usually increased, and in some instances is notably decreased. Soon the urine becomes bloody, with a greater or less quantity of albumen, and there may be bloody casts with excess of epithelium from the uriniferous tubules. If turpentine is the cause, the urine will have the characteristic odor of violets, and the turpen-

tine can be recognized by its odor in the breath. Remember that all this may be caused by the *inhalation* of turpentine: if due to this substance, the symptoms will abate on its removal. If the irritation has been caused by cantharides, so much fibrine will be poured out, in consequence of the irritation of both kidney and bladder, that often clots, large enough to interfere with micturition, form in the bladder. When due to this poison, the symptoms *do not* disappear upon the removal of the exciting cause; but the urine may continue to be bloody and contain bloody casts for weeks.

The *diagnosis* of these cases is simple, since the symptoms follow so soon upon the cause, and the condition of the urine attracts attention.

The *treatment* consists in abstention from the noxious agent, and in taking an excess of drink to cleanse the kidneys. In severe cases, absolute rest in bed, morphia, mucilaginous drinks, with mild oleaginous purgatives are recommended.

(2.) Hyperæmia caused by diseased conditions of other organs.

This may be considered as only a symptom of some other affection; and necessarily caused by some disturbance of the circulation.

There may be general venous stasis caused by valvular lesions of the heart, or by certain abnormal states of the lungs; or the congestion may be local and due to some obstruction in the vena cava ascendens above the entrance of the renal veins. The former cause is the more frequent and the condition of the kidney which is produced and is known as *cyanotic induration*, is described by Klebs as follows: "The kidneys are larger than they should be, and are surrounded by a capsule which is provided with very little fat; the capsule proper can be stripped off easily, and the surface of the organs looks vascular, but perfectly smooth—the venous radicles, or star-like commencements of the veins, appearing enlarged and filled to distension. The whole organ is considerably firmer than it should be, and does not diminish in firmness after the blood has drained off from it. Upon section, both medullary and cortical substances are seen to be highly vascular,

although the medullary cones are the more deeply colored of the two, by reason of the specially marked engorgement of the vasa recta.

In the cortical substance the vascularity is generally diffused throughout the capillaries, the glomeruli not appearing over distended. Microscopical sections show the hyper distension of the veins and capillaries extending back even to the Malpighian tufts. The epithelium of the tubuli is ordinarily unaltered; on the other hand, the interstitial tissue is unwontedly tough, but not broader, or only very little broader, than normal; even in the fresh state, it admits of easy demonstration by brushing out the cells, and displays its fibrous texture more clearly than in the normal condition.

This change may exist for a long while without causing any disturbance of the renal functions, although a very little increase of the arterial tension may suffice to allow albumen or blood to escape from the glomeruli, owing to the fact that the outflow of the venous blood takes place with difficulty. Another danger, however, exists, that, namely, of secondary disease of the kidney provoked by its malnutrition, for the blood, thus stagnating in its capillaries, is poor in oxygen and over-loaded with carbonic acid, and does not provide proper nutrition for the organ; under these circumstances, a granular degeneration of the epithelial cells takes place, chiefly affecting those which line the curling cortical tubes; the cortical substance appears then of a pale grayish-red color, and stands out in marked contrast with the deeply cyanotic medullary substance. This peculiar color of the cortical substance is due to the fact that the swelling of the epithelium in the cortical portions squeezes the blood out of the capillaries surrounding them, and then, on section the glomeruli—from which the outflow per vasa efferentia has been impeded—stand out against the surrounding pale parts as dark red points, and not unfrequently some of their capillaries burst in consequence of this obstruction to the outflow, and discharge their blood into the tubuli uriniferi.”

In considering the *symptoms*, we shall confine ourselves mostly to those manifested in the kidney. Of course there will be distension of veins leading to cyanosis, and diminished arterial

pressure, causing a feeble and at times an almost imperceptible pulse. These two points, venous distension and arterial weakness, must be constantly held in mind, for they are the corner stones of every rational explanation of the renal symptoms. In consequence of the diminished arterial pressure, the daily quantity of urine passed will be small, frequently not more than from 400 to 600 c. c.

The urine will be albuminous; the albumen being forced from the inter-tubular capillaries into the uriniferous tubules, by the venous congestion.

With the albumen more or less blood corpuscles often pass through. There will be a heavy deposit of urates because the amount of water is too small to hold them in solution, and because the absolute quantity of urates is increased by imperfect oxidation.

The urea will be in excess in proportion to the water, but deficient absolutely.

From the above mentioned facts, it will be seen that the specific gravity is necessarily high, usually ranging from 1030 to 1035.

In these cases, the treatment must be directed to the primary diseases of which the renal affection is only a symptom; consequently, the discussion of the treatment does not belong here. Suffice it to say that when the trouble is caused by valvular disease of the heart, the urinary symptoms often abate, and in some cases, the urine becomes perfectly normal under the skillful use of digitalis. The digitalis may be given in combination with the acetate of potash, which will be oxidized, during its passage through the body to the carbonate, acting as a diuretic and also preventing a deposit of uric acid or urates, which is likely to occur from the great excess of these substances present.

PARENCHYMATOUS INFLAMMATION OF THE KIDNEY.

This form of kidney disease is also best discussed under the two heads of (1) acute and (2) chronic.

The acute form may follow upon scarlatina, diphtheria, small pox, etc., or it may be produced by sudden exposure to cold

when the body is over-heated, or, again, it may be the result of an injury. It is supposed by some that the nephritis following scarlatina is due to an accumulation of poisons, which irritate the kidneys. Be this as it may, it is well known that true scarlatinal nephritis does not appear until, on an average, the twentieth day after the first appearance of the rash. (Bartels.)

In a case of nephritis following diphtheria, Cœrtel claims to have found micrococci in the substance of the kidney; but others have been unable to discover any, after many examinations.

The symptoms are well marked, and the diagnosis easily made, when we have the history, the appearance of the patient and the examination of the urine all before us. The history and symptoms of a typical case may be best illustrated from the following:

A field laborer, a very strong, and before this attack, a healthy man, was at work saving the grain from an approaching storm, during one of the warmest days of last summer. His exertions had overheated his body and covered it with perspiration when the storm came on, and he was thoroughly drenched with rain. During the following night he complained of chilly sensations, which were manifested by his shivering; also, of pain in the small of the back. The subsequent morning, the eyelids were swollen and soon the feet and ankles became œdematous. The urine was scanty and bloody, with a deposit of bloody casts, urates, uric acid, and oxalate of lime. Under proper treatment recovery soon followed. The improvement was indicated by an increased quantity of urine, and the gradual disappearance of the blood, together with the entire deposit. The treatment consisted in rest, drastic purgatives, and the use of hot air bath to produce diaphoresis. All irritant diuretics *must* be avoided.

Chronic nephritis is caused by repeated attacks of the acute form, or by constant irritation, as that produced by oxalate of lime. We judge of the extent of this disease by the quantity of urea daily excreted, and by the kind and number of casts.

In the first stages of chronic parenchymatous inflammation, the urine may be normal in amount and may contain a normal proportion of urea, the only indication of a diseased state of

the kidney being the presence of albumen with the occasional or constant appearance of the narrow or hyaline casts, and often of blood and bloody casts. As the disease progresses, the amount of water increases on the average, but often fluctuates from day to day; thus, one day the quantity of urine may be normal, or even less than the average amount passed by a healthy person of the age, size and sex of the patient; while the next day, a great excess of urine may be passed. The specific gravity and per cent. of urea vary inversely with the quantity of urinary water. As the disease progresses, granular casts begin to appear and increase in number day by day. If the disease is not arrested, the granular casts soon outnumber those of the hyaline variety; while the latter may entirely disappear. As the disease progresses, the granular casts become darker in appearance and wider; while waxy casts begin to appear. The waxy casts then increase in number and become wider and often are quite yellow and glisten like wax.

Besides the granular casts, there will often be observed, in the deposit, a large quantity of what are known as granule cells. These are aborted epithelial cells from the tubules of the kidney and may be washed out, as such, or they may result from the breaking up of the granular casts into their constituent granules. Moreover, these granules may contain oil, or may consist principally of globules of oil. The globules of oil will also be observed in the casts not unfrequently. Albumen is always present in chronic parenchymatous inflammation of the kidney and may be found in quantities as large as 5 per cent.

RENAL CIRRHOSIS.

Under this head, I wish to describe the true contracting kidney. Of all the forms of renal disease, this is the most difficult of diagnosis. There are no conditions of life that are exempt from it. Many cases are upon record, where the renal lesion was not suspected until uraemic poisoning manifested itself. This disease may exist for a long while without attracting any serious attention, either from the patient or his physician. The daily excretion of urine is generally increased: this, however, is not, by any

means, always true. Remember that only frequent, I might say constant, examinations of the urine will enable us to detect this condition of the kidney; because, for days and weeks together, the urine may be perfectly normal. Albumen may or may not be found; though it is generally present, but in a small quantity, during some stage of the disease. If any casts are found, which, so far as my experience goes, is rather the exception than the rule, they are of the hyaline variety. The most constant factor in the analysis of the urine, is the small per cent. of urea, with an increase in the amount of water. As the disease progresses the symptoms may become somewhat more marked; for then there may be dyspeptic pains with nausea and vomiting, after a meal, also an obstinate diarrhœa often precedes death. The extent of dropsy is always, when this disease alone exists, very slight. The feet and ankles may be somewhat swollen after standing or walking, and the eye-lids puffy on rising in the morning, but farther than these there are no dropsical effusions. Often disordered vision, due to structural changes of the retina, is the first thing to attract the attention of the patient. The physician should make it his rule to, frequently and thoroughly, examine the urine in all doubtful cases.

In a typical case of renal cirrhosis, the amount of albumen is very small, only sufficient to produce a slight opalescence when the urine is tested with heat and nitric acid. The amount of urine, for the 24 hours, generally measures from 2000 c. c. to 3000 c. c. The urine may be almost colorless, is generally feebly acid in reaction, and seldom contains a heavy deposit. In such a specimen, the greatest care is needed in order to detect any casts that may be present. It is best to pour from 30 to 40 ounces of the urine into a conical glass and allow to stand for several hours and then examine a drop of the urine taken from the bottom of this vessel. Although the amount of albumen in this disease is generally very small, I have known it to be present in as great a quantity as 2 per cent.

AMYLOID DEGENERATION.

The term amyloid was given to this condition of the kidney for this reason; if a preparation of the diseased organ be mois-

tended with a solution of iodine and examined under the microscope, it will be found to be stained reddish-brown, which is farther changed to violet on the addition of a drop of dilute sulphuric acid. This test, which resembles so closely the well-known reactions of starch, caused Virchow to believe that it was due to a deposit of some amylaceous substance; but on a chemical analysis, it was found to contain nitrogen, and to belong to the albuminates. This amyloid deposit differs, however, from other albuminous substances by being insoluble in gastric juice.

The amyloid deposit may be removed from an organ by the following process: First, an organ which contains much of the amyloid substance (as shown by the readiness and extent to which it colors, when microscopical sections are tested with iodine and with iodine and sulphuric acid) is needed. Remove, as completely as possible, the blood-vessels, and also the gall-bladder, if the liver be under examination. Cut the organ into fine pieces; wash these with cold water, removing the water either by filtration or decantation; boil the residue with water, in order to loosen the connective tissue; treat the residue first with alcohol and then with ether in order to remove fat and cholesterine. Boil the residue, which consists of amyloid substance and cell membranes, with alcohol acidified with hydrochloric acid. This forms a gelatinous mass of the other albuminous substances, but is without effect upon the amyloid. Now digest the yet insoluble residue with gastric juice for some hours at 40°. The other albuminous substances are digested and may be removed from the amyloid, which remains insoluble.

Amyloid is soluble in concentrated hydrochloric acid, forming an acid-albumin, or syntonin. From its solution in strong hydrochloric acid, the syntonin is precipitated on the addition of water. In the caustic alkalis, amyloid dissolves, forming albuminates.

The *cause* of this disease is chronic suppuration. It does not matter in what part of the body the pus is formed; but amyloid degeneration follows only when the air gains access to the pus. Pus may be poured into the pleural cavity, for instance, for a long while and not cause amyloid degeneration of any organ, but as

soon as an opening is made so that the air has access to the pus, this peculiar degeneration occurs. It is supposed that by the action of air on the pus, a new chemical substance is formed which, as it circulates through the body, lowers the vitality of certain organs and causes this degeneration. The kidney is not the only organ that undergoes this change, but the suprarenal capsule, spleen, various lymphatic glands and the liver, one or all, are found, on post mortem, to present the same pathological aspect. While it is true that amyloid degeneration may follow chronic suppuration in any person, it must be borne in mind that there are certain predisposing conditions; these are syphilitic, scrofulous, and tuberculous.

Dickinson thinks that the formation of this amyloid, or, as he prefers to call it, lardaceous substance is due to the withdrawal of alkalis, especially of potash, from the body. Dr. Dupre found (see Transactions of the Pathological Society of London, Vol. XXII,) that three healthy livers contained an average of .283 of one per cent. of potash; while three lardaceous livers yielded an average of .131 of one per cent. of this alkali. In cases of lardaceous degeneration of the spleen, the potash contained in this organ has been found, as an average in five cases, to be .196 of one per cent. of potash; while a healthy spleen contains .311 of one per cent. of potash. Now, it is supposed that, since the white corpuscles of the blood are rich in potash, and these corpuscles are withdrawn in suppuration, for the formation of pus, the body suffers from a deficiency of alkalis; and the consequent excess of acid forms this lardaceous matter. According to this theory, the administration of salts of potash should be found beneficial in this disease; but it has not proven of as great practical value as was supposed that it should. However, this may be due, as Dickinson has supposed (see London Lancet, April 29, 1876,) to the inability of the system to avail itself of the potash which may be furnished as a medicine. Consequently, this distinguished physician gives the salts of potash with iron and quinia. Iodide of potash, long continued, has been found very beneficial in this disease.

During the first stages of this disease, the urine is increased

in quantity, is clear and contains much albumen. Microscopical examination reveals hyaline casts and epithelial cells from the uriniferous tubules; the epithelium will give the reaction with iodine and sulphuric acid, but the casts are only stained yellow. The dropsy is marked, but confined to the abdomen and lower extremities.

As the disease progresses, the amount of urine is decreased, until often the daily quantity does not measure 100 c. c.; the specific gravity goes up as high as 1035 to 1040; the per cent. of urea is increased to 4 or 5, while the daily excretion of urea does not vary much from the physiological standard. The urine becomes dark colored and contains a visible deposit, which on microscopical examination is found to consist of hyaline, granular and large waxy casts.

COLORING MATTERS.

The variations in the color of the urine have already been referred to, and it only remains to briefly describe some of the most important coloring matters which appear in the urine either in health or in disease. To separate the various coloring principles of the urine is a difficult task, and it is probable that some of them are more or less modified during the processes of separation.

Indigogen.—This substance, also known as uroxanthine, is found as a normal constituent of the urine of mammals, being especially abundant in the urine of the horse. Dilute acids (slowly in the cold, more rapidly when warmed) decompose indigogen with the formation of indigo-blue and indigo-glucin. The former is deposited from the solution in blue granules, or forms a bluish pellicle upon the surface of the fluid. The indigo-glucin is a syrupy fluid which reduces copper, but is incapable of the alcoholic fermentation. Jaffe gives the following methods of detecting and estimating the indigogen in the urine:

(1) *In urine rich in indigogen, as the urine of the horse.* To 10 c. c. of the urine, add an equal volume of strong hydrochloric acid, then to this mixture add, drop by drop, a saturated solution of chlorinated lime, when the color becomes successively red, violet, green, and blue. Care must be used in the addition of the solution of chlorinated lime; for if too little of this reagent

be added, the blue color will not be produced, while if too much be added, the indigo-blue will be oxidized. The exact quantity of the solution of chlorinated lime necessary to produce the blue coloration should be ascertained by repeated experiments. If it be desired to estimate the quantity of indigogen, from 200 c. c. to 300 c. c. of the urine is treated with an equal volume of strong hydrochloric acid, and the requisite amount of the saturated solution of chlorinated lime. The mixture is allowed to stand for 24 hours, and then the residue is collected upon a filter, which has been washed with hydrochloric acid, dried and weighed. The residue on the filter is washed with hot water which dissolves the hippuric and benzoic acids, while the indigo remains insoluble. The indigo is now washed, first with dilute ammoniac hydrate, then with water, after which the filter with its contained indigo is dried at from 100° to 110° , and weighed. The weight of the filter and contents, less the weight of the filter, will be the weight of the indigo obtainable from the indigogen of the urine taken.

(2.) *Urine which is poor in indigogen.* To 1500 c. c. of human urine, add sufficient milk of lime to produce an alkaline reaction, then add chloride of calcium which throws down the phosphates and sulphates. After standing for from 12 to 24 hours, the supernatant fluid is removed by filtration. The filtrate is concentrated (at first on the sand-bath, and then on the water-bath) to a syrup. During the process of evaporation, the reaction of the urine should be tested from time to time, and its alkalinity retained, by the addition of sodic hydrate, if necessary. The syrupy residue is now treated with 500 c. c. of strong alcohol and the mixture is warmed for a few minutes; then poured into a clean beaker and allowed to stand for 24 hours. From the solution, the alcohol is now removed by distillation; the residue is dissolved in water and treated with a very dilute solution of ferric chloride as long as a precipitate forms, but avoiding an excess of the precipitant. The precipitate is removed by filtration; the filtrate is treated with ammoniac hydrate which precipitates the excess of iron; the mixture is boiled and the precipitated oxide of iron is removed by filtration. The filtrate is concentrated

to 200 c. c., filtered, if necessary, and the indigo is precipitated, freed from impurities, dried and weighed as given in (1).

According to Jaffe, one litre of the urine of the horse yields an average of .152 of a gramme of indigo; while a litre of human urine yields only .0066 of a gramme.

Besides the test given for indigogen by Jaffe, there are several others for the detection of this substance. One given by Stokvis is as follows: Warm some urine with two parts of commercial nitric acid at from 60° to 70° , then shake the mixture with chloroform. If indigogen be present in considerable quantity, the chloroform will be immediately colored blue; while spectroscopic examination of the chloroform solution will reveal the absorption band of indigo-blue between C and D.

To from 4 c. c. to 6 c. c. of strong hydrochloric acid in a test-tube, add from 20 to 40 drops of the urine under examination and gently heat the mixture, when, if indigogen be present, a violet or blue color will be developed. (Heller's test.)

Indigo-blue. $C_{16}H_{10}N_2O_2$.—This substance is not found in normal urine but results from the decomposition of indigogen. It is not unfrequently observed deposited in blue granules in urine, to which nitric or hydrochloric acid has been added in order to precipitate the uric acid. It may also result from indigogen-during the spontaneous decomposition of urine. It generally appears in granules, though sometimes in small plates, and at other times in fine needles.

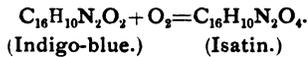
The preparation of indigo-blue from the urine has been given; it may also be obtained from the indigo of commerce. Put some powdered indigo and grape sugar into a clean flask or bottle, add some concentrated solution of caustic soda, then fill the vessel to overflowing with warm dilute alcohol; carefully fit the cork so as to exclude the air. After the mixture has stood for some hours, a clear, yellow solution is obtained, the indigo-blue having been changed to indigo-white, $C_{16}H N_2 O_2$. If now the clear solution be decanted and left exposed to the air, oxygen is taken up, indigo-blue is again formed and is deposited in crystals. Instead of grape sugar and alcohol, ferrous sulphate and warm

water may be used in the preparation of indigo-blue ; but in this case, the deposit will be amorphous.

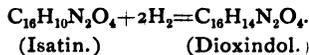
Indigo-blue dissolves in concentrated sulphuric acid, especially on being warmed, forming a deep blue solution of *indigotindisulphonic acid* and *indigotinmonosulphonic acid*. On diluting this solution with water, the latter acid falls as a blue precipitate and may be removed by filtration. If a solution of indigo-blue in sulphuric acid be neutralized with sodic or potassic carbonate, a blue precipitate is formed. This precipitate is soluble in pure water and forms the indigo-carmine sold in the shops.

If indigo-blue be boiled with water, and nitric acid be gradually added to the boiling mixture until all the blue color is destroyed, *isatin*, $C_{16}H_{10}N_2O_4$, is obtained and forms, on cooling, in beautiful prisms with a red lustre.

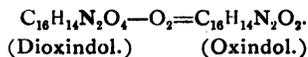
Indol has already been referred to as being the source of the normal odor of fæces and as being obtained from indigo (see p. 58). But since an understanding of the physiology of the indigo-forming substances in the urine will depend upon our knowledge of the chemistry of indol, this subject demands more detailed consideration. Indol is obtained by the reduction of indigo. It has been shown above that isatin results from the oxidation of indigo-blue : this change is shown in the following equation :



Now if an alkaline solution of isatin be treated with sodium-amalgam, and the solution be evaporated, the sodium compound of *dioxindol* is formed, and deposited in glistening crystals. The isatin has been reduced :



If now dioxindol be farther treated with tin and hydrochloric acid or with sodium-amalgam, farther reduction takes place and *oxindol* is formed :



If oxindol be heated with powdered zinc, a farther reduction

that ligating the small intestines of a dog increased the quantity of indigogen. In catarrh of the intestines and in diffuse peritonitis, the amount of indigogen has been found increased. It is a notable fact that the first urine passed after a cholera collapse contains a great excess of indigogen. Thudichum gives the following very reasonable explanation of this fact: "In the course of the choleraic process, large quantities of albuminous matters in the muscles and organs lose their colloid state; and having in this process of liquefaction absorbed the necessary amount of heat, and thus produced the low temperature observed in all cases of collapse, pass into the blood, and from this into the intestinal canal. Here they are immediately subjected to a fermentative process, which resembles in many respects the kind of putrefaction, modified by pancreatic ferment, which we have described above."

It must be remembered that the color of the urine is no indication as to the quantity of indigogen present; thus, large quantities are not unfrequently detected in pale urines.

We thus see that indigo may be obtained from the urine, then reduced artificially to indol; then the indol may be injected subcutaneously and will again appear in the urine as indigogen, from which indigo and indol may again be obtained. Can any one study this subject, and then say, honestly, that the changes going on in the body are produced wholly by "vital force," and do not depend upon the laws of chemistry?

Urobilin, $C_{32}H_{40}N_4O_7$.—This coloring matter was first discovered by Jaffe, who named it urobilin; afterwards, Maly showed that it was identical with hydro-bilirubin and could be obtained by the action of sodium-amalgam on bilirubin (see p. 49). It is prepared from bilirubin by the following method: To bilirubin suspended in water, a piece of sodium-amalgam is added; the whole is allowed to stand exposed to an ordinary temperature for a short while, and then gently warmed on the water-bath. The supernatant fluid is then poured off from the mercury and treated with either hydrochloric or acetic acid. The acid precipitates the urobilin or hydrobilirubin as a reddish-brown powder, which may be collected, washed with water and dried.

Jaffe's method of preparing urobilin is as follows: Treat the urine with basic acetate of lead; collect the precipitate which forms; wash it with water; dry it; then pulverize it and boil with spirits of wine, then add absolute alcohol acidified with sulphuric acid. This acidified alcohol dissolves the urobilin forming a wine-red solution which is separated from insoluble substances by filtration. The filtrate is treated with an excess of ammonia and again filtered. This filtrate is diluted with an equal volume of water, and then treated with chloride of zinc as long as a reddish-brown precipitate falls. This precipitate is collected upon a filter and washed first with cold, and then with hot water, then dried. The dried precipitate is placed in a beaker and treated with alcohol acidified with sulphuric acid. This again dissolves the coloring matter, and the solution is filtered. To the filtrate add half its volume of chloroform, then an excess of water; shake well and remove the chloroform, which now contains the urobilin. The chloroform is washed well with water, and then the chloroform is evaporated, when urobilin remains. In the highly colored urine of fever, the urobilin may be precipitated by the direct addition of ammonia and chloride of zinc.

Urobilin is a brownish-red, amorphous powder; soluble in alcohol, ether and chloroform, forming solutions which are red, brownish-red, or yellow according to the degree of concentration. The chloroform solution of urobilin, obtained from the urine, exhibits a marked green fluorescence; while the solutions of that obtained from bilirubin do not manifest this property until they have been rendered alkaline with ammonia, and then treated with a few drops of a solution of chloride of zinc; after this treatment, the fluorescence is very marked. Urobilin is very sparingly soluble in water, freely soluble in alkalis, and in alcohol acidified with sulphuric acid. Spectroscopical examination of an acid solution of this coloring matter reveals a band, not very sharply defined, between b and F. In alkaline solutions, the band is more sharply defined and lies nearer b. If to an ammoniacal solution of urobilin, chloride of zinc be added until the precipitate, which first appears, is redissolved, then this

solution be examined with the spectroscope, the above mentioned band will be much more sharply defined.

Urobilin readily combines with alkalis forming compounds which are soluble in water and in an excess of the alkali. It is precipitated from neutral solutions on the addition of the chloride of zinc. This salt is reddish-brown in color, and is freely soluble in ammonia. Thudichum claims that urobilin is a mixture of several substances and that it is not identical with hydrobilirubin; but Hoppe-Seyler thinks that the *uromelanin*, *paramelanin*, *omicholin*, *omicholic acid*, etc., of Thudichum need further investigation, (See Handbuch, S. 217.)

Physiology.—Maly thinks that urobilin results from the reduction of bilirubin and that this reduction takes place in the intestinal canal. Hoppe-Seyler has prepared a substance, which has the properties of this coloring matter, by the action of hydrochloric acid and tin on haematin. This author also thinks that urobilin, as such, is not contained in normal urine, but that it is formed during the process of separation by the oxidation of other substances. It seems evident that this coloring matter results from that of the blood and represents the disintegration of the red corpuscle; whether it first passes from the blood into the bile and there exists as bilirubin and other bile-pigments is not known.

Pathology.—Urobilin is increased in fevers and the amount excreted, in a given time, varies with the heat of the fever. In the highly colored urine of fever, this coloring matter may often be detected by spectroscopic examination of the filtered, acid urine; or by adding an excess of ammonia, filtering, adding chloride of zinc and then applying the spectroscopic test; by precipitating the urine with the basic acetate of lead, washing this precipitate with water, dissolving it in alcohol acidified with sulphuric acid, rendering alkaline with ammonia, adding chloride of zinc and examining with the spectroscope. (Hoppe-Seyler).

Uroerythrin is the name given by Heller to the coloring matter which so often causes a deposit of urates to have a pink or reddish tint. It is abundant in cases of acute rheumatism and is

identical with the murexide of Prout and with the purpurin of Golding-Bird.

GRAPE SUGAR,— $C_6H_{12}O_6$.

To the chemist, the term sugar, is somewhat indefinite and indicates the class rather than the individual. The sugar, which is a constant constituent of the blood, is identical with grape sugar. It is prepared on a large scale by boiling starch for several hours with dilute sulphuric acid, then neutralizing the acid with carbonate of lime, filtering and evaporating, when the grape sugar crystallizes. It may also be obtained in a very pure state from diabetic urine by the following process: Concentrate the urine to a syrup on the water-bath; allow the syrup to stand for several days when the solid constituents will have crystallized; wash the crystalline mass with a little cold alcohol, which dissolves the urea; treat the residue with alcohol; filter while hot, and allow to stand when the sugar crystallizes in warty granules. These may be redissolved in hot alcohol, filtered and allowed to recrystallize.

Sometimes four-sided prisms can be obtained, but generally, only granules form. Grape sugar is slowly soluble in water and on being dissolved in water, it loses its property of crystallization; for if an aqueous solution be evaporated, only an amorphous mass remains. The crystals of grape sugar contain water, and are represented by the formula, $C_6H_{12}O_6 + H_2O$. If chloride of sodium be present, it combines with the sugar, forming large, six-sided, double pyramids, which have the composition represented by the formula, $2 C_6H_{12}O_6 + Na Cl + H_2O$.

(1.) *Trommer's test*.—When grape sugar is boiled with an alkaline solution of a cupric salt, the copper is reduced and deposited as a red or yellowish precipitate of the suboxide, Cu_2O . Upon this fact, depends Trommer's test for sugar which is applied as follows: To a solution of grape sugar, or to diabetic urine, add a few drops of a dilute solution of sulphate of copper, render the mixture alkaline, and heat, when the suboxide of copper is precipitated. If a solution of grape sugar in pure water be used for this test, the precipitate will be colored red; while if the test be applied to diabetic urine, the color of the

precipitate will vary between a red and a yellow, depending upon the amount and kind of organic coloring matters present. Instead of applying the solution of copper and the alkali separately, in this test, we now use Fehling's solution. This consists of a solution of the sulphate of copper, mixed with a solution of Rochelle salts (tartrate of potash and soda) in sodic hydrate. The sodic hydrate added to a solution of the sulphate of copper would throw down a precipitate of the black oxide, Cu O ; consequently, the alkaline tartrate is added in order to hold the black oxide in solution.

In the application of Trommer's test to the urine in searching for sugar, some caution must be used. In the first place, it must be remembered that the disappearance of the blue color of the Fehling solution alone is no proof of the presence of sugar. The blue is caused to disappear by the action of the alkali upon the organic matter present. Not only must the blue color disappear, but there must be a distinct precipitate of the suboxide of copper. Now this precipitate is to be recognized by its color; because, since Fehling's solution is alkaline, it will throw down a dirty white precipitate of phosphates, which is to be distinguished from that caused by sugar.

It is also quite essential to regard the amount of Fehling's solution that is added to the fluid under examination for sugar. If too much of this test fluid be added, the blue color of the excess may hide from view a small quantity of the suboxide which may be formed. If too small a quantity of Fehling's solution be added, the small amount of suboxide, which may be formed, may be hidden by the color of the urine or other fluid under examination. If there be albumen in the fluid, it must be removed before the application of Trommer's test for sugar. If the fluid be already acid, and contains but little albumen, this is removed by heat and filtration. If the fluid be not acid, it should be rendered so by the addition of acetic acid (an excess of the acid is to be avoided), then heated and filtered. If this is done the filtrate should be rendered alkaline before the application of the test for sugar; and it is well to remark here that any strongly acid fluid should be neutralized or rendered alkaline, before the

application of Trommer's test. If there be a large quantity of albumen in the fluid to be tested for sugar, the albumen is best removed by adding to the solution an equal weight of crystals of sulphate of soda, boiling and filtering. Thus, in testing blood for sugar, a weighed portion of the blood should be mixed with an equal weight of crystallized sodic sulphate, then a hot saturated solution of sulphate of soda is added; the mixture is then boiled and filtered, when, if sugar be present, it may be found in the filtrate on the application of Trommer's test.

(2.) *Moore's test.*—If a solution of grape sugar be boiled with sodic or potassic hydrate, the sugar is decomposed, while the solution becomes colored brown. Upon this reaction, depends Moore's test for sugar. This test is best applied as follows: To a small test tube about one-half or two-thirds full of the fluid under examination, add sufficient sodic or potassic hydrate to render strongly alkaline; holding the test tube by the bottom, heat the upper part of the fluid in the flame; the sugar contained in the heated portion will be decomposed and produce a brown coloration. The advantage to be derived from heating the upper portion of the fluid is that decomposition of the sugar takes place only in the heated portion and the contrast between this and the unchanged portion renders the color much more distinct. If but little sugar be present, the color produced, on the application of this test, will be a light yellow; this deepens as the amount of sugar is increased and may be quite black.

(3.) *Böttcher's test.*—To a solution of grape sugar or to some diabetic urine, add an equal volume of a concentrated solution of sodic carbonate, then add a small piece of the basic nitrate of bismuth and boil the mixture. The bismuth is reduced to a suboxide, the reduction being indicated by the change of the color of the bismuth to gray, and then, if sufficient sugar be present, to black. If only traces of sugar be present, but little bismuth must be added. Instead of the basic nitrate of bismuth, a preparation obtained by the following process may be used: To nitrate of bismuth, add a large excess of potassic hydrate; collect the precipitate which forms, and add sufficient tartaric acid to dissolve

it. If a drop of this solution be boiled with one of grape sugar, the bismuth falls as a black precipitate.

(4.) *Mulder's test.*—Render a solution of indigo alkaline by the addition of carbonate of sodium: boil this with a solution of grape sugar, when the blue color disappears and the fluid becomes yellow. If but little sugar be present, the solution will become purple instead of yellow, and even when much sugar is present, the purple may be noticed as a transitory color. Now if the yellow fluid be shaken with free access of air, the original blue color again appears. This test is not very suitable for application to the urine, unless considerable sugar be present.

(5.) *Fermentation test.*—If a test tube be filled to overflowing, with a feebly acid solution of grape sugar, a small piece of yeast be added, and the tube closed with a tightly fitting cork, or a rubber stopper, which has a small glass tube passing through it, with one end extending down into the solution and the other bent at right angles, vinous fermentation will take place and the carbonic acid gas will rise to the top of the test tube and, by its pressure, will force some of the solution out through the small tube. The test tube should be left in a warm place for several hours, when the stopper may be removed and the carbonic acid tested with a lighted match.

Physiology.—Sugar is present in the blood, chyle and lymph; whether it be a constituent of normal urine or not, is a question which has long been under investigation by physiologists, and seems to be now quite positively decided in favor of the affirmative by the experiments of Pavy. This untiring investigator has labored in this field for years and has done much in bringing out new facts in regard to the physiology and pathology of sugar. Pavy's method of testing normal urine for sugar may be expressed as follows: To some normal urine (100 c. c. or more), add the normal acetate of lead in an excess. This throws down a heavy precipitate consisting of the chloride, sulphate, phosphate, urate and probably other constituents. This precipitate is removed by filtration. To the clear filtrate, which contains an excess of the acetate of lead, ammoniac hydrate is added. Another copious precipitate falls, and among other things, con-

tains the sugar combined with the oxide of lead. Sugar does not combine with the oxide of lead in an acid solution, and for this reason, it escapes precipitation on the first addition of the acetate of lead to the urine. The precipitate produced with the acetate of lead and ammonia is washed with hot water, at first by decantation and is then collected upon a filter and the washing is continued until the filtrate is no longer found alkaline when tested with red litmus paper. However, excessive washing is to be avoided, because the sugar may be removed. (Pavy.) The washed precipitate is now suspended in a little distilled water and treated with a current of hydrosulphuric acid gas for some hours. The precipitated sulphide of lead is removed by filtration, and the filtrate, containing the sugar, is then heated until the hydrosulphuric acid is driven off. The fluid is now concentrated to a small volume, either on the water-bath or in vacuo. This concentrated fluid contains the sugar which will respond to Trommer's test or to any of the other tests as already given. (For Pavy's description of this test, see *London Lancet*, March 30, 1878.)

Not only has Dr. Pavy obtained the qualitative test for sugar, in normal urine, but he has estimated the quantity excreted, and found that it varies from .09 to .5 of a part per 1000. When a quantity of sugar greater than .5 of a part per 1000 is present in the urine, it can generally be detected by the ordinary applications of the tests and then the person is said to have diabetes.

The old doctrine of Bernard is that the liver is a sugar-forming organ; the experiments of Pavy go to prove that it is not a sugar-forming, but is a sugar-assimilating organ. According to the former, the office of the liver is to furnish the blood with sugar; according to the latter, the office of the liver, in this respect, is to prevent the passage of the sugar into the blood. It was formerly supposed that the sugar was formed in the liver, then was carried out with the blood, and was oxidized during its passage through the lungs. But analyses of the blood, taken from both sides of the heart, showed that the sugar was not perceptibly lessened during the passage of the blood through the lungs. It was then supposed that the sugar was consumed or

absorbed during the passage of the blood through the systemic capillaries. Indeed, Bernard made quite a number of analyses of arterial and venous blood, which seemed to prove that the former contained more sugar than the latter (*Lecons sur le Diabete*). But Pavy, employing another process (and evidently a much more reliable one) of estimating the amount of sugar, and using more care in collecting the blood, has shown that the difference between the amount of sugar in arterial and that in venous blood is very small. Pavy found as the average for eleven estimations (four made upon the arterial and venous blood collected simultaneously from the dog immediately after death; and seven made upon the arterial and venous blood collected simultaneously from the dog during life) that the arterial blood contained 0.941 of a part of sugar, and the venous blood, 0.938 of a part of sugar per 1000 of blood.

From these experiments, it seems quite evident that there is no considerable destruction of sugar in the systemic capillaries. Then the question arises, what does become of the sugar of the food? Some of it probably passes through the absorbents into the thoracic duct and then into the general circulation; but the greater part of the sugar is absorbed into the portal system and carried to the liver. After reaching this organ, probably more of the sugar passes on unchanged into the general circulation; but the greater part of the sugar is transformed into glycogen. Then the question arises, what becomes of the glycogen that is formed in the liver from the carbohydrates and albumen of the food? The old glycolytic theory held that this glycogen of the liver was transformed into sugar, which passed out in the blood. But Pavy and Tscherinoff have shown that the blood of the hepatic vein contains no more sugar than does that of the portal vein or that of the heart. Moreover, if the glycogen is transformed into sugar, what becomes of the sugar thus formed? We have already seen that the sugar is not oxidized either in the lungs or in the systemic capillaries. In fact it is not known how the blood can oxidize sugar. Then to answer the question, what is the fate of the glycogen? It may be answered that the destination of the glycogen is not positively known. It evidently serves as a reserve

which is stored up during digestion and is afterwards called upon, during the hours of fasting, to yield its supply of force. It is also evident that the ultimate products of the oxidation of the sugar of the food or of the glycogen of the liver are carbonic acid and water. The most plausible theory with regard to the fate of the glycogen, is that it is converted into fat. Animals fed upon starchy food fatten more rapidly than when this kind of food is withheld; but how the glycogen is transformed into fat is not known.

It must be borne in mind that the idea of the physiology of sugar, as given above, does not invalidate the statement that the carbohydrates are valuable force-producing foods. The final products of this kind of food are water and carbonic acid; and the transformation of the sugar into glycogen and of the latter into fat (if such a transformation does take place) does not lessen the amount of force furnished by the sugar.

Pathology.—Pavy teaches that whenever the glycogenic function of the liver, as taught by Bernard, is established, diabetes results. In health, the liver prevents the larger portion of the sugar reaching the blood and thus prevents diabetes. If the sugar reaches the blood, as sugar, it can not be oxidized and consequently is excreted in the urine. Therefore if the liver fails to arrest the sugar and to transform it into glycogen, the former passes on, unchanged, into the blood and is carried to every part of the body and a proportional amount will be excreted in the urine. Again, it is well known that from post mortem changes in the substance of the liver, a ferment is generated and converts the glycogen into sugar. This ferment, or a similar one, may be generated in certain diseased states and may cause the conversion of the glycogen into sugar. Pavy found that diabetes could be produced by surcharging the blood with oxygen by means of artificial respiration. It is also well known that the inhalation of carbonic oxide, (C O), causes diabetes. Now it is supposed that either the carbonic oxide gas, itself, or the carbonic oxide-haemoglobine, which results from the combination of the gas with the coloring matter of the blood, abnormally stimulates the liver, and causes the conversion of the glycogen into sugar.

In diabetes mellitus, the amount of urine is increased; but the increase is seldom so great as that of diabetes insipidus. The specific gravity is high, unless there be albumen present. In a case of diabetes mellitus coexisting with albuminuria, I found the specific gravity as low as 1010. In this disease, the total quantity of urea for the 24 hours is greatly increased; for, as has been stated, the escape of the sugar from the body, without yielding any force, causes a greater consumption of the nitrogenous food and tissues. The amount of urinary water may be so great as to cause a deficiency of urea in proportion to the water; but the total quantity for the 24 hours will be excessive.

The high specific gravity is due to the presence of the sugar, and in a marked case of diabetes mellitus, the density is seldom less than 1030 and may be as high as 1060. The total quantity of sulphates, phosphates, and chlorides are often increased. This is also due to the excessive destruction of nitrogenous food and tissue. The amount of sugar varies from a barely perceptible trace to 600 grammes for the 24 hours. Remember that sugar is always present in the urine, but that it is only when the quantity is sufficient to be detected by the ordinary tests that it is abnormal. The excretion of sugar in diabetes varies with the kind of food, being increased when much starchy or saccharine food is taken; but the sugar does not disappear from the urine when the food consists entirely of albuminous substances. However, the patient, who excretes 10 grammes of sugar per day, when living entirely upon animal food, is to be regarded as in a more serious condition than he who, while living upon starchy food, excretes 100 grammes of sugar per day.

QUANTITATIVE ANALYSIS OF URINE.

ESTIMATION OF UREA.

(1.) *By Liebig's Method.*—This depends upon the fact, that in neutral and alkaline solutions, mercuric nitrate precipitates urea, and that as soon as an excess of the mercury solution has been added, the excess will be shown by placing a drop of the mixture on a glass slide with a drop of carbonate of soda, when a yellow coloration will be produced.

Preparation of the Mercury Solution.—Dissolve 77.2 grammes of pure red oxide of mercury, or 71.5 grammes of the metal, in strong nitric acid. Apply heat and add more acid, if necessary, until all the mercury has been converted into *mercuric* nitrate, which will be indicated by the failure to produce a precipitate in some of the solution diluted with water on the addition of a solution of chloride of sodium; because *mercurous* chloride is insoluble, while *mercuric* chloride is soluble. Then drive off excess of acid and dilute to 1000 c. c. If on diluting, any precipitate should appear, nitric acid must be added drop by drop, sufficient to dissolve the precipitate, but avoiding any excess. Each c. c. of this solution will precipitate .01 gramme of urea.

Preparation of the Baryta Mixture.—Before the urea can be precipitated from the urine by the mercury solution, the sulphates and phosphates must be removed. This can best be done by precipitating them with a mixture of two volumes of cold, saturated solution of baric hydrate and one volume of ditto baric nitrate. This is known as the “baryta mixture.”

Application to the Urine.—To 20 c. c. of urine add 10 c. c. of the baryta mixture; filter; to 15 c. c. of the filtrate in a small beaker, add the mercuric nitrate solution, slowly from the burette, until a drop from the beaker placed on a glass slide, with a drop of a solution of sodic carbonate, turns from a white to a yellow color. Read off from the burette the amount of the mercury solution used. Each c. c. of this will indicate .01 of a gramme of urea in every 10 c. c. of urine. From this, the total amount in the twenty-four hours' urine may be calculated.

In some cases, certain errors arise from this method of estimating urea. I will not enter into detail, but will mention some of the more important errors with the best methods of avoiding them.

If the urine contains more than 10 grammes of sodic chloride in every 1000 c. c., 2 c. c. must be deducted from the number of c. c. of mercury solution used for the 10 c. c. of urine; because *that* much of the solution would be taken up by the chlorides.

If the urine contains albumen, this must be removed by heat, acetic acid and filtration.

If part of the urea has been decomposed into ammoniac carbonate, this will interfere with the estimation of the remaining urea, and must be disposed of by evaporating to dryness; when the ammonia will be driven off, and the residue may be redissolved in water; and the urea, which it contains, estimated as before. The alkalinity caused by the ammonia may be estimated with a normal acid solution, and the amount of urea calculated from this.

(2) *Estimation of Urea by conversion into Nitrogen Gas.*— Bearing in mind the objections to Liebig's method, Russell and West invented an apparatus for decomposing the urea and measuring the liberated nitrogen. This depends upon the fact, that if a solution of urea be treated with an alkaline solution of hypochlorite, or hypobromite of soda, urea is at once decomposed and nitrogen gas given off. It only remains to construct an apparatus suitable for this reaction. Many modifications have been proposed, but one of the simplest and cheapest is described by Sutton as follows: "The tube for decomposing the urine is about 9 inches long, and about half an inch inside diameter; at 2 inches from its closed end an elongated bulb is blown, leaving an orifice at its neck of three-eighths of an inch in diameter; the bulb should hold about 12 c. c.; the mouth of this tube is fixed into the bottom of a tin tray about $1\frac{3}{4}$ inches deep, which acts as a pneumatic trough; the tray is supported on legs long enough to allow of a small spirit lamp being held under the bulb tube; the measuring tube is graduated so that the amount of gas read off expresses at once what may be called the percentage amount of urea in the urine experimented upon, i. e., the number of grammes in 100 c. c., 5 c. c. being the quantity of urine taken in each case."

The *hypobromite* solution is best made by dissolving 100 grammes of caustic soda in 250 c. c. of water and adding to this, when cool, 25 c. c. of bromine. This solution must be kept in the dark, and will become unfit for use within two or three weeks under any circumstances. If only a few estimations are to be made at a time, it would be better not to make the full quantity as given above, but to take proportional parts.

Application to the Urine.—Pour 5 c. c. of the urine into the

bulb and fill up to the top of the constriction with water, in order to exclude all air; but the water must not extend much above the constriction. Take a solid glass rod as long as the bulb tube, with a piece of thin rubber tubing drawn over one end, which should fit tightly into the upper part of this constriction. Place this tube, which acts as a stopper, in position, and fill the upper part of the bulb tube with the hypobromous solution. Fill the trough half full of water. Fill the measuring tube with water, and with the thumb over its open end, invert it in the trough. If any air rises in this tube, again fill with water and invert, repeating, if necessary, until no air remains in the tube after inversion. Remove the stopper and place the open end of the measuring tube over the bulb tube. As soon as the stopper is removed, the hypobromite passes down into the bulb, coming in contact with the urine and liberating the nitrogen, which rises into the measuring tube, from which the per cent. of urea is read off.

If the urine is albuminous, the albumen should be removed by heat and acetic acid, as given under Liebig's method. The albumen effects the operation only so far as it takes a longer time for the bubbles of gas to subside, so that the per cent. may be read off.

If the urine under examination contains a great excess of urea, so that the measuring tube will not hold all the gas liberated, dilute a certain quantity of the urine with an equal bulk of water and use 5 c. c. of this solution. In this case, the amount of gas as read off, must be doubled in order to have the correct percentage.

ESTIMATION OF CHLORIDES.

(Calculated as Chloride of Sodium.)

Liebig's Method.—This depends upon the fact that, if a solution of mercuric nitrate be added to one of sodic chloride, mercuric chloride is formed and the solution remains clear. Now if urea be present it will precipitate the mercury as soon as all the chlorides have been taken up. Consequently, in estimating the chlorides in the urine with mercuric nitrate, the process is complete as soon as a permanent cloudiness appears.

Standard Solution of Mercuric Nitrate.—It is necessary

that this solution should be as pure as possible, and especially that it should not contain any silver or lead, as these would precipitate the chlorides and interfere with the test.

Take 18.42 grammes of pure red oxide of mercury, dissolve in nitric acid, converting it all into the *mercuric* salt as under urea. Any excess of acid must be avoided. Dilute to one litre. Each c. c. of this solution will take up .01 of a gramme of chloride of sodium.

Application to the Urine.—To 20 c. c. of urine add 10 c. c. of the baryta mixture (same as used in estimating urea); filter; to 15 c. c. of the filtrate neutralized or rendered feebly acid with nitric acid, add slowly from the burette the mercuric nitrate solution, until a permanent cloudiness appears. Read off from the burette the amount of this solution used. Each c. c. will indicate .01 of a gramme of chloride of sodium in each 10 c. c. of the urine; from which, the amount in the twenty-four hours' urine can be calculated.

Example: Suppose that it requires 6 c. c. of the mercurial solution to produce the cloudiness and that 1200 c. c. were passed during the twenty-four hours; then the total amount of chloride of sodium would be found by the following proportion; 10 c. c. : 1200 c. c. :: .06 grammes : X—7.20 grammes.

ESTIMATION OF SULPHURIC ACID.

(Estimated as SO_3 .)

Standard Baric Chloride.—Dissolve 30.5 grammes of pure crystallized chloride of barium in some distilled water and dilute to one litre. Each c. c. of this solution will equal .01 gramme of SO_3 . A dilute solution of sodic or magnesian sulphate will also be required.

Application.—50 c. c. of clear urine are poured into a beaker, acidified with hydrochloric acid, and heated on the sand-bath. As soon as the solution boils, the lamp is removed and the baric chloride is allowed to flow slowly from the burette into the beaker, and it must continue to flow as long as the precipitate is seen to increase. The precipitate is allowed to subside, then more of the baric chloride is added, and this process repeated until

no farther precipitate is produced. Much time and labor will be saved by filtering a few drops of the solution every now and then, and allowing these to fall into a test tube containing some of the dilute sodic or magnesian sulphate. As soon as an excess of baric chloride has been added, a precipitate will appear in the test tube. Read off from the burette the amount of baric chloride used; each c. c. of which will indicate .01 of a gramme of SO_3 in each 50 c. c. of urine, and from this the total amount may be calculated.

ESTIMATION OF PHOSPHORIC ACID.

(Estimated as P_2O_5 .)

By Uranic Acetate.—This method is based upon the fact that when a solution of acetate of uranium is added to a solution containing soluble phosphates, sodic acetate and free acetic acid, all the phosphoric acid will be precipitated as phosphate of uranium. This precipitate is of a light yellow color, insoluble in acetic, but soluble in hydrochloric acid. The point of completion of the reaction may be ascertained by placing a drop of the yellow mixture upon a piece of filter paper, which has previously been moistened with ferro-cyanide of potash and dried. As soon as there is the slightest excess of the uranic acetate, the paper will be stained *brown*, due to the formation of ferro-cyanide of uranium. The following solutions will be needed:

(1.) *Solution of Ferro-cyanide of Potash*: about one part of the salt to twenty parts of water. The test papers are to be moistened with the solution and dried; they may be kept for months and still give the color on the application of a drop of dilute uranic acetate.

(2.) *Solution of Sodic Acetate* is prepared by dissolving 100 grammes of sodic acetate in distilled water, diluting to 900 c. c., and then adding 100 c. c. of acetic acid.

(3.) *Standard Solution of Disodic Hydric Phosphate*, made by dissolving 50.4 grammes of the crystallized salt in water and diluting to one litre. Each c. c. of this solution contains .01 of a gramme of P_2O_5 .

(4.) *Uranic Acetate*.—Since this can not be obtained sufficiently pure to be weighed out and used directly, we make a

solution of it of indefinite strength and standardize it with the other solutions.

It has been found best to make the solution of uranic acetate of such a strength that each c. c. will precipitate .005 of a gramme of P_2O_5 . Now each c. c. of the sodic phosphate solution contains .01 of a gramme of P_2O_5 . Consequently, every 2 c. c. of the uranic acetate should be made equal to every 1 c. c. of the sodic phosphate: or upon adding 20 c. c. of the uranic acetate to 10 c. c. of the sodic phosphate, and then touching the paper which has been moistened with the ferro-cyanide of potash, with a drop of the mixture, we should just get the brown color.

Put 10 c. c. of the sodic phosphate with 5 c. c. of the sodic acetate solution into a beaker. To this, add slowly from the burette the uranic acetate, testing, occasionally, for the color on the paper. Suppose that on the addition of 8 c. c. from the burette, the color is obtained, then 8 c. c. of the uranic acetate are as strong as 20 c. c. should be, and for every 8 c. c. of the uranic solution that we have, 12 c. c. of water should be added. If it should require more than 20 c. c. to produce the color, the uranic solution must be concentrated by evaporation or more of the solid salt added. The solution has now been graduated.

Application.—50 c. c. of clear urine, with 5 c. c. of the sodic acetate solution, are poured into a beaker and heated; to this, the uranic acetate is slowly added from the burette. The mixture is constantly stirred with a glass rod, which should be applied frequently to the test paper. As soon as the brown color is obtained the process is completed. Read off from the burette the amount of uranic acetate solution used. Each c. c. will indicate .005 of a gramme in every 50 c. c. of urine.

ESTIMATION OF PHOSPHORIC ACID COMBINED WITH EARTHY BASES.

The method just given determines the total amount of phosphoric acid, but the physician often desires to know the amount of phosphoric acid existing as earthy phosphates. To 100 c. c. of clear urine, add ammonia to a slight alkaline reaction; set aside for 12 hours. At the expiration of this time, the earthy phosphates will have subsided; the clear fluid is decanted through a

filter, the phosphates collected on the same filter and washed with distilled water, containing a little ammonia; then dissolved in acetic acid; the solution is diluted, sodic acetate added, and the phosphoric acid estimated with the standard solution of uranic acetate. Each c. c. of the uranic acetate used will represent .005 of a gramme of phosphoric acid in each 100 c. c. of urine; from this, the amount of phosphoric acid existing in combination with the earthy bases, in the total urine for the 24 hours, may be calculated. This subtracted from the total amount of phosphoric acid in the urine will give the amount of phosphoric acid in combination with the alkaline bases.

ESTIMATION OF CALCIUM AND MAGNESIUM.

To 200 c. c. of urine add sufficient ammoniac hydrate to produce a strongly alkaline reaction. Allow this to stand for some time and then collect the precipitate, which has formed, and which consists of the earthy phosphates, upon a filter. Dissolve this precipitate in acetic acid. To this solution, add a solution of oxalate of ammonia, which throws down the calcium as an oxalate; while the magnesium remains in solution. Collect the oxalate of lime upon a filter (reserving the filtrate for the estimation of magnesium). Dissolve the oxalate of lime in dilute hot hydrochloric acid. To this solution, add a few drops of dilute sulphuric acid and then alcohol in large excess. The precipitated sulphate of lime is collected upon a filter (the filtrate being further tested by the farther addition of dilute sulphuric acid and alcohol to insure the precipitation of all the lime) dried, ignited and weighed. This gives the weight of CaSO_4 obtainable from 200 c. c. of urine; from this, the amount of calcium, in the 200 c. c. and then in the total urine for the 24 hours, may be calculated.

To the filtrate from the oxalate of lime, add ammoniac hydrate to a strongly alkaline reaction, when the magnesium is thrown down as ammonio-magnesian phosphate. This precipitate is allowed to subside, which it readily does. It is then washed by decantation with water containing a little ammonia, transferred to a platinum dish, heated to redness, cooled and weighed as the pyrophosphate

of magnesium, $Mg_2P_2O_7$. From this, the amount of magnesium in the 200 c. c. and in the 24 hours' urine may be calculated.

ESTIMATION OF URIC ACID.

—The volumetric method of estimating uric acid is open to so many objections and is, consequently, so unreliable in any but the most experienced hands, that the gravimetric only will be given here.

To 200 c. c. of urine in a beaker, add 10 c. c. of nitric acid; mix well, cover with a piece of glass, and set in a cool place for twenty-four hours; at the end of this time, uric acid crystals will be observed on the bottom and sides of the beaker. Decant the supernatant fluid through a filter paper which has been, previously, dried and weighed; or through tarred filter papers; collect the crystals on the same filter, dry at 100° and weigh. The difference between the weight of the paper alone, and that of the paper with the crystals, will be the amount of uric acid in 200 c. c. of urine. More or less coloring matter adheres to the crystals and influences the weight, causing a slight error.

ESTIMATION OF FREE ACIDS.

The acidity of the urine is, without doubt, due to several substances, among which may be mentioned acid phosphate of soda, lactic, kryptophanic, and other organic acids. This estimation is made with a solution of caustic soda, which has been graduated so as to just neutralize a standard solution of oxalic acid of 10 grammes of the pure crystallized acid, dissolved in water and diluted to one litre.

Application.—100 c. c. of urine are poured into a beaker and the standard alkali allowed to fall into this slowly, until a drop of the mixture, taken up with a fine glass rod or a feather and streaked across some delicate blue litmus paper, produces no change of color. The amount of the alkali is read off and the degree of acidity is registered as being equal to so much oxalic acid. Each c. c. of the alkali used is equivalent to .01 of a gramme of oxalic acid.

ESTIMATION OF SUGAR.

The most common method of estimating sugar is with a

solution of sulphate of copper, and is based on the fact, that if this salt be heated with a solution of tartrate of potash and soda, in sodic hydrate, no reduction occurs; but as soon as some grape sugar is added to the heated mixture, the copper is reduced to the suboxide which is deposited as a red or yellow precipitate. In pure water, the precipitate would always be red, but in the urine it has a yellow color. Many different preparations of copper for this test have been proposed, but the best, and the one almost exclusively used is Fehling's solution, which is prepared as follows:

Weigh out 34.65 grammes of pure crystallized sulphate of copper, pulverize in a mortar and dissolve in 200 c. c. of distilled water.

Dissolve caustic soda in 500 c. c. of water until the solution has a sp. g. of 1.14; then dissolve, in this solution of caustic soda, 173 grammes of crystallized Rochelle salts.

Gradually mix the two solutions, stirring with a glass rod. The mixture will have a deep blue color, and must be diluted to one litre; when 10 c. c. of it will just be decolorized by .05 of a gramme of grape sugar.

Application.—Measure into a clean porcelain dish 10 c. c. of Fehling's solution with an equal bulk of water; 10 c. c. of the urine are diluted to 100 c. c. with distilled water, and the burette filled with this solution, which is allowed to fall into the *boiling* dish of Fehling's solution until the blue color is entirely destroyed. Read off from the burette the amount used, one-tenth of which is urine, and contains .05 of a gramme of grape sugar.

For Knapp's method of estimating sugar with mercuric cyanide, see Sutton's "Volumetric Analysis." The only advantage that the mercuric cyanide has over Fehling's solution is that it will keep longer without deterioration. If the Fehling's solution is not fresh, it is always best to boil it alone for some time, and if the red or yellow precipitate is not thrown down, it is still fit for use.

ESTIMATION OF ALBUMEN.

By weight.—Pour 50 c. c. of distilled water into an evaporating dish, acidify with a drop or two, not more, of acetic acid,

place on the sand-bath and boil. To this, while boiling, add slowly 50 c. c. of the clear filtered urine. While adding this, test, frequently, the contents of the dish, with the blue litmus paper, and if the reaction is not acid, add a drop or two of acetic acid, always avoiding any excess. The albumen will be coagulated and must be collected upon a filter, which has been previously dried and weighed; the precipitated albumen is washed on the filter with distilled water (until, on evaporating a few drops of the filtrate to dryness, no residue is left) dried at 110° and weighed. Deducting from this, the weight of the filter paper, we have the amount of albumen in 50 c. c. of the urine, and from this the amount in the twenty-four hours urine may be calculated.

Clinical Method.—The physician often does not care to know how many grammes of albumen his patient passes in twenty-four hours; but he is anxious to know whether the daily amount is on the increase or decrease. He desires to find out the proportion between the amount passed on one day and that passed on another. For this purpose the following method is applicable.

Dilute the twenty-four hours' urine to 3000 c. c. Precipitate a definite part of this, in a test tube, with heat and a few drops of nitric acid: allow the coagulum to completely subside, and mark the tube so as to indicate the bulk of albumen, or if the next estimation is to be made within a few days, leave the albumen in the tube with the supernatant fluid. When the next estimation is made, proceed exactly in the same manner; diluting the twenty-four hours' urine to the same quantity; taking the same definite part of this and precipitating in the same tube, or in one of the same size. By a comparison of the bulk of coagula obtained in the two instances, it is easily ascertained whether the albumen has increased or decreased in amount.

THE RESULTS OF A QUANTITATIVE ANALYSIS.

After a quantitative analysis of a specimen of urine has been made, it is desirable to present the results in some compact form. For the analysis, the 24 hours' urine should be obtained

and measured as has been directed (see p. 154 et seq.). The specific gravity of the mixed urine should be ascertained and the total weight of the urine and of the solids calculated according to the rules given on pages 173 and 174. Then each of the constituents should be estimated.

The results of the analysis may be represented as in the table on the following page. It is well to represent the quantity of urine in terms of both the French and English measures. In this, 30 c. c. are considered as equivalent to one fluid ounce; this, it is true, is not the exact equivalent, but since the amount in cubic centimetres is exact, that in ounces is only used as an indication of the approximate equivalent in the English measure. The total weight of the urine, solids and each constituent is also represented in both grammes and grains; in this, it is considered that 15.43 grains are equivalent to 1 gramme.

A person weighing 200 lbs. will probably consume more nitrogenous food and excrete more urea than one, in the same state of health, who weighs but 100 lbs. Consequently, one column is given showing the proportion in grammes per kilogramme, and another showing the proportion in grains per pound of the body weight. One kilogramme is considered as the equivalent of 2.2 pounds.

In the sample table for exhibiting the results of a quantitative analysis, all of the constituents of the urine are not given; but if others are to be added, they are to be reported as those given in the table. The greatest care should be used in this quantitative work. Remember that the man, who is not conscientious, neat and exact in all his work, is not fit for a chemist nor a physiologist, and all analyses made without due care will be of no value. If one goes through the work in an awkward manner, and then *guesses* that it is about right, such a person should have guessed at first and not disgraced the work which he pretends to do. Every thing should be done with that accuracy required by a *scientific* conscience.

SPECIMEN TABLE FOR REPORTING THE RESULTS OF A QUANTITATIVE ANALYSIS.

	C. C.	Ounces.	Specific gravity.	Total grammes.	Total grains.	Grammes per kilo., body weight.	Grains per lb., body weight.
Urine	900	30	1025	922.5	14234	14.2	99.6
Total Solids.....				58.25	898.80	.896	6.285
Urea.....				20	308.6	.31	2.16
Total Phosphoric acid (P_2O_5).....				3	46.29	.046	.324
Phosphoric acid combined with earthy bases				1	15.43	.0154	.108
Phosphoric acid combined with alkaline bases				2	30.86	.0306	.216
Chlorides (NaCl).....				6.5	100.295	.1	.701
Sulphuric acid.....				2.2	33.946	.034	.237
Uric acid.....				.4	6.172	.006	.043

PRELIMINARY EXAMINATION OF URINE.

Before the student begins to analyze specimens of urine for diagnostic purposes, he should become perfectly familiar with the reactions of normal urine. Moreover, it will be necessary for him to be able to recognize those substances which may be present in the urine from accidental causes, also to prepare and study both the normal and abnormal constituents of the urine. The student will find it to his advantage to add sugar and other abnormal constituents to normal urine, and then test for them; in this way, should he study as thoroughly as possible every substance which may possibly be present in the urine. If one does this work well, he will have no difficulty in making analyses of the urine of his patients.

ACCIDENTAL CONSTITUENTS.

(1.) Examine under the microscope all of the most common starches; as wheat, corn, potato, rice, arrow-root, tapioca, and sago.

(2.) Also examine hair, cotton and woolen fibres, bits of
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feathers, pieces of pine shavings and striated muscular fibre.

(3.) Take some saliva from the mouth and examine under the microscope for epithelial scales and salivary corpuscles.

BEHAVIOR OF NORMAL URINE WITH ORDINARY REAGENTS.

(1.) Heat some normal urine in a test tube ; if it be strongly acid, no change occurs ; if it be but feebly acid, the phosphate of lime will be precipitated and may be redissolved by the addition of a drop of nitric acid.

(2.) Heat some normal urine with nitric, hydrochloric, or acetic acid ; observe that a peculiar odor is given off, and that the color of the urine becomes darker.

(3.) To some normal urine, add ammoniac hydrate, when ammonio-magnesian and calcic phosphates will be precipitated. Allow the precipitate to subside and then examine it microscopically, when prismatic or stellate crystals of triple phosphates will be observed. This deposit is soluble in acetic and the mineral acids.

(4.) Render normal urine alkaline by the addition of either sodic or potassic hydrate ; an amorphous precipitate of the phosphates of calcium and magnesium will fall and will be found soluble in acetic and the mineral acids.

(5.) Add nitrate of silver to some urine acidified with nitric acid, when a precipitate of the chloride of silver forms. This precipitate is amorphous, and insoluble in nitric acid, soluble in ammonia (see p. 220).

(6.) To some urine acidified with hydrochloric acid, add chloride of barium, when an amorphous precipitate of the sulphate of barium falls. This precipitate is insoluble in acids (see p. 213).

(7.) To normal urine, add either uranic acetate or ferric chloride, when a yellowish-white precipitate of the phosphate of uranium or of iron forms : either of these will be found insoluble in acetic acid, soluble in hydrochloric acid.

(8.) Add to normal urine, oxalic acid, or ammoniac oxalate when calcic oxalate will be precipitated (see p. 225).

(9.) To normal urine, add absolute alcohol or chloroform,

when a faint cloudiness is produced either immediately or after standing for some time. This precipitate consists of a kind of albumen normal to the urine (see p. 243) and disappears on the addition of water.

(10.) The addition of mercuric nitrate to urine produces a precipitate. On the addition of the first few drops of the mercury solution, a precipitate forms and soon redissolves; while on farther addition of mercuric nitrate, a permanent precipitate forms. As the mercury solution first falls into the urine, it unites with the urea forming a precipitate which is immediately decomposed by the chlorides present, forming mercuric chloride. As soon as all the chlorides have been taken up, then any further precipitate formed by the combination of mercury and urea remains undissolved.

UREA.

(1.) Prepare crystals of pure urea from the urine (see p. 177).

(2.) Prepare and study the crystals of nitrate of urea, as obtained from the urine (see p. 179).

(3.) Obtain the crystals of oxalate of urea from either the urine or from an aqueous solution of urea which has been prepared artificially (see p. 179).

URIC ACID.

(1.) Prepare uric acid from human urine and study the forms of the crystals and their solubility in various reagents (see p. 190).

(2.) Prepare uric acid from either the urine of serpents or from guano (see pages 189 and 190).

(3.) Prepare crystals of alloxan and of nitrate of urea from uric acid (see p. 191).

(4.) Make the murexide test with some uric acid (see page 192).

(5.) Prepare allantoin from uric acid (see pages 192 and 193).

(6.) Prepare and study the acid urates of soda, potash, ammonia, and lime (see pages 193 and 194).

HIPURIC ACID.

(1.) Prepare hippuric acid from the urine of the horse (see pages 198 and 199).

(2.) Take a dose of benzoic acid at night and test the urine passed on rising next morning for hippuric acid (see page 200); or eat greengages and collect the urine passed during the next 24 hours and examine it for hippuric acid (see p. 201).

PHOSPHATES.

(1.) To some normal urine, add ammoniac hydrate; allow the precipitate, which forms, to subside and examine it under the microscope, when stellate or pennate crystals of ammonio-magnesian phosphate will be observed (see p. 205).

(2.) Set some normal urine aside until the urea gradually decomposes and the urine becomes alkaline, then examine the deposit under the microscope and observe the prismatic crystals of ammonio-magnesian phosphate (see p. 205).

(3.) To some normal urine, add potassic or sodic hydrate sufficient to produce an alkaline reaction, when an amorphous precipitate of the phosphates of calcium and magnesium will be thrown down and will be found soluble in acetic and the mineral acids (see pages 206 and 207).

(4.) Prepare crystals of the acid phosphate of lime (see page 207).

(5.) Separate the earthy from the alkaline phosphates and precipitate the phosphoric acid of the latter as ammonio-magnesian phosphate (see p. 208).

(6.) Obtain sodic phosphate, Na_2HPO_4 , from the urine (see p. 208).

(7.) Prepare crystals of the acid phosphate of sodium from the urine. Also prepare the same artificially (see p. 208).

SULPHATES.

(1.) To normal urine, acidified with hydrochloric acid, add chloride of barium, when a white, amorphous precipitate of the sulphate of barium will be thrown down and will be found insoluble in acids (see p. 213).

CYSTINE.

(1.) Examine a prepared specimen of cystine under the microscope, studying its crystalline form.

CHLORIDES.

(1.) To some normal urine, acidified with nitric acid, add a few drops of a solution of nitrate of silver. The chloride of silver is precipitated and should be tested as recommended on page 220.

(2.) Prepare crystals of chloride of sodium from the urine (see pages 220 and 221).

OXALATES.

(1.) Prepare and study the crystals of oxalate of lime (see p. 225).

(2.) Set some normal urine aside and examine it from day to day, and note the length of time elapsing between the emission of the urine and the appearance of the crystals of calcic oxalate.

XANTHINE.

(1.) Prepare xanthine from muscular tissue according to the method of Stædeler (see pages 234 and 235).

(2.) Prepare the precipitates of xanthine with nitrate of silver (see p. 234).

GUANINE.

(1.) Prepare guanine from Peruvian guano (see p. 239.)

ALBUMEN.

(1.) Obtain egg-albumen by beating the whites of eggs with a glass rod, then adding an equal volume of water and filtering through cloth. This albumen is by no means pure, but may be used for the purpose of becoming familiar with the reactions of albuminous urine.

(2.) To normal urine, add some of the albumen, prepared as above, and apply the heat and nitric acid test. The student should add the albumen in various proportions and become acquainted with the limits of the reaction. (For the method of applying the tests for albumen, see pages 242 and 243.)

BLOOD.

(1.) Take a drop of blood from the finger, place it on a glass slide, add a drop of urine, cover with the thin glass, and examine under the microscope.

(2.) Obtain a greater quantity of blood from a vein or from a cat or dog, and add it in varying proportions to the urine, and then apply the tests. Examine under the microscope, also by means of the spectroscope, and also apply Heller's test for blood pigment (see pages 244 and 245). Also test for albumen in the urine which contains blood.

PUS.

(1.) Obtain pus from some suppurating wound, and examine it under the microscope. The addition of dilute acetic acid to the pus corpuscle will bring out from one to five, generally three, nuclei. This test with acetic acid is seldom of any value in the identification of pus in the urine; consequently, the student must become perfectly familiar with the appearance of the pus corpuscle. It should be added to the urine in varying proportions, the urine set aside for a while in order to allow the pus to subside, and then examined under the microscope. It is quite necessary that the student should become expert in the detection of minute traces of pus. Urine containing pus is always albuminous; this is necessarily true; because, the liquor puris contains albumen; but it must be remembered that the quantity of albumen due to traces of pus may not be detected. The test for pus by means of the microscope is much more delicate than the test for albumen. Consequently, if no albumen be found by the ordinary test, this is not proof sufficient of the absence of pus. This question will often arise, is there more albumen in a specimen of urine, than can be accounted for by the pus or blood present? This can only be answered from the experience and judgment of the analyst. Consequently, the student should add various quantities of pus and blood to normal urine, then compare the abundance of the corpuscles in the deposit, with the bulk of albumen thrown down by heat and nitric acid.

EPITHELIUM.

(1.) Kill a cat or a dog, remove the urinary organs and examine the epithelium from the various parts.

SUGAR.

(1.) Dissolve some grape sugar in water (if grape sugar can not be obtained, a substitute may be obtained by dissolving cane sugar in water, acidifying the solution strongly with either hydrochloric or sulphuric acid, and boiling for a few minutes. This solution, when neutralized, readily reduces the copper of Fehling's solution) and apply all of the tests given for sugar (see page 266 et seq.) Then add the solution of grape sugar to normal urine and apply all of the tests for sugar ascertaining the delicacy of each.

(2.) To normal urine, add a little sugar and much albumen and test for the former (see pages 267 and 268).

INDIGOGEN.

(1.) Prepare indigo from the urine of the horse according to the method of Jaffe (see p. 258).

(2.) Prepare pure indigo-blue from the indigo of commerce (see p. 260.)

UROBILIN.

(1.) Prepare urobilin from the highly colored urine of a fever patient.

CHOLESTERINE.

(1.) Prepare cholesterine from human gall-stones (see p. 46.)

(2.) Apply the various tests given for cholesterine on page 47.

BILE.

(1.) Dilute some ox-bile (obtained from the slaughter-house) with an equal volume of water, filter and apply Pettenkoffer's test for bile-acids (see p. 34.) To various dilutions of the bile with water, apply the same test.

(2.) To urine, add ox-bile in various proportions and apply Pettenkoffer's test.

(3.) To some human, or dog-bile apply Gmelin's test for bile-pigment (see p. 48.)

(4.) To urine containing bile, apply Hoppe-Seyler's modification of Gmelin's test (see p. 48.)

(5.) To the urine of a jaundiced patient, apply the following modification of Gmelin's test: Warm 100 c. c. of the urine, and render it feebly alkaline with baric hydrate. Collect the precipitate, which forms, upon a filter, and dry it. Place a small piece of the dried precipitate in a clean porcelain dish and add a drop of nitrous (fuming nitric) acid, when the series of colors of Gmelin's test will be developed.

(6.) To some normal urine, add ox-bile and apply the modification of Pettenkoffler's test as given on page 35.

TYROSINE AND LEUCINE.

(1.) Prepare tyrosine and leucine from horn or hair as directed on pages 110 and 111.

(2.) To normal urine, add tyrosine and leucine. Concentrate the urine on the water-bath; allow the syrup to cool, and examine it with the microscope. Tyrosine will be found crystallized in needles, which are readily soluble in ammoniac hydrate. The leucine appears in brownish discs or globules.

CREATINE AND CREATININE.

(1.) Prepare creatine as given on page 132, and study the form and solubility of the crystals.

(2.) Prepare creatinine from creatine (see p. 133.)

(3.) Obtain creatinine from the urine according to the method of Neubauer (see pages 134 and 135.)

INOSITE.

(1) Prepare inosite from muscle as recommended on pages 138 and 139.

OIL.

(1) To some urine, add a drop of milk, or of an emulsion, shake the urine with ether; allow the ethereal layer, which contains the oil, to separate. By means of a pipette, place a few drops of the ether upon a glass slide. Allow the ether to evaporate; add a drop of water to the residue and examine under the

microscope for oil globules. These must not be confounded with air bubbles.

EXAMINATION OF URINE SUSPECTED TO BE ABNORMAL.

Collect the urine for the 24 hours, mix and measure it. Ascertain the specific gravity and reaction. Set a portion aside in a clean glass vessel (better, a conical one) and allow the deposit to subside for microscopical examination, as given in the following tables (A,) (B,) and (C.) Filter another portion and test the clear filtrate according to table (D.)

MICROSCOPICAL EXAMINATION OF URINARY DEPOSITS.

Allow the urine to stand in a glass vessel undisturbed for some time; then by means of a small pipette or dipping rod, take a drop from the bottom of the vessel; place the drop on a glass slide, cover with a thin glass and examine under a microscope which magnifies from 300 to 500 diameters. The objects seen under the microscope may be either crystallized, amorphous, or anatomical. The same substance may appear at one time in crystals, and at another in the amorphous form, and may thus indicate different pathological results; consequently, the following tables are given:

(A.) CRYSTALS ARE FOUND IN THE DEPOSIT.

Name.	Reaction of the urine in which the deposit occurs.	Form, or forms of the crystals.	Chemical Tests.	Pathological Indications.
Ammonio-magnesianic phosphate.	Ammoniacal.	(1.) Stellate or pennate.	Soluble in acetic acid.	The stellate and pennate forms have no pathological import. They can result only from the immediate addition of ammonia to the urine, and if present, they indicate the addition, either intentional or accidental, of ammonia to the urine after emission (see p. 205).
Acid phosphate of lime.	Needle-shaped, prismatic, and rhombic.	(2.) Prismatic.	Soluble in acetic acid. The needle-shaped and rhombic varieties are to be distinguished from uric acid by the absence of coloring matter, and from both uric and hippuric acids by the ready solubility of the phosphate in hydrochloric acid. The prismatic form can not be distinguished by optical examination from the corresponding form of the triple phosphates, but the triple phosphate occurs as a deposit only in ammoniacal urine, while this form of calcic phosphate is deposited only in acid urine.	The prismatic form of the triple phosphates indicates that the urine has gradually become ammoniacal from the decomposition of urea. This decomposition may have taken place in the body, which occurs in retention and in inflammation of the bladder. If the decomposition has taken place outside of the body, the crystals will, of course, be indicative of no pathological condition (see pages 169, 170, 171, 205). The appearance of these crystals in the urine is probably due to an excess of acids over the bases in the blood. This salt not unfrequently is deposited in the kidney and bladder, forming calculi. Hassall has made a study of this substance and finds that deposits of crystallized phosphate of lime are of frequent occurrence and that they are of greater pathological importance than triple phosphates.

<p>Oxalate of lime. Acid, rarely neutral, or alkaline.</p>	<p>Octohedrons, diamonds, dumb-bells, and discs.</p>	<p>Insoluble in acetic acid and alkalis, soluble in hydrochloric acid.</p>	<p>If oxalate of lime be found in the urine, within 48 hours after emission, it shows that there is an excess of oxalic acid in the system. This may arise from the kind of food, or from imperfect oxidation. Oxalates frequently form calculi, and more frequently lead to structural disease of the kidney. For further particulars concerning the pathology of oxalate of lime, see p. 227 et seq.</p>
<p>Acid, rarely neutral, or alkaline.</p>	<p>In a great variety of forms, all of which are modifications of the rhombic plate. The crystals are generally more or less colored.</p>	<p>Insoluble in dilute acids, soluble in alkalis. Collect the crystals either by filtration or decantation, wash them with alcohol and apply the <i>muresside</i> test (see p. 192).</p>	<p>Free uric acid may be deposited from either of the following causes: (1) the urine may be unduly acid, the stronger acids taking up the bases and setting the uric acid free; (2) there may be an absolute excess of uric acid formed, so that the normal amount of bases is not sufficient to take up all the acid; (3) the proportion of alkaline bases may be abnormally small. If a deposit of uric acid be due to the first and third of these causes, the treatment should consist in the administration of alkalis; if due to the second cause, acid tonics and other oxidizing agents should be used (see p. 196, et seq.).</p>
<p>Acid, the urate of ammonia is not unfrequently found in ammoniacal urine.</p>	<p>Urates are seldom in crystals; the urates of ammonia and lime form needles, often arranged in bundles and balls.</p>	<p>Disappear on the application of heat. Soluble in caustic potash and soda; are decomposed on the addition of an acid with the formation of crystals of free uric acid; collect the deposit by either filtration or decantation, wash with a little alcohol, remove to a clean porcelain dish and</p>	<p>Urates are the most common constituents of urinary deposits. They vary in color, from white to crimson; the higher the color, the more serious the indication. An occasional deposit of urates may appear from very trivial causes, as from a change in diet, in the amount of exercise taken, and in the temperature. A deposit of urates occurring in acute inflammatory diseases is an indication for the better,</p>

(A.) CRYSTALS ARE FOUND IN THE DEPOSIT.—Continued.

Name.	Reaction of the urine in which the deposit occurs.	Form, or forms of the crystals.	Chemical Tests.	Pathological Indications.
Carbonate of lime.	Neutral, alkaline, or feebly acid.	Dumb-bells and discs.	<p>apply the murexide test (see p. 192).</p> <p>Soluble in acetic acid with effervescence. The dumb-bells and discs of the carbonate are distinguished from those of oxalate of lime, by the insolubility of the latter in acetic acid.</p>	<p>showing that so much of the poison has been eliminated. But a constantly recurring deposit of urates is to be regarded as indicative of some disease of the heart, liver, lungs, or spleen. (See p. 197, et seq.)</p> <p>This substance is seldom found in the urine of man; but is a constant constituent of the urine of some herbivorous animals. When present in human urine, it is due either to the addition of some carbonate to the urine after emission, or to the decomposition of the urea either in the body, or after emission. The decomposition of the urea yields carbonate of ammonia, the carbonic acid of which unites with the lime.</p>
Tyrosine.	Acid, neutral or alkaline.	In needles often arranged in bundles.	<p>Freely soluble in ammoniac hydrate. The crystals of tyrosine resemble those of sulphate of lime, but the latter are insoluble in ammonia.</p>	<p>Tyrosine is found in the urine in cases of serious disorder of the liver, having been especially observed in the so-called yellow atrophy of this organ. It has also been observed in typhus and typhoid fevers and in small-pox.</p>
Leucine.	Acid, neutral or alkaline.	In brownish discs or globules.	<p>The discs and balls of leucine resemble urates from which they are distinguished by the weak refractive power of the leucine. They may also</p>	<p>Leucine occurs frequently with tyrosine; but since the former is more freely soluble in the urine, it is seldom obtained only after concentration.</p>

Cystine.	Acid, neutral, or alkaline.	Six-sided plates.	be mistaken for oil globules, but the leucine is insoluble in ether. Freely soluble in ammonia, insoluble in carbonate of ammonia; soluble in the mineral acids and in oxalic acid, insoluble in acetic and tartaric acids.	The presence of cystine in the urine is due to the imperfect oxidation of the organic sulphur-containing constituents of the food. The quantity of cystine excreted in a case of cystinuria varies with the kind of food and with the amount of oxygen which the patient receives. Nitro-muriatic acid and other oxidizing agents are indicated (see p. 217, et seq.).
Xanthine.	Acid.	In small oval crystals.	Soluble in ammonia; also soluble in strong sulphuric acid, from which it is not precipitated on dilution and thus distinguished from uric acid.	Denotes an imperfect degree of oxidation of the nitrogenous constituents of food and tissue. Xanthine is especially abundant in the blood and urine in leucocytæmia (see p. 236).
Cholesterine.	Acid, alkaline, or neutral.	In large rhombic plates, clear and thin, and with a characteristic notch in one corner.	Soluble in boiling alcohol, from which it crystallizes on cooling. Cholesterine is sometimes found in the urine in globules resembling oil, when it should be collected and dissolved in boiling alcohol, and allowed to crystallize.	Cholesterine forms the principal constituent of biliary calculi; it has been found in the urine in cases of obstruction of the bile-duct, and in fatty degeneration of the kidney.
Sulphate of lime.	Acid.	In fine needles resembling tyrosine.	The needles of sulphate of lime dissolve on the addition of an excess of water. They are distinguished from those of tyrosine by the insolubility of the sulphate in ammonia.	Crystals of sulphate of lime have been found spontaneously deposited in human urine in a single case (Valentiner Med. Centralbl. S. 913). These crystals may be obtained from the urine of the horse. (See p. 213.)
Hippuric acid.	Acid.	In needles or rhombic prisms.	Easily dissolves on the application of heat.	A deposit of hippuric acid indicates an excess of this constituent, and is generally due to the kind of food.

(B.) AMORPHOUS SUBSTANCES ARE IN THE DEPOSIT.

Name.	Reaction of the urine in which the deposit occurs.	Chemical Tests.	Pathological Indications.
Phosphates of lime and magnesium.	Alkaline.	Soluble in acetic acid, insoluble in alkalis; distinguished from amorphous phosphates by the fact that the phosphates do not disappear on the application of heat, and by the failure to obtain crystals of free uric acid by the addition of acetic acid to the phosphates, also by the failure of the deposit of phosphates to respond to the murexide test; distinguished from amorphous oxalates by the insolubility of the latter in acetic acid.	If the deposit consists wholly of the amorphous phosphates of lime and magnesium, the urine is alkaline from a fixed alkali. This deposit often occurs in the urine soon after a meal, and is then due to the excess of alkalis in the food. However, if such a deposit constantly appears in the urine, it indicates an excess of bases over the acids in the blood. Urine constantly alkaline from a fixed alkali denotes a low state of vitality. In the treatment of these cases, two objects may be kept in view. These are (1) to relieve as speedily as possible any irritation of the bladder, and (2) to increase the vitality of the patient, and in this way to remove the cause of alkalinity (see p. 168).
Oxalate of lime.	Acid, rarely neutral or alkaline.	Insoluble in acetic acid, soluble in hydrochloric acid; distinguished from amorphous phosphates by the solubility of the latter in acetic acid; distinguished from amorphous urates by the solubility of the urates in alkalis and on being heated, also by the failure of the oxalates to respond to the murexide test and to yield crystals of free uric acid on being treated with acetic acid.	Amorphous oxalates are very seldom observed; they may arise either from the addition of oxalic acid to the urine after emission, or from the administration of large quantities of oxalic acid (see p. 226).
Urates.	Acid, the urate of	Determine the presence of urates as in	Urates are the most common constitu-

ammonia is not un- (A). It now remains to determine the elements of urinary deposits. They vary in frequently found in base with which the uric acid is com- color from white to crimson: the higher ammoniacal urine. bined; collect the urates upon a filter; the color the more serious the indication. place some of this collected deposit upon An occasional deposit of urates may ap- a piece of platinum and heat in the flame pear from very trivial causes: as from a of a Bunsen burner or spirit lamp; (1) it change in diet, in the amount of exercise communicates an intense yellow color to taken and in the temperature. A deposit the flame, *sodium urate*: (2) it imparts a of urates occurring in acute inflammatory violet color to the flame, *potassium urate*. diseases is an indication for the better, Remove another portion of the collected showing that so much of the poison has deposit to a clean porcelain dish, add a been eliminated. But a constantly recur- little potassic hydrate and then heat; if ring deposit of urates is to be regarded the vapor of ammonia is given off, and as indicative of some disease of the heart, colors a red litmus paper held over the liver, lungs, or spleen. (See p. 197, et dish, the deposit contains *ammonium* seq.) *urate*.

(C) ANATOMICAL OR FORMED CONSTITUENTS ARE FOUND.

Name.	Reaction of the urine in which the deposit occurs.	Form.	Means of Detecting.	Pathological Indications.
Pus.	Acid, neutral, or alkaline.	In corpuscles.	By microscopic appearance.	The presence of pus shows undue inflammation. If the pus be from the bladder, it will generally contain much mucus, giving the deposit aropy consistency, and the urine will frequently be alkaline from a volatile alkali. In suppurative cystitis, the greater part of the pus and mucus will be passed after the water; while in pyelitis the pus will be distributed through the urine, which will generally be of an acid reaction. In urethritis, the first urine passed will contain all, or the greater part of the pus. (See page 245.)
Blood.	Acid, alkaline, or neutral.	In corpuscles, or disintegrated.	By the microscopical detection of the corpuscles, or by spectroscopical examination, or by Heller's test for blood-pigments. See page 245.	The presence of blood in the urine may be due either to a physiological (as in menstruation) or to a pathological hæmorrhage. If there be clots large enough to be visible to the unaided eye, the blood must have passed into the urine below the secreting structures. If from the bladder the clots will often be quite large, and may obstruct the passage through the urethra. If the coagulation has taken place in the ureters, the shape and size of the clots will so indicate. When from the pelvis of the kidney, the coagula are much smaller than those from the bladder, and may preserve the shape of the calices. If from the substance of the kidney only, the clots will be

<p>Epithelium. Acid, alkaline, or neutral.</p>	<p>Pavement and columnar.</p>	<p>By microscopical examination.</p>	<p>microscopic in size, having been formed in the tubules, and the urine will generally have a smoky tint. (See page 244.)</p> <p>(1) Epithelium from the uriniferous tubules consists of small circular pieces, with a large nucleus in the center.</p> <p>(2) Pieces from the pelvis of the kidney are triangular and polyhedral, thicker than, but not so large as, those from the bladder and vagina.</p> <p>(3) From the ureters, the pieces are conical or triangular, with a nucleus near the base; they are smaller than those from the urethra.</p> <p>(4) From the bladder, large and small spheroidal pieces, the small ones more nearly circular, the larger ones are polyhedral, and often elongated at one corner.</p> <p>(5) From the urethra (male) columnar, conical, with a nucleus near the base.</p> <p>(6) Vaginal epithelium is pavement, polyhedral, more oval than those from the bladder. If the pieces of epithelium are normal in appearance but unduly abundant, only an excessive desquamation is indicated; but if they contain globules of oil, or give the amyloid reaction (see p. 256) the organ is undergoing degeneration.</p>
<p>Caests. Acid, neutral or alkaline.</p>	<p>In cylinders.</p>	<p>Examine the deposit under a microscope which presents a clear field.</p>	<p>(1) <i>Hyaline</i> casts are smooth, structureless, and may be detected by the addition of a solution of iodine in iodide of potash, or of a dilute solution of carmine, when they will be stained yellow or red, respectively. These casts are formed by the coagulation of albumen in</p>

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(C) ANATOMICAL OR FORMED CONSTITUENTS ARE FOUND—Continued.

Name.	Reaction of the urine in which the deposit occurs.	Form.	Means of Detecting.	Pathological Indications.
Mucus.	Acid, neutral or alkaline.	In corpuscles and in a gelatinous mass.	There is no difference between mucus and pus corpuscles. Mucus is gelatinous andropy.	<p>the uriniferous tubules, and simply indicate albuminuria.</p> <p>(2) <i>Epithelial</i> casts are cylinders or pipes, formed by the removal of the epithelia of the tubules in mass. They are caused by inflammation of the mucous membrane of the kidney.</p> <p>(3) <i>Granular</i> casts consist of masses of aborted epithelia, differing from the epithelial casts in the fact that the individual cells are not fully developed. They indicate a more inflamed state of the mucous membrane of the kidney than is indicated by the epithelial casts.</p> <p>(4) <i>Bloody</i> casts consist of coagulated albumen with blood corpuscles entangled, and are formed in hematuria.</p> <p>(5) <i>Waxy</i> casts have the appearance presented by melting a piece of wax, dropping it upon a glass slide, and allowing it to cool. They are formed by an abnormal secretion from the kidney, and indicate a more serious condition of this organ than is indicated by any other form of casts.</p> <p>An excess of mucus shows undue irritation of some part of the urinary tract. The source of the mucus is to be ascertained as given under pus.</p>

Spermatozoa.	Acid, neutral, or alkaline.	About .045 of a millimetre long, with one extremity enlarged, flattened, triangular, and termed the 'head.' The other extremity the 'tail,' is long and slender.	Examine under a microscope which magnifies at least 300 diameters.	Their occasional appearance is of little or no importance; but their continued or frequent presence shows that there is something either mental or physical which unduly excites the glands.
Oil.	Acid, neutral, or alkaline.	In globules, either floating through the fluid, or contained in casts and epithelium.	Agitate some urine in a test tube with ether; remove some of the ether and place on a glass slide, allow the ether to evaporate, add a drop of water to the residue, cover with a thin glass and examine under the microscope for oil globules.	Oil is often accidentally present in the urine from bottles not thoroughly cleansed, use of catheter, etc. Casts and epithelium containing oil globules are found in fatty degeneration of the kidney.
Sarcina Venetriculi. (Merismopediæ punctata.)	Acid, neutral, or alkaline.	Appear as small cubical moving masses. They often appear greenish or brownish. In small oval spores which may be found single or attached to each other.	By microscopical examination; they are distinguished from vibrios by the difference in shape. By microscopical examination. Some times one spore will be observed just budding out from another.	These algæ may be so abundant in the urine as to cause a visible grayish deposit. They have been found in catarrh of the bladder.
Torulæ.	Acid.			These fungi appear only in urine which contains sugar; and show that the sugar is undergoing the process of fermentation.
Penicillium.	Acid.	In spores, and interlacing branches which are seen to be composed of separate parts.	By microscopical examination. The separate spores cannot be distinguished from the torulæ; but the thallus of the well-developed penicillium differs from that of the sugar fungus.	These fungi may appear in any acid urine on standing. It was formerly supposed that albumen was necessary for their development; but this theory has been proven to be false. However, they are more likely to occur in urine which contains either albumen or sugar than in any other.
Vibrios.	Acid, neutral, or alkaline.	Small moving threads, or filaments.	By microscopical examination. These little bodies differ greatly in length and in other respects.	They arise from the decomposition of organic matter, and may be formed in the bladder in cystitis.

(D.) EXAMINATION OF THE CLEAR FILTRATE.

Name.	Reaction of the urine in which the substance occurs.	Influence of the constituent upon the physical properties of the urine.	Chemical Tests.	Pathological Indications.
Urea.	Generally strongly acid when there is an excess of urea; is an excess of urea; and feebly acid, neutral, or alkaline when there is a deficiency of urea.	When there is an excess of urea, the specific gravity is high and the coloring deep; when there is a deficiency of urea the specific gravity is low and the coloring is light unless there be some abnormal coloring matter present.	The absolute amount of urea excreted in a given time can be ascertained only by a quantitative analysis. (See p. 273.) Whether the urea is in excess or deficient in proportion to the water may be ascertained by the following tests: <i>Test for excess.</i> —Place a drop of urine on a glass slide and add a drop of nitric acid; leave in a cool place. If within 5 minutes an abundant crop of crystals of nitrate of urea appear, the urea is in excess. <i>For deficiency.</i> —Evaporate some urine to half its bulk. Take a drop and proceed as in excess. If no crystals are formed in 5 minutes, there is deficiency of urea.	There is an excess of urea in most febrile diseases and in diabetes. The increase of urea in fever is generally in exact proportion with the temperature of the body. (See p. 186.)
Chlorides.	Acid, neutral, or alkaline.	A deficiency of chlorides may appear in either pale or highly colored urine; or the specific gravity may be	Whether there be a deficiency of chlorides or not can be ascertained only by a quantitative analysis. (See p. 276.)	With regard to chlorides, we may say that an excretion of less than one gramme for the 24 hours is abnormal. In pneumonia, typhus fever, acute rheumatism and erysipelas, common

Phosphates.	<p>Acid, neutral, or alkaline.</p>	<p>either high or low.</p>	<p>salt is diminished and frequently is not found at all in the urine. (See p. 221, et seq.)</p>
	<p>No characteristic effect upon the physical properties of the urine.</p>	<p>Whether there be an excess or deficiency of phosphates can be ascertained only by a quantitative analysis. (See p. 278.)</p>	<p>There is an excess of phosphates in the urine in inflammatory diseases of the nervous system and in rickets and osteomalacia. There is deficiency in indigestion and in structural diseases of the kidney. (See p. 211.)</p>
Sulphates.	<p>Urine with an excess of sulphates is generally acid.</p>	<p>Urine containing an excess of sulphates is of high specific gravity.</p>	<p>An increase of sulphates arises either from an excess of sulphates in the food, or from the use of medicinal sulphates. Otherwise, sulphates vary with the degree of oxidation and are increased in febrile diseases and decreased in skin diseases and in all cases of imperfect oxidation. (See p. 214, et seq.)</p>
Carbonates.	<p>Generally ammoniacal.</p>	<p>There is always a deposit in urine which contains carbonates in any appreciable quantity.</p>	<p>Ammonic carbonate results from the decomposition of urea.</p>
Leucine and tyrosine.	<p>Acid, neutral, or alkaline.</p>	<p>Urine containing these constituents is frequently colored with bile.</p>	<p>Leucine and tyrosine occur in the urine in severe structural diseases of the liver,—see table (A).</p>
Cystine.	<p>Acid, neutral, or alkaline.</p>	<p>Fresh urine containing cystine has a sweet-briar odor, when decomposed with ammonia.</p>	<p>Cystine results from the imperfect oxidation of the sulphur which exists in organic combination in the food, as in eggs and</p>

(D.) EXAMINATION OF THE CLEAR FILTRATE, — Continued.

Name.	Reaction of the urine in which the substance occurs.	Influence of the constituent upon the physical properties of the urine.	Chemical Tests.	Pathological Indications.
Blood.	Acid, neutral, or alkaline.	Urine containing much blood is more or less colored. It may be blood-red, or smoky, or even black.	To the solution apply Heller's test for blood-pigment (see p. 245). Also examine with the spectroscope.	See table (C). If the blood be from the kidney, the urine will be smoky or dark; while if the blood be from the bladder, or the urethra, the color of the urine will be red.
Bile.	Generally acid, though it may be neutral or alkaline.	Dark-red or greenish.	Apply Fetteskoffler's test for bile-acids (see p. 34), and Gmelin's test for bile-pigments (see p. 48).	Bile appears in the urine in obstruction of the bile-duct, and in excessive formation of bile.
Urobilin.	Generally acid.	Highly-colored.	Examine with the spectroscope (see p. 265).	Urobilin is increased in all febrile diseases and causes the high color of the urine of fever patients.
Indigogen.	Acid, neutral, or alkaline.	The urine may be highly colored or pale.	To from 4 to 6 c. c. of strong hydrochloric acid in a test tube, add from 20 to 40 drops of the urine and heat gently, when, if indigogen be in excess, a violet first passed after a cholera color blue color will be developed. (See p. 260.)	Indigogen is in excess in the urine in cases of obstruction of the intestines, also in catarrh of the intestines, and in the urine first passed after a cholera color blue color will be developed. (See p. 260.)

<p>Albumen.</p>	<p>Acid, neutral, or alkaline.</p>	<p>Urine containing much albumen is heat and by nitric acid. Each generally pale and of these should be applied, first frequently of low separately and then together. specific gravity. (See p. 242.)</p>	<p>The albumen may come from the bladder, as in cystitis, from the pelvis of the kidney, as in pyelitis, and from the serum of the blood directly, as in structural diseases of the kidney. (See p. 242, et seq.)</p>
<p>Sugar.</p>	<p>Generally acid.</p>	<p>Urine containing much sugar is increased in amount, if these are not sufficiently pale and of high isfactory, add the fermentation test (see p. 269)</p>	<p>Diabetes mellitus has been known to follow upon (1) injury to the head, with or without fracture of the skull; (2) clot in the pons varioli; (3) softening at the base of the brain; (4) disease of the sympathetic nerve; (5) excessive brain work; (6) uterine disease; (7) disordered digestion; (8) exposure to cold, etc. (Harley.)</p>

Examination of Urine.

For John Little at the request of
Dr. Victor Vaughan

Physical and Chemical Characters.

Total quantity for 24 hours 906 cc

Color Yellow Odor Pungent

Reaction Acid Sp. Gr. 1022

Deposit, quantity and general appearance Small Part Deposit

Urea 1 cc Urates 10%

Phosphates 1% Sugar 2%

Albumen not any

Microscopical Examination.

Crystals Rhomboidal

Anatomical elements Bile

Other morphological elements Not

any

Pathological Indications.

Bright's Disease

Dated Feby 13th 1886

DETECTION OF MEDICINAL SUBSTANCES IN THE URINE.

This is a branch of the analysis of urine to which no great attention has been given, and it may, at first, seem unnecessary to discuss it here; but let us consider its importance. It is well known that some of the most common medicines often produce strange, and at times, injurious effects. This is sometimes due to an accumulation of the medicine in the system: one dose is given, and if it does not produce certain effects in a given time, the physician administers another, without knowing whether the first has been either entirely or partially eliminated. I have made quite a number of experiments in this line with iodide and bromide of potash, and with morphia. In some cases, I have found that after administering a medicinal dose of these substances, they appeared in the urine within less than an hour's time, and disappeared within twenty-four hours; while in other cases, they can not be detected in the urine until the expiration of twenty-four hours. Now suppose that these medicines are given at certain intervals of time to two patients; in one, the substance is rapidly eliminated; in the other, it is unduly retained; the doses are repeated, giving one as much as the other, the system of the first contains only the ordinary dose, while that of the second may contain three, or four times the medicinal dose. I have no doubt but the life of many a patient could have been saved from the cumulative action of medicinal poisons by a timely examination of

the urine ; and this is the only apology I will offer for introducing this subject.

MORPHIA, $C_{17}H_{19}NO_3H_2O$.

Concentrate the urine to one-tenth its bulk, render it alkaline with ammonia, and shake well with amylic alcohol. Separate the alcohol and evaporate it to dryness ; to a portion of the residue, add two or three drops of concentrated H_2SO_4 , and heat on the water-bath for one hour, then add a drop of HNO_3 which will produce a deep red color if morphia be present. Treat a second portion of the residue with iodic acid and bisulphide of carbon. The morphia liberates iodine, which colors the bisulphide.

STRYCHNIA, $C_{22}H_{24}N_2O_2$.

Concentrate the urine to a syrup, render strongly alkaline with KHO , and agitate well with chloroform. Separate the chloroform and evaporate it to dryness on the water-bath ; to the residue add strong H_2SO_4 , and heat on the water-bath for one hour, then neutralize with carbonate of sodium, and render alkaline with KHO ; agitate again with chloroform ; separate the chloroform and evaporate to dryness in a small porcelain dish on the water-bath ; dissolve the residue in a few drops of H_2SO_4 , then slowly move a small crystal of bichromate of potash through this solution ; if strychnia be present, the crystal will produce a purple coloration.

VERATRIA, $C_{32}H_{52}N_2O_8$.

Concentrate the urine to a syrup, render alkaline with KHO , agitate with chloroform, remove the chloroform and evaporate it to dryness on the water-bath ; treat the residue with ether, remove the ether and evaporate it to dryness. To a portion of the residue, add a few drops of concentrated H_2SO_4 , and heat on the water-bath, when a crimson color will be produced, if veratria be present. Dissolve the remaining part of the residue in HCl ; this solution is colorless, when cold, dark red when warm.

ATROPIA, $C_{17}H_{23}NO_3$.

Evaporate the urine to dryness on the water-bath, add a few drops of KHO , and agitate with ether ; remove the ether, evap-

orate it to dryness ; dissolve the residue in chloroform ; remove the chloroform and evaporate it to dryness ; dissolve the residue in water, and place a drop of this solution in the eye ; if atropia be present the pupil will be dilated.

SANTONINE, $C_{15}H_{18}O_3$.

Santonine imparts a deep red color to alkaline urinae. If the urine, when passed, be of normal reaction, no peculiarity of color will be observed, but upon the addition of an alkali, the characteristic color will be produced ; this color disappears after standing, or after being agitated with oxygen.

IODINE AND BROMINE.

When iodides or bromides are administered in medicinal doses, they may be detected in the urine, upon the addition of chlorine water and bisulphide of carbon.

ARSENIC AND ANTIMONY.

Evaporate the urine to dryness ; to the residue add fuming nitric acid, and heat on the sand-bath until all the organic matter is destroyed. Dissolve the residue in water strongly acidified with HCl. Treat this solution with H_2S gas for 24 hours ; collect and wash the precipitate, and remove it to a porcelain dish or crucible. cover with fuming nitric acid, and heat to dryness. Treat the residue with water, which will dissolve the arsenic but not the antimony. Test the water solution for arsenic by Marsh's test. If the substance be antimony, it can be dissolved in dilute HCl, and precipitated with H_2S gas, the precipitate having the characteristic orange-red color.

For details of this method for detecting and separating arsenic and antimony, see a paper by the author, in the American Chemist for August, 1875.

MERCURY.

Evaporate the urine to dryness and destroy the organic matter with nitric acid as given under arsenic and antimony. Mix the residue with carbonate of soda and bichromate of potash, put this into a tube which is opened at one end and has a bulb blown at the other. Shake the mixture into the bulb and heat, keeping the open end of the tube cool. The mercury is vaporized, and collects in small globules upon the upper and cool extremity of the tube.

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