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OUTLINES OF THE CLINICAL CHEMISTRY OF URINE

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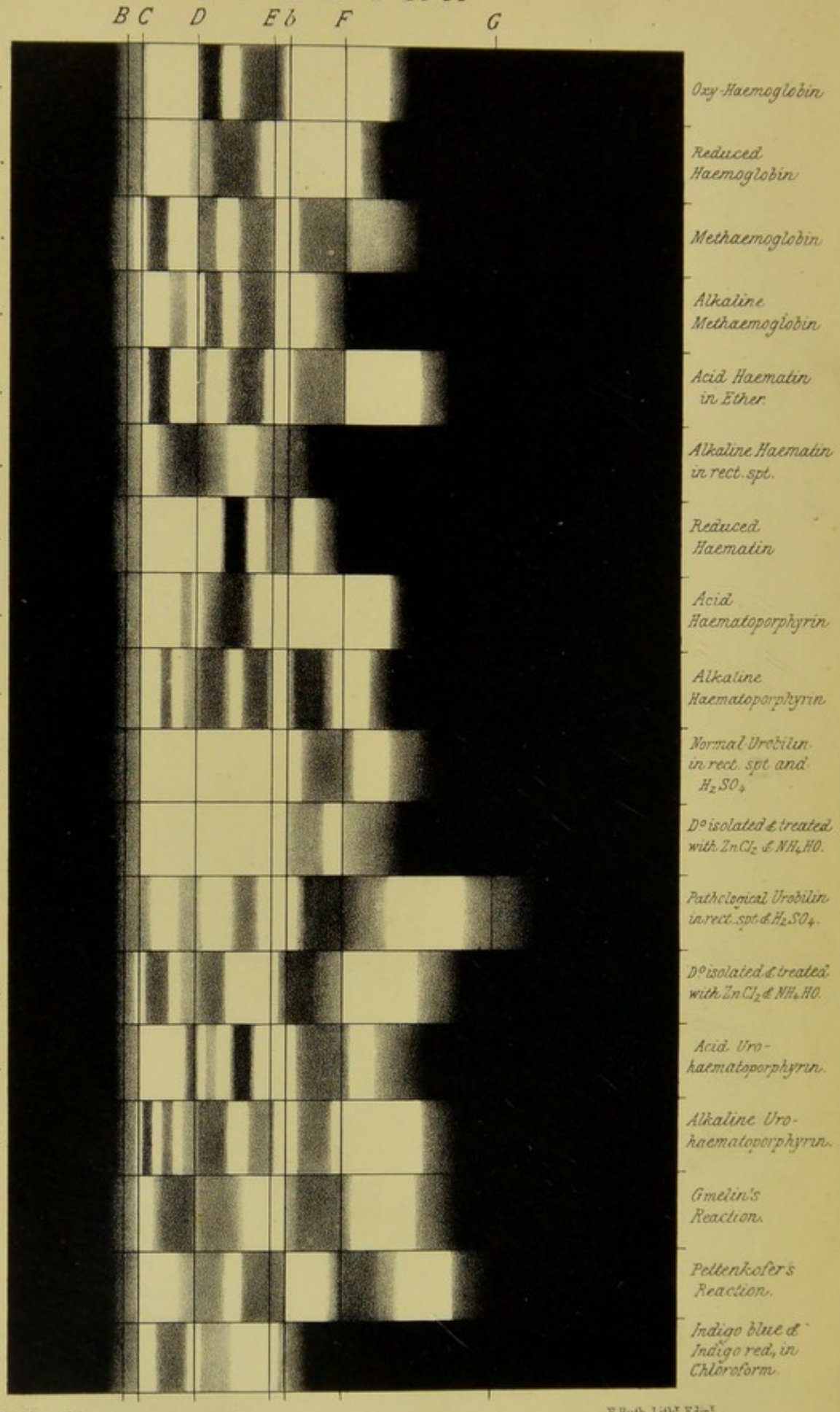

C.A.Mac Munn del.

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OUTLINES

OF THE

CLINICAL CHEMISTRY OF URINE

BY

C. A. MAC MUNN, M.A., M.D. (DUB.)

With 64 Woodcuts and Plate of Spectra



LONDON
J. & A. CHURCHILL
11, NEW BURLINGTON STREET
—
1889

UNIVERSITY
OF BRISTOL
MEDICINE

To

MY FRIEND,

T. L. W.,

I Dedicate this Book.

PREFACE.

This book contains a brief but, I hope, accurate account of the chemistry of urine in health and disease. The busy practitioner has not time to read up large text-books, and requires in small compass such information as will enable him to perform an analysis of urine, or to judge intelligently of the results of one made by another. I have endeavoured to meet this want, and, in addition, to supply an account of the more recent advances in the subject, which at present are difficult of access, owing to their being published in German and other text-books and periodicals. Where I have failed to give an intelligent account of any subject, I have endeavoured to remedy the deficiency by copious references, so that anyone anxious to know what has been done can follow up the literature for himself.

I think I ought to mention that I should never have run the risk of exposing my efforts to criticism if I had not been asked to do so by some friends, who were good enough to listen to the post-graduate lectures included in these pages, which were given at the Birmingham Medical Institute during the early part of this year. Even if I should happen to be roughly handled, I shall still feel that

I have done my best; and the knowledge that the effort was made under such disadvantages as beset the path of an individual like myself, engaged in general practice, may help to disarm criticism.

My task has been lightened considerably by the kind help of Dr. Halliburton, who has made many valuable suggestions and a few corrections, and I here give him my warmest thanks. To Mr. A. E. Johnson I am also indebted for a revision of those parts of the book which deal with volumetric estimations.

I have made free use—at times, perhaps, without due acknowledgment—of the following:—Salkowski and Leube's "Die Lehre vom Harn," Hoppe-Seyler's "Physiologische Chemie," and "Handbuch der physiologisch- und pathologisch-chemischen Analyse," also his "Zeitschrift für physiologische Chemie," Neubauer and Vogel's "Guide to the Qualitative and Quantitative Analysis of the Urine" (American translation), Tyson's "Practical Examination of Urine," Gamgee's "Physiological Chemistry of the Animal Body," Sutton's "Volumetric Analysis," Foster's "Text-book of Physiology," McKendrick's "General Physiology," Landois and Stirling's "Text-book of Human Physiology," Krukenberg's "Grundriss der medicinisch-chemischen Analyse," Brunton's "Pharmacology, Therapeutics, and Materia Medica" and "Disorders of Digestion," Ralfe's "Clinical Chemistry" and "Practical Treatise on Diseases of the Kidneys," Charles's "Physiological and Pathological Chemistry," and the works of Sir W. Roberts, Dr. Saundby, and others.

I beg to thank Messrs. J. and A. Churchill, Professor McKendrick, Messrs. Longmans and Co., Messrs. Gibbs,

Cuxon and Co., and Messrs. Southall Bros. and Barclay, for the loan of blocks for the wood-cuts. Some of these wood-cuts are familiar to many, but they illustrate the text as well as new ones might have done, and their use has saved expense. I have not added a final "e" in the case of animal alkaloidal bodies, as its omission is of no importance except to the hypercritical.

I should like to have referred to some more recent discoveries, such as that of the synthesis of uric acid by Drs. Behrend and Roosen, described briefly in "Nature" for May 16th of this year, and to others; but if this book should prove useful I hope to do so by-and-by.

CHAS. A. MAC MUNN.

Oakleigh,
Wolverhampton,
June, 1889.

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EXPLANATION OF CHART OF SPECTRA.

(Frontispiece.)

Spectrum 1.—Oxyhæmoglobin : moderately dilute aqueous solution.

,, 2.—Reduced hæmoglobin.

,, 3.—Methæmoglobin.

,, 4.—Alkaline Methæmoglobin : sometimes the band before D is much closer to that line, and seems to merge into the next band.

,, 5.—Acid Hæmatin in Ether : got by adding acetic acid to a little blood, and agitating with ether.

,, 6.—Alkaline Hæmatin : got by treating a little blood with rectified spirit and caustic soda.

,, 7.—Reduced Hæmatin, or Hæmochromogen : got by adding ammonium sulphide to the last solution.

,, 8.—Acid Hæmatoporphyrin : got by dissolving hæmatoporphyrin in rectified spirit and sulphuric acid.

,, 9.—Alkaline Hæmatoporphyrin : got by dissolving hæmatoporphyrin in rectified spirit and ammonia.

,, 10.—Normal Urobilin : rectified spirit and sulphuric acid extract from the precipitate obtained by treating normal urine with neutral and basic acetate of lead.

,, 11.—Normal Urobilin : isolated by agitating the last solution with chloroform, evaporating, dissolving the residue in rectified spirit, and treating the latter solution with *zinc chloride and ammonia*.

,, 12.—Pathological Urobilin : rectified spirit and sulphuric acid extract of the lead precipitate from urine of a case of tubercular peritonitis.

,, 13.—Pathological Urobilin : isolated as described under Sp. 11, dissolved in rectified spirit, and treated with *zinc chloride and ammonia* (Combination spectrum).

, 14.—Acid Urohæmatoporphyrin : rectified spirit and sulphuric acid extract of the lead precipitate from urine of case of acute rheumatism.

Spectrum 15.—Alkaline Urohæmatoporphyrin : isolated from urine as described under Sp. 11, dissolved in rectified spirit, and treated with ammonia.

,, 16.—Gmelin's Reaction : a chloroformic solution of bilirubin is cautiously treated with common nitric acid until a blue, or blue-violet, colour appears, the acid is then removed with a pipette. The bands are different in each stage of the reaction, as described in the text.

,, 17.—Pettenkofer's Reaction with Human Bile Salts, after the solution has stood some time. At first, another somewhat narrow and feeble band (as described by me in the "Spectroscope in Medicine," p. 165) is seen, but this disappears, and is replaced by the band at D. The bile salts were isolated in the usual manner, dissolved in distilled water, and then the test applied. This spectrum appears to vary considerably under unknown conditions.

,, 18.—Indigo-blue and Indigo-red (?) from Normal Urine. The urine was boiled with an equal bulk of common fuming hydrochloric acid, and, when cold, agitated with chloroform. The same spectrum may be obtained by treating the urine by Jaffé's method, and agitating with chloroform. The bluer the chloroform, the darker is the band before D ; the nearer it approaches red, the darker that after D. A third band at F is generally seen also, due to the presence of urobilin.

OUTLINES

OF THE

CLINICAL CHEMISTRY OF URINE.

PART I.

NORMAL URINE AND ITS CONSTITUENTS, AND THEIR VARIATION UNDER DISEASED CONDITIONS.

CHAPTER I.

FUNCTION OF THE KIDNEY AND MECHANISM OF ITS SECRETION.

In the kidney three distinct processes are carried on:—(1) Waste products arising from the wear and tear of the various organs and tissues are got rid of, as well as products absorbed from the intestinal canal. (2) Water holding these in solution is excreted. (3) A portion of this water is again absorbed for the needs of the economy, at least under certain conditions.

General Structure.—The kidney is a compound tubular gland, and like such glands carries on a true secretion, while at the same time it contains a complicated filtering apparatus, differing from an ordinary filtering apparatus in this, that it has the property of keeping back certain albuminous bodies held in solution in the blood. The *secreting* part of the kidney is represented by the glandular epithelium lining the uriniferous tubules, while the Malpighian tufts are concerned mainly in *filtering* off the water. At the same time we must remember that some salts, such as

chloride of sodium, accompany the water filtered through the Malpighian tufts. Let us examine the structure of the kidney for a few moments. On making a section (Fig. 1) parallel to its flat surface and through its middle, it is seen to be differentiated into two distinct parts—a cortical beneath the surface and a medullary within it; the medullary

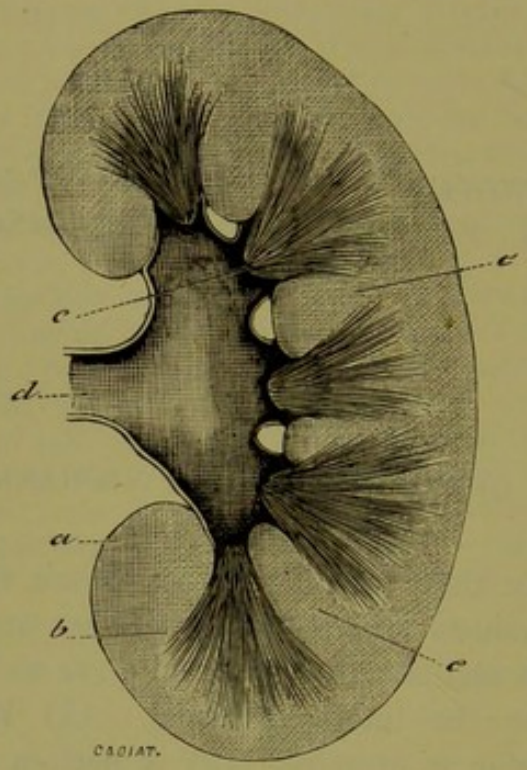


FIG. 1.—SECTION OF KIDNEY OF MAN.

a, Cortical substance composed chiefly of convoluted tubules; the portions between the medullary pyramids form the columns of Bertin, *e*. *b*, Pyramids of medullary substance, composed of straight tubes, &c., radiating towards cortex to form the pyramids of Ferrein. *d*, Commencement of ureter leading from renal pelvis. *c*, Papillæ, where the tubes open into pelvis.—(Yeo's "Physiology," *after Cadiat.*)

is known also as the pyramidal part, being composed of about a dozen irregularly shaped pyramids,* whose apices project into the hollow part of the kidney, known as the pelvis of the kidney. Each pyramid is composed of bundles of tubes, which open by very small orifices at its apex, or papilla, into the pelvis. The spaces into which

* Malpighian pyramids.

these pyramids project are known as the calices, cups, or infundibula. The pelvis of the kidney is continuous, by means of a funnel-shaped opening, with the ureter. As Lauder Brunton remarks:—"The kidney may be looked upon as a large filtering apparatus, and the funnel into which the filtrate drains is the ureter."*

The cortex of the kidney, a little less than a quarter of an inch in width, is of a light brown colour, and has a granular appearance, the granules being due to the presence of the Malpighian corpuscles; it also shows striæ, which are due to the outward prolongations of the medullary rays, known here as the pyramids of Ferrein. The *boundary zone* of the medullary portion is darker in colour, and is striped with alternate streaks of clear and opaque lines, the former being due to blood-vessels, the latter to the uriniferous tubes. The papillary region of the medullary part is lighter in colour than the rest of the medulla and is striated. The straight tubes of the medulla run out in bundles towards the cortex, forming medullary rays, these ending in the cortex as the pyramids of Ferrein. The part of the cortex between the latter is called the labyrinth, on account of the complicated arrangement of the tubes in it.

Renal Tubules.—If we commence with the tubules (Fig. 2), we find that each begins in the cortex as a dilatation, embracing a tuft of blood-vessels. This dilatation is known as Bowman's capsule, and the little tuft of blood-vessels is the glomerulus: both of them constituting a Malpighian body, or corpuscle. These are only found in that part of the cortex known as the labyrinth. The tubes then run a very complicated course up and down the cortical and medullary parts, being joined by other tubules on the way, until they form collecting tubes, which finally open by small apertures on the apices of the papillæ, where they may be seen by means of a lens. They widen and narrow here and there during their course, but on an average are

* Lauder Brunton: "Practitioner," vol. xxvii., August, 1881.

the $\frac{1}{600}$th of an inch in diameter. They are made up of a nearly homogeneous basement membrane, lined by an epithelium, which differs in the various parts of the tube (as shown in Fig. 2), and leaves a space or lumen for the flow of urine through the tubes.

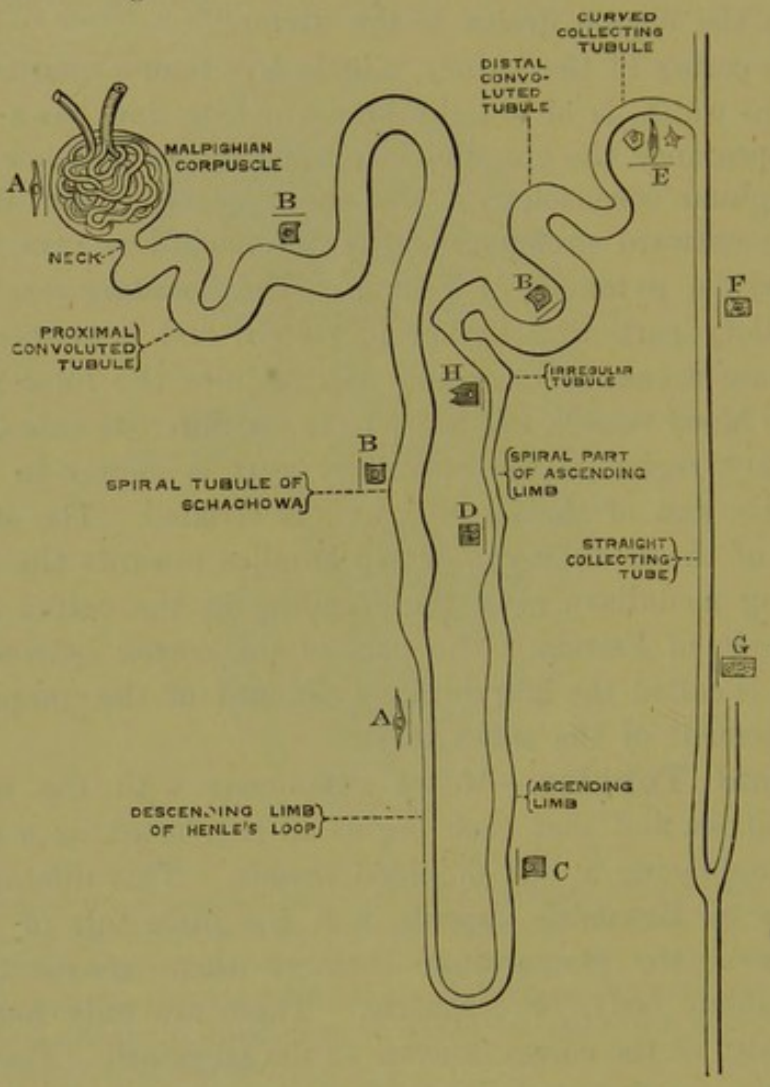


FIG. 2.—DIAGRAM SHOWING COURSE OF A URINIFEROUS TUBULE WITH THE EPITHELIUM CELLS LINING THE VARIOUS PORTIONS OF IT.

The latter being represented more highly magnified than the tubes which contain them.—(Gray's "Anatomy.")

The capsule of Bowman, or dilated extremity of a uriniferous tube, is composed of a hyaline basement membrane, thickened by fibrous tissue, and lined with flattened epithe-

lium; the tuft embraced by it is also covered by epithelium (Fig. 3), and it is this which prevents—at least to a great extent—the albuminous constituents of the blood from escaping into the tube. At all events, after ligature of the renal arteries, and subsequent restoration of the renal circulation, the urine was found to be albuminous, and at the same time this epithelial lining was found affected. The capsule embracing the glomerulus, or tuft of blood-vessels, is then (see Fig. 2)—(1) The dilated end of a uriniferous tube, which consists, in

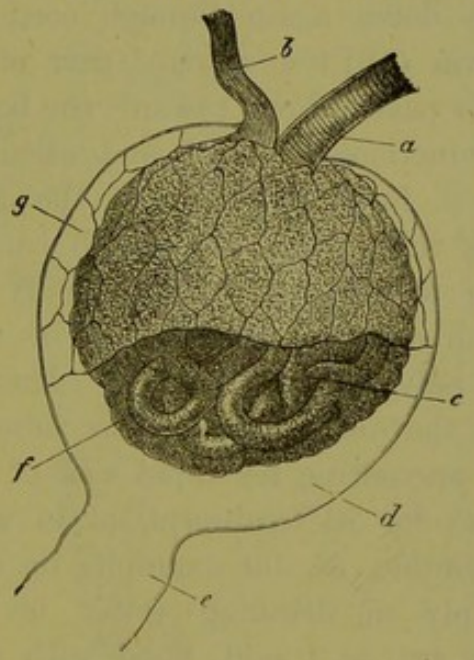


FIG. 3.—GLOMERULUS FROM KIDNEY OF RABBIT.

a, *Vas afferens*. *b*, *Vas efferens*. *c*, Glomerulus. *d*, Undermost part of capsule shown without epithelium. *e*, Neck. *f*, Epithelium of the glomerulus. *g*, Epithelium of internal surface of capsule after treatment with nitrate of silver.—(Frey's "Histology and Histo-Chemistry.")

addition, of the following parts:—(2) The neck (lined with cubical epithelium), being the narrow part of the tube before it spreads out to form the capsule; (3) the proximal convoluted tubule, which, beginning wide and tortuous, becomes straight as it approaches the medullary ray and somewhat spiral, forming (4) the spiral tube of Schachowa; the latter suddenly narrows at the junction of cortex and medulla forming (5) the descending limb of Henle's loop, which passes

down to the base of a pyramid, then turns back again, forming (6) the ascending limb of Henle's loop, which widening out again in the boundary zone, but again becoming narrow in the cortex, passes up in a medullary ray. It then becomes (7) the irregular tubule, which has a somewhat angular outline, and then forms the (8) distal convoluted tubule, which is somewhat dilated. The latter then narrows again to form (9) the first part of the collecting tubule or curved collecting tubule, which opens at an angle into a straight tube; this runs down again through cortical and medullary part to form (10) the straight part of the collecting tubule. As this passes down towards the boundary zone, it receives other junctional tubules, and when it reaches the boundary zone it forms (11) one of the collecting tubes. These collecting tubes then unite with each other in the papillary region to form (12) the excretory tubes or ducts of Bellini, which open on the summit of a pyramid into a calix of the pelvis of the kidney. Lauder Brunton is of opinion that the *constrictions* in the tubules "may serve the purpose of preventing too rapid exit of the water, and thus allow time for its reabsorption in cases where its retention is desirable, as, for example, on a hot day, and when the supply of drinking water is very limited."* All the tubules are, as I said, lined with epithelium, and it is this epithelium which separates the solid constituents of the urine from the blood brought to the tubules by the surrounding plexus of blood-vessels.

Blood-vessels.—It is now necessary to describe the course of the blood-vessels, which I shall do very briefly.

The renal arteries are large in comparison with the size of the kidney. Each renal artery enters the kidney in four or five branches, which pass in at the *hilus*, and are embedded in fat among the infundibula. They enter the *substance* of the kidney between the papillæ, and run between the pyramids of Malpighi; they then divide and subdivide, and

* Lauder Brunton: "Pharmacology, Therapeutics, &c.," chap. xv.

at the bases of the pyramids in the boundary zone they form arches between cortex and medulla. From these arches *interlobular* arteries are given off, which pass up between the medullary rays in a nearly straight course towards the surface of the cortex. From these interlobular arteries (Fig. 4) short branches spring, which, without dividing, go straight to the glomerulus, the latter being embraced by the capsule, or

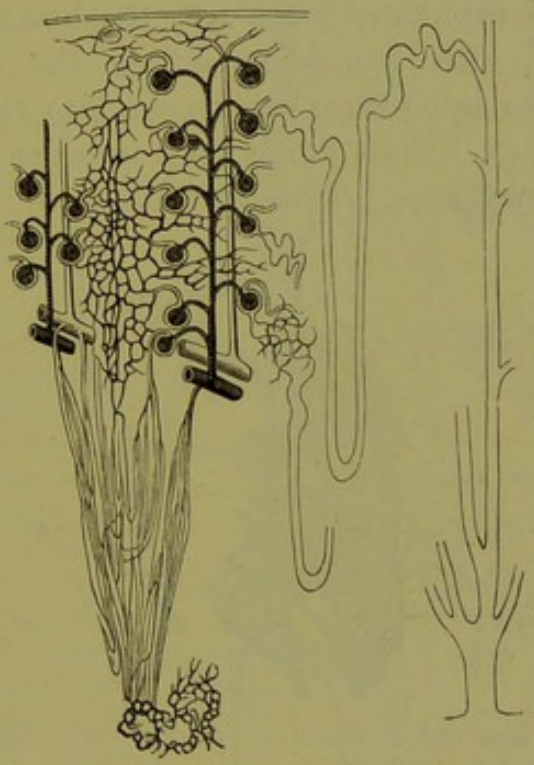


FIG. 4.—DIAGRAM SHOWING RELATION OF BLOOD-VESSELS TO TUBULES OF THE KIDNEY.

The upper half corresponds to the cortical, the lower to the medullary part of the organ. The tubes are shown on the right, and some are left out. The darkly-shaded arteries send off straight branches to the pyramid and larger interlobular branches to the glomeruli, the efferent vessels of which form the plexus around the convoluted tubes.—(Yeo's "Physiology.")

dilated extremity of the uriniferous tubule. The branch, which goes into the glomerulus, is the *afferent* artery (Figs. 3, 4, and 5). It breaks up within the capsule into a looped plexus of capillaries. From the centre of this plexus a small vein arises, known as the *efferent* vein: this leaves the

capsule close to the point where the afferent artery enters it. This vein, however, after leaving the tuft, does not join with others to form larger venous trunks, but breaks up into a number of branches which ramify over and among the uriniferous tubules. All the efferent veins do not, however, behave in this manner, as those from the lowermost glomeruli form straight vessels (false *vasa recta*), which pass into the medulla and break up into a plexus in the papillæ. The plexus of capillaries formed by the breaking up of the efferent vein, after ramifying among the tubules again collect together into veins, which open into the interlobular veins (*b*, Fig. 6). These finally open into the venous trunks in the boundary zone. Their further course is described on

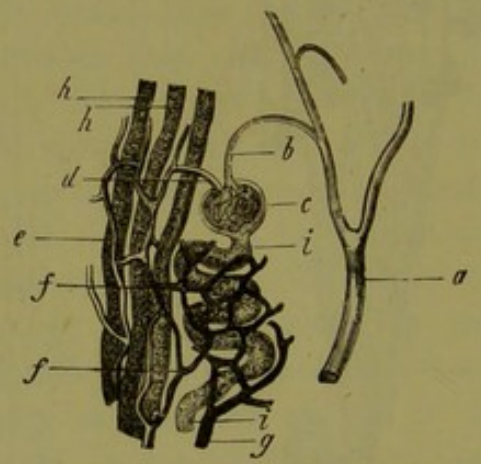


FIG. 5.—FROM THE KIDNEY OF THE PIG (*semi-diagrammatic*).

a, Arterial twig. *b*, Afferent vessel of the glomerulus, *c*. *d*, *Vas efferens*. *e*, Plexus formed by the subdivision of the latter. *f*, Meshes of capillaries around the convoluted tubes. *g*, Radicle of a venous twig.—(*Frey.*)

page 10. Hence there are two sets of capillaries in the kidney—those *within* the capsules forming the tuft, and those formed by the efferent veins *outside* of the tufts (Fig. 5). The latter have been compared to the portal system in the liver.

But besides these interlobular arteries there are others which are given off to the medulla. They arise at the junction of cortical and medullary parts, and are called true *vasa recta* (Fig. 4); these do not end as tufts, but run down into the

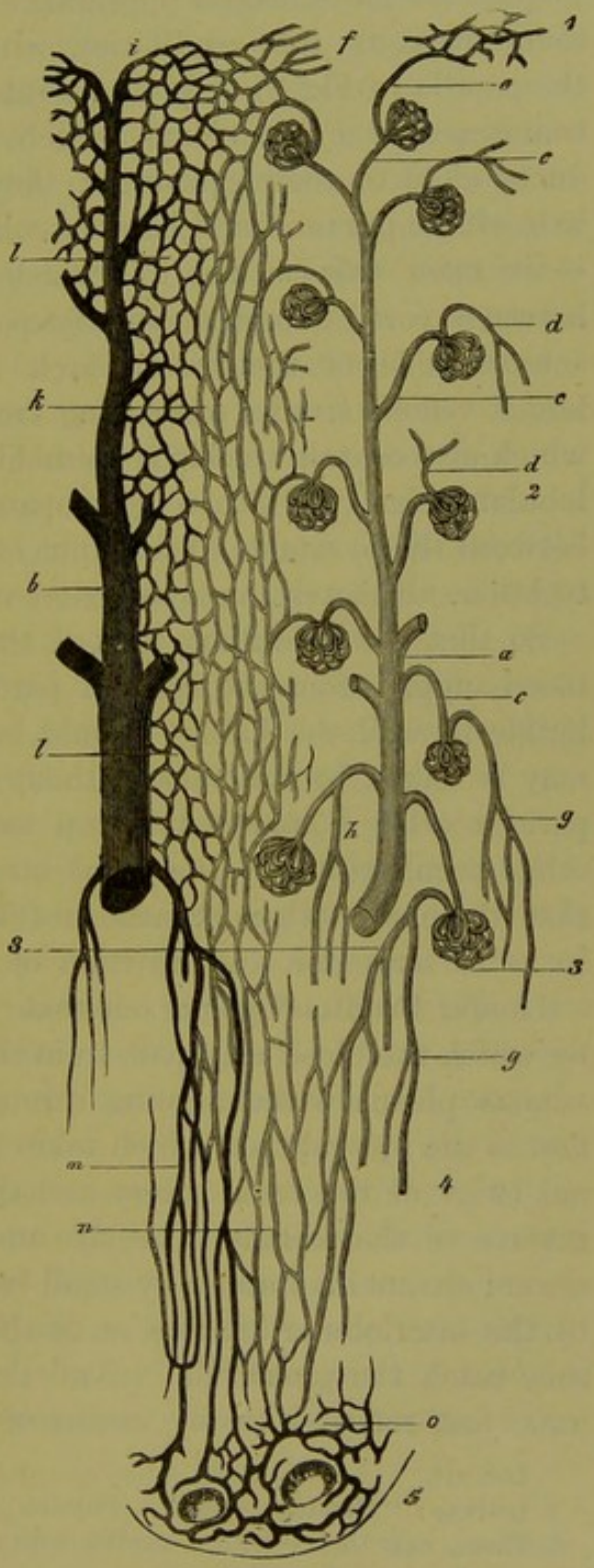


FIG. 6.

PLAN OF THE CIRCULATION OF THE KIDNEY (*much shortened*).

1, External portion of cortex. 2, Cortex. 3, Boundary layer. 4, Medulla. 5, Apex of papilla. *a*, Arterial twig. *b*, Vein. *c*, *Vas afferens*. *d*, *Vas efferens*. *e*, *Vas efferens* and capillary network (at 1) of surface. *g*, *Vas efferens* of a deeply placed glomerulus. *h*, *Arteriola recta*. *i*, Venous radicle of the surface. *k*, Capillaries of the medullary process. *l*, Of the convoluted tubules. *m*, *Venulæ rectæ*. *n*, Medullary capillaries. *o*, Network around the openings of the uriniferous tubes at the papillæ. —(*Frey.*)

pyramids, where they help, with the uriniferous tubules, to produce the streaked appearance in the pyramid. They then break up into capillaries, which end at the apex of the papilla (*o*, Fig. 6). At that point the veins of the pyramid commence as a fine plexus, which surrounds the excretory ducts close to their openings; they then run towards the base of the pyramid and become collected into larger vessels —the *venæ rectæ* (*m*, Fig. 6)—which run up to the boundary between cortical and medullary parts, and open into the concave side of the venous arch in that situation. The larger venous trunks proceeding from the venous arch, and which also contain the blood poured into them from the interlobular veins (p. 8), then (accompanied by the arteries) run between the pyramids to the sinus of the kidney, and, joining together, ultimately form the renal vein.

So that the medullary part of the kidney has a separate blood-supply from the cortical part, and it becomes intelligible how, if the kidney should be congested, a short cut may be taken by the blood without its entering the cortical part at all. I cannot take up more space in describing other peculiarities of the renal circulation, but I may say that there are other branches of blood-vessels distributed so as to serve for the nutrition of the kidney itself.

Lauder Brunton* points out that "there are three channels by which the blood may pass from the renal arteries into the venous plexus without going through the glomeruli. The first is the inosculation which takes place between the terminal twigs of the renal artery and the venous plexus on the surface of the kidney, directly under the capsule.† The second channel is formed by small branches given off directly by the interlobular arteries or by the afferent arteries, before they reach the glomeruli." And the third is that which I have just referred to, by means of the true *vasa recta*.‡

* Loc. cit.

† Ludwig: "Handworterbuch d. Physiol.," v. R. Wagner, Bd. ii.

‡ These, near their origin, inosculate with the venous plexus surrounding the convoluted tubules (Brunton).

Nerves.—The nerves of the kidney accompany the artery, and contain a number of ganglia. When the nerves are cut the blood-vessels of the kidney dilate, and when they are stimulated the blood-vessels contract. These nerves come from the renal plexus and the lesser splanchnic nerve, and contain filaments derived from the sympathetic and cerebro-spinal systems.* When the splanchnics are divided the vessels of the kidney generally dilate, and by irritation of the splanchnics they are caused to contract. It is supposed that the nervous centre for the renal arteries is situated—like the principal vaso-motor centre for the body—in the medulla; but there are reasons for supposing that there are other centres in the spinal cord and in the solar and mesenteric plexuses.† When a puncture is made in the floor of the fourth ventricle the quantity of urine increases considerably, although it is at first diminished. Stimulation of the vaso-motor centre in the medulla, such as is produced by venous blood or certain drugs—for example, digitalis—causes the renal blood-vessels to contract, like those in other parts of the body. But if, under these circumstances, the renal nerves are divided, then the arteries dilate and the amount of urine is very much increased, its increase being caused not only by the dilatation of the renal vessels, but by the increased arterial tension in other parts of the body. The nitrites are said to paralyze the muscular fibres in the *vasa afferentia*, while digitalis increases the arterial tension all over the body; hence Lauder Brunton suggests that by combining spirit of nitrous ether (for example), which contains nitrite of ethyl, with digitalis, we should obtain a much freer flow of urine than if we give digitalis alone.‡

Theories of Secretion.—As I said above, two distinct processes take place in the kidney, namely (1) modified filtration and (2) a true secretion. Bowman maintained that

* Quain's "Anatomy," 9th ed., vol. ii., p. 659.

† Lauder Brunton: Loc. cit.

‡ Lauder Brunton: "Practitioner," vol. xxxii., 1884. Cf. also Leech: Nitrite of Ethyl, "Med. Chronicle," vol. ix., 1888, p. 177.

the glomeruli were entirely concerned in the filtering off of water from the blood, the water containing only the highly diffusible and soluble salts; while the specific urinary constituents were secreted by the epithelium lining the tubules, and carried off by the water flowing through the tubules. Ludwig supposed that filtration and reabsorption took place, a dilute solution of urea and salt being poured out from the Malpighian corpuscles, and the fluid becoming more concentrated as it flowed along the tube; but Heidenhain's experiments have confirmed, to a great extent, Bowman's theory. He showed that sulph-indigotate of soda, when injected into the blood, after section of the medulla, which causes lowering of the blood-pressure within the kidney, was taken up by the cells of the convoluted tubes (and by those only) so that they became blue. That Ludwig was, however, correct in supposing that absorption of water takes place from the tubes is rendered probable by the observations of Hüfner on the kidney of fishes, frogs, tortoises, birds, and mammals, as in these the tubes are long or short according as the animal requires water to be reabsorbed in each case. Ribbert, by direct experiment, has given support to this view, as he succeeded in extirpating the medullary substance of the kidney while leaving the cortical part. He collected the urine which passed through the Malpighian bodies only, before it had reached Henle's loops, and he found it much more watery than that secreted by the entire kidney.*

Owing to the peculiarities of the renal circulation in the frog and newt, which possess the so-called renal-portal vein, these animals are peculiarly fitted for such experiments. In them there are two distinct blood-supplies to the kidney—the Malpighian bodies being supplied by the renal artery, the convoluted tubes by the renal-portal vein. Nussbaum took advantage of this. By ligaturing the renal artery, he stopped the functional activity of the glomeruli, and by ligaturing the renal-portal vein that of the tubules. By

* Ribbert: "Virchow's Archiv," July, 1883, S. 189.

then injecting a substance into the blood, after ligature of either one or the other vessel, and determining the presence of this substance in the excretion, he could tell whether it was got rid of by the glomeruli or the tubules. He found that sugar, peptones, and albumin pass out exclusively through the glomeruli, as they were not found when the renal artery was ligatured; whereas indigo-carmine, when injected after ligature of the renal artery, was found in the epithelium lining the tubules, and it was not accompanied by any water. Urea, also, was found to be excreted exclusively by the renal epithelium, but it was accompanied by water supplied by the venous plexus. Hence, as Brunton remarks, "The excretion of water takes place in a double manner: it passes out through the glomeruli when the renal arteries are free, and it passes out from the venous plexus along with urea, even although the renal arteries are tied."*

Hence, the greater part of the water is filtered off through the glomeruli, being accompanied by certain inorganic salts, and is dependent entirely upon blood-pressure, whereas the *secretion* proper takes place in the epithelium lining the tubules, and is practically independent of blood-pressure. This secretion may, however, be accompanied by the elimination of water, owing to the chemical stimulation of the renal epithelium by the urea, &c.

It would lead us too far to enter more fully into a description of the physiology of the kidney, but it may not be out of place to enumerate briefly how the secretion of urine may be increased and how it may be diminished. By the use of Roy's oncometer and its accompanying registering oncograph, much more accurate experiments than formerly can now be made on the changes in the volume of the kidney.

"A. The secretion of the urine may be increased:—

a. By increasing the general blood-pressure; by

(1) Increase of the force or frequency of the heart-beat.

* Loc. cit.

(2) Constriction of the small arteries of areas other than that of the kidney.

b. By increasing the blood-pressure in the kidney, by *relaxation of the renal artery, without compensating relaxation elsewhere;* by

(1) Division of the renal nerves (causing polyuria).

(2) Division of the renal nerves and stimulation of the cord below the medulla (causing greater polyuria).

(3) Division of the splanchnic nerves; but the polyuria thus produced is less than in (1) and (2), as these nerves are distributed to a wider area, and the dilatation of the renal artery is accompanied by dilatation of other vessels, and therefore with a somewhat diminished general blood-supply.

(4) Puncture of the floor of the fourth ventricle or mechanical irritation of the superior cervical ganglion of the sympathetic, possibly from the production of dilatation of the renal arteries.

"B. Secretion of urine may be diminished:—

a. By diminishing the general blood-pressure; by

(1) Diminution of the force or frequency of the heart-beats.

(2) Dilatation of capillary areas other than that of the kidney.

(3) Division of the spinal cord below the medulla, which causes dilatation of the general abdominal area, and urine generally ceases being secreted.

b. By increasing the blood-pressure, by stimulation of the spinal cord below the medulla, the constriction of the renal artery which follows not being compensated for by the increase of general blood-pressure.

c. By constriction of the renal artery, by stimulating the renal or splanchnic nerves, or the spinal cord."*

Besides the causes which diminish the secretion of urine just mentioned, it may be noticed that another cause is liga-

* Copied from the 12th ed. of Kirkes's "Physiology" (after M. Foster).

ture or blockage of the ureter, or compression of the renal vein (Meyer, von Frerichs). In the latter case, the venous stand-still compresses the capillary loops within the Malpighian capsule, so that filtration cannot take place.* A very similar condition may occur in the venous stasis resulting from cardiac or pulmonary disease.

In connexion with this subject we may briefly consider the action of *diuretics*.

"Diuretics may act in several ways. They may act:—

"A. On the circulation in the kidney, raising the pressure in the glomeruli.

(1) *Locally* (*a*) by contracting the *efferent* vessels, or the arterial twigs which pass directly to the capillary plexus; (*b*) by causing dilatation of the renal arteries, and thus increasing the supply of blood to the kidney. This they may do, also, in more ways than one, for they may either paralyze the vaso-motor nerves of the kidney, or act on vaso-dilating mechanisms.

(2) They may raise the blood-pressure *generally* by causing the contraction of vessels in other parts of the body.

"B. Other diuretics may act on the secreting cells of the tubules, and may increase both the amount of water and the amount of solids excreted by them."

This quotation is from Lauder Brunton, and the paper from which it is taken is well worthy of study, as the haphazard manner in which we are apt to use diuretics, often does more harm than good in cases of renal disease.†

* Landois and Stirling, vol. ii., 1885, p. 573.

† Lauder Brunton: "Practitioner," vol. xxxii., 1884.

CHAPTER II.

GENERAL CHARACTERS OF NORMAL HUMAN URINE.

Normal human urine is a lemon-yellow, or amber-yellow coloured, transparent watery fluid, of acid reaction, and having a peculiar smell sometimes described as aromatic. It is said to have a saline and bitter taste. Its specific gravity varies from 1015 to 1025, reckoning distilled water as 1000. The average quantity is 50 ounces, or 1419 cubic centimeters, in the twenty-four hours. The colour of urine is influenced by the amount of water present; thus concentrated urine is high-coloured, while very dilute urine has the slightest yellow tint.

Reaction.—The reaction of normal urine is acid, due, not to the presence of free acid, but to acid sodium phosphate. That no free acid is present is proved by the fact that urine gives no precipitate with sodium hyposulphite (von Voit, Huppert); also, as recently shown by Brücke, by its behaviour with Congo red.* One part of free hippuric acid in 55,000 of distilled water causes this reagent to become violet, or inky, but urine gives no change of colour. Therefore, the absence of free acid is finally proved. The acid sodium phosphate is derived from the basic sodium phosphate, owing to the uric, hippuric, and sulphuric acids and CO_2 taking up part of the soda, so that the phosphoric acid forms an acid salt (Landois and Stirling).† The acid reaction is increased by the use of acids, by a purely flesh diet, after

* Brücke: "Monatsh. Chem.," viii., 95—100; and "Journ. Chem. Soc.," 1887, p. 986; "Monatsh. Chem.," viii. 632—637; Ibid., ix., 31—41.

† Landois and Stirling, 3rd ed., p. 394.

prolonged muscular exertion, and by ammoniacal salts, which are changed in the body into nitric acid combinations. The urine passed in the morning is strongly acid. Sometimes free acids, such as formic and acetic, are present, and the so-called acid fermentation, which is really said to be an oxidative change, and which takes place in urine after it has stood some time, is accompanied by the presence of these acids. They are sometimes present in excess under diseased conditions, and such a condition has been called "*lipaciduria*" by von Jaksch (see page 89). In lipaciduria due to liver disease he found 0·06 gram of fatty acids present in the urine of twenty-four hours.*

The urine becomes less acid or alkaline on a vegetable diet, as in the herbivora, in which it is normally alkaline (although it becomes acid in fasting herbivorous animals), by the use of caustic alkalies, alkaline carbonates or alkaline salts of the vegetable acids, by mixture with alkaline blood or pus, by alkaline fermentation occurring in the bladder, by the presence of calcium or magnesium carbonates, and in cases where the gastric juice is made to escape through a fistula. During digestion the urine may become alkaline (so-called *alkaline tide*).

The reaction is determined by means of blue and red litmus paper; turmeric paper is of very little use. Sometimes urine shows the so-called amphoteric reaction, blueing red litmus paper and reddening blue. According to Krukenberg this is due to insufficient delicacy of the litmus paper.†

Estimation of the Acidity.—The *acidity* of urine can be estimated by determining the amount of caustic soda necessary to produce a neutral reaction in 100 c.c. of urine. There are required: (1) Standard oxalic acid solution, got by dissolving 1 gram pure oxalic acid which has not effloresced, in distilled water, and diluting to 100 c.c.; 10 c.c. of this solution contain 100 milligrams of oxalic acid. (2) Tincture

* Von Jaksch: "Zeits. physiol. Chemie," x., 536–560; "Journ. Chem. Soc.," 1886, p. 1056.

† "Grundriss d. med. chem. Analyse," S. 78.

of litmus and blue litmus paper. (3) Sodic hydrate solution, the strength of which must be determined by the oxalic acid solution. Each cubic centimeter must indicate 10 milligrams of oxalic acid.

Ten c.c. of the oxalic acid solution are accurately measured by a pipette and run into a small beaker, and a few drops of tincture of litmus added, which colours the fluid red. The sodic hydrate solution is then dropped into this until the fluid becomes blue. Suppose 6 c.c. of the sodic hydrate solution have been required, these correspond to 100 milligrams of oxalic acid; 400 c.c. of water are therefore added to 600 c.c. of the sodic hydrate solution, and a liter of fluid thus obtained, each cubic centimeter of which exactly corresponds to 10 milligrams of oxalic acid.

To apply this to urine:—Litmus paper must be used as indicator on account of the colour of the urine. 50 or 100 c.c. of urine are measured off into a beaker, and the standard sodic hydrate solution added, until a drop of the mixed fluid makes the blue litmus paper no longer red. A drop is then put on red litmus paper, and if it is made blue, the amount of sodic hydrate solution used is read off. The experiment is then repeated with a fresh quantity of urine, but a few drops less NaHO solution are added until the right point is reached. This gives the amount of acidity corresponding to so much oxalic acid (Neubauer and Vogel). Violet litmus paper may be used, or cochineal in solution, or rosolic acid in alcohol; the last is said to be preferable (Charles).*

This estimation may be sometimes required, as there is a marked increase of acidity in the urine in some diseases; for example: diabetes mellitus, pyrexia, acid dyspepsia, &c.

The urine may be *alkaline* from either *fixed* or volatile alkali. This is determined by simply heating the litmus

* Sutton advises a decinormal, $\frac{N}{10}$, solution of caustic soda, using a violet litmus paper as indicator; the degree of acidity is then registered as being equal to the quantity of $\frac{N}{10}$ alkali used.—("Volumetric Analysis," fifth ed., 1886, p. 327. (Cf. Salkowski and Leube, S. 20—22.)

paper until dry, after it has become blue by the alkaline urine: if the blue disappears, then volatile alkali (ammonium carbonate) is present; if it persists, then fixed alkali. The alkalinity from *fixed* alkali is due either to excess of alkaline carbonates of potassium and sodium, or to the alkaline phosphates. If alkaline carbonates are present the urine will effervesce on adding mineral acids.

The amount of *alkalescence* of urine is determined by means of a titrated solution of oxalic acid, of which 1 c.c. is equal to ·01 gram of sodium hydroxide; but this method cannot be recommended. Urine alkaline from *volatile* alkali is met with in cases of chronic cystitis, in which, owing to the introduction of micro-organisms from without into the bladder, the urea undergoes a transformation into ammonium carbonate. This is generally brought about by the action of an organism resembling in many respects the yeast-plant: it is known as the *Torula ureæ*.

Alkaline and Acid Fermentation.—Urine itself on standing, after its separation from the body, undergoes two changes—(1) acid fermentation, or, more correctly, oxidation; and (2) alkaline fermentation.

(1) The so-called *acid fermentation or oxidation* begins to increase soon after the urine is passed, and reaches its maximum about the third day.* At the same time a sediment appears, consisting of uric acid, acid urate of sodium in amorphous granules, and calcium oxalate. It is probable that a fungus and the mucus of the bladder (Scherer) play an important part in this change, and, according to Pasteur, oxygen is at the same time absorbed by the urine. After a longer time the urine undergoes—(2) *the alkaline fermentation*, which is due to the action of an organized ferment, the *Torula ureæ*. At the same time the urea is split up by the influence of the product of activity of this micro-organism into ammonia and carbonic acid. Müller,†

* Ralfe: "Clinical Chemistry," p. 111.

† Müller: "Journ. f. prakt. Chem.," Bd. lxxxi., 1860, S. 467.

Pasteur,* and Van Tieghem† have shown that the alkaline fermentation of urine is due to the presence of this micro-organism, the *Torula ureæ*. "But just as later researches showed that a part at least of the alcoholic fermentation is produced by an unorganized ferment, isolable from the yeast, so also have subsequent experiments shown that the complete fermentative hydration of urea can be brought about by a soluble ferment obtainable from the fermenting urine"‡ (Sheridan Lea).

Musculus showed in 1874 that if urine undergoing active alkaline fermentation be filtered through paper, and the paper washed with water until free from alkaline reaction, and dried at 35°—40° C., it retains a ferment capable of exciting the alkaline fermentation of urea.§ But, while Musculus attributed the fermentative power of this paper to the presence of the *Torula* in its pores, Dr. Sheridan Lea has shown it is not due to these, but to a special unorganized ferment which he has succeeeded in isolating, and which is secreted by the *Torula*. There is no doubt that the same decomposition of urea into ammonium carbonate may be brought about within the bladder by the introduction of micro-organisms on dirty catheters.

The change which takes place is thus chemically shown:—Urea : $CO(NH_2)_2 + 2H_2O$ = ammonium carbonate : $(NH_4)_2CO_3$.

In urine which has undergone this alkaline fermentation a turbidity ensues; a part of the ammonia escapes; a part forms, with uric acid, acid urate of ammonia, which separates out in small granules covered with spines, or a part becomes combined with phosphoric acid (H_3PO_4) and magnesium, as triple phosphate; the latter forming the "coffin lid" crystals. Amorphous phosphates of lime and bacteria are also present.

* Pasteur: "Compt. Rend.," t. l., 1860, p. 869.
† Van Tieghem: "Compt. Rend.," t. lviii., 1864, p. 210.
‡ Dr. Sheridan Lea: "Journ. of Physiology," vol. vi., No. 3.
§ Musculus: "Compt. Rend.," t. lxxviii., 1874, p. 132.

The Transparency of the Urine.—Diminished transparency may be due to three causes:*—(1) mucus, this may be detected by adding acetic acid, which produces slight increase in the opacity; (2) the earthy phosphates of calcium and magnesium: these disappear with nitric acid (heating alone precipitates them, but the precipitate disappears on adding acetic or a mineral acid); (3) the so-called mixed urates of sodium, potassium, calcium, and magnesium, which are deposited when the urine cools, but disappear on heating.

In the case of urine containing pus and in chylous urine the transparency is diminished. Where much pus or mucus is present the urine may be viscid or glutinous.

The Colour of the Urine may vary in healthy conditions, owing to concentration or dilution; or in disease, owing to the presence of blood, bile, or other pathological pigments; or it may be altered by drugs, or by extraneous colouring matters added by the patient in order to deceive the doctor. The urine of diabetes, of hysteria, anæmia, convulsions, and granular kidney is generally pale, while in fever and febrile states it is high-coloured, owing to the diminution in the quantity of water. Blue and brown urine may be seen, the former due to the presence of indigo blue, and the latter to methæmoglobin.

The following very useful table is from Krukenberg† :—

PATHOLOGICAL AND OTHER URINE-COLOURS (KRUKENBERG).

COLOUR OF URINE.	CAUSE OF COLOURATION.	PATHOLOGICAL CONDITION.
Pale yellow to colourless	Diminution of normal pigments	Anæmia, chlorosis, diabetes, and after nervous attacks
Dark yellow to brown-red: easily depositing a sediment	Increase of normal or occurrence of pathological pigments	Acute febrile diseases

* Tyson: "Practical Exam. of Urine," 5th ed., 1886, p. 19.

† "Grundriss d. med. chem. Analyse," 1884, S. 78.

Colour of Urine.	Cause of Colouration.	Pathological Condition.
Yellowish and milky	Fatty drops floating in the urine	Chyluria
	Suspended pus-corpuscles	Pyelitis, or other purulent disease
Green or yellow-green	Bile colouring-matters	Jaundice
Greenish - yellow, later greenish-brown, or approaching black	Decomposed hæmoglobin, and various chromogens. Carbolic urine is similar	Hæmorrhages into the kidneys, long-continued intermittent fever ; also melanotic cancer
Red or reddish	Unchanged hæmoglobin	Hæmorrhages, or hæmoglobinuria ?
	Pigments which are taken up with the food (*e.g.*, madder, bilberries, logwood, fuchsin)	
Brown	Bile colouring matters and methæmoglobin	
Brown - yellow to red-brown, becoming blood-red on adding alkalies	Substances which are introduced into the organism with senna, rhubarb, and chelidonium	

Vogel has drawn up a scale in which the colours are divided into: (1) pale yellow, (2) bright yellow, (3) yellow, (4) reddish yellow, (5) yellowish red, (6) red, (7) brownish red, (8) reddish brown, and (9) brownish black.* It is as well, when describing the colour of urine, to use this nomenclature.

Acid urine is generally deeper in colour than alkaline,

* Neubauer and Vogel : "Guide to Analysis of Urine," American trans., 1879.

and on acidulating an alkaline urine it becomes deeper in colour.*

Santonin and chrysophanic acid both colour the urine yellow: to distinguish them, G. Hoppe-Seyler recommends, after adding caustic soda, which in each case colours the urine red, to agitate the urine with amyl alcohol. The santonin colouring matter goes into this and gradually changes, in contact with the oxygen of the air, into yellow, whereas that of the other is insoluble or almost insoluble in amyl alcohol, and its colour persists for a long time. The spectroscope also shows differences.†

The special colouring matters of normal and pathological urine will be described by-and-bye.

Quantity of Urine and its Specific Gravity.—The average amount of urine of a healthy male adult is said to be 1,500 c.c. or about 50 fluid ounces in twenty-four hours. Tyson thinks this is slightly too high, and puts it at from 40 to 50 ounces, or from 1,200 to 1,500 c.c.‡ With increased action of the skin its amount is diminished. The smallest amount is secreted between 2 and 4 a.m., the greatest between 2 and 4 p.m. (Weigelin). Women pass less urine than men, from 900 to 1,200 c.c. in twenty-four hours (Landois and Stirling).

The specific gravity varies, under normal conditions, from 1015 to 1025, though by excessive draughts of water it has been known to sink to 1002, and after great sweating, as after forced marches, it has risen to from 1035 to 1040.§ In infants it varies from 1003 to 1006. Special vessels are used for collecting the urine of twenty-four hours.

A healthy adult male excretes about 70 grams or 2½ ounces of solids daily in his urine.

* The urine of digestion (*urina cibi*) may be reddish-yellow, and after free potations (*urina potus*) it may be nearly colourless. In diabetes it may be pale green, in melanæmia dark brown, and in melanuria black.

† G. Hoppe-Seyler: "Chem. Central.," 1886, S. 746.

‡ Tyson: Loc. cit., p. 29.

§ Dr. W. G. Smith has recorded a specific gravity of 1065.

In diabetes mellitus the specific gravity may reach 1050 and one above 1028 should excite suspicion of that disease. But urine of low specific gravity, even as low as 1010, may contain sugar* (Tyson). The specific gravity is increased in the first stage of fevers, and in the first stage of acute Bright's disease, while it is diminished in diabetes insipidus, in hysterical and spasmodic hydruria, and in granular kidney. In all stages of Bright's disease (except in the first stage of acute) the specific gravity is decreased owing to the diminished excretion of urea. As a rule, diminished specific gravity means less urea, and increased specific gravity means more urea, *i.e.*, if sugar be absent.

The specific gravity is calculated either by means of a urinometer, or, as it is called in Germany, an aräometer, or, if greater accuracy is preferred, by a specific gravity bottle, or as it is called in Germany, a picnometer. The temperature should be noted at the same time, and increased or diminished as required, as each instrument is graduated for a certain temperature. The vessel in which the urine is placed should be large enough to prevent contact of the urinometer with its sides, and should be placed on an even surface, and the eye brought on a level with the upper surface of the fluid in taking the reading on the stem of the urinometer. Air-bubbles should be removed by means of blotting-paper. If the stem is greasy, little drops of water are apt to cling to it; and if it is put into the fluid wet, it sinks too low. Usually, urinometers are incorrectly graduated; the graduations should not be equal, but get closer towards the bulb, because allowance must be made for the weight of the stem above the level of the fluid. Every instrument should be checked against a correct one, or by means of a specific gravity bottle. It should sink to 0° (usually taken as 1000) of its scale when immersed in distilled water at the temperature for which it was graduated.

* This would be likely to occur in cases of granular kidney accompanied by glycosuria.

If too little urine is available the method of mixture with distilled water may be adopted. Thus, suppose it is necessary to add three times as much water as urine to enable the urinometer to float (there are four volumes present), and that the specific gravity of the mixture is 1003, then the specific gravity of the urine $= 1000 + (3 \times 4) = 1012$. But if the instrument is incorrect, the error is magnified by the number of volumes used. Hence it is not advisable to rely too much on this method (Tyson). For small quantities of fluid, Wilson's specific gravity balls may be used. These are simply small hollow balls of glass, and the specific gravity of a fluid is determined by putting different balls into it until one will float. Balfour Stewart and Gee describe how they are made, and state that they will "show a difference in the third place (of decimals) in the density of a liquid."*

In taking the specific gravity with the specific gravity bottle a chemical balance is required. The absolute weight of a given volume of fluid divided by the absolute weight of an equal volume of distilled water gives the specific gravity.

In taking the specific gravity, the urine of twenty-four hours should be collected, and a sample selected from it.

Estimation of Total Solids.—By means of a simple formula, known in this country as Christison's formula, and abroad as that of Haeser or Trapp, we can determine approximately the amount of solid matters excreted in the urine in twenty-four hours. The last two figures of the specific gravity expressed in four figures are to be multiplied by 2·33 (Haeser and Christison), or by 2 (Trapp), or 2·2 (Loebisch). Thus, if a person passes 1,200 c.c. of urine in twenty-four hours, and the specific gravity of a sample taken from it is 1022, then

$$22 \times 2{\cdot}33 = 51{\cdot}26 \text{ grams in } 1{,}000 \text{ c.c.},$$

$$\text{or in } 1{,}200 \text{ c.c.} = \frac{51{\cdot}26 \times 1{,}200}{1{,}000} = 61{\cdot}51 \text{ grams.}$$

Although this method answers for rough clinical estima-

* Stewart and Gee: "Lessons in Elementary Pract. Physics," 1885.

tion, it is not accurate enough for scientific results. In the latter case the solids must be estimated by a chemical balance, after evaporation of all the water, as follows (Neubauer and Vogel)* :—10 to 15 c.c. of urine (from a twenty-four hours' collection) are weighed or measured into a small accurately-weighed porcelain crucible, which can be closed by a cover, and the urine evaporated to dryness on a water bath. The residue is then dried at 100° C. in an air bath for one or two hours, and then placed in an exsiccator to cool over strong sulphuric acid and weighed. It is then heated again to 100° C. and weighed a second time. If it has not sensibly decreased in weight, the operation is finished, and after subtracting the weight of the crucible the weight of the residue is obtained. If this weight is subtracted from that of the quantity of urine taken, the weight of water in this is also known.

Example.

Amount of urine in twenty-four hours = 1,000 c.c. of sp. gr. 1·024. 10 c.c. were evaporated to dryness, and the residue dried at 100° C.

Crucible + residue = 24·891 grams.
Crucible alone = 24·350 „
Residue = 0·541 „

0·541 gram of residue is contained in 10 c.c. urine, so that in 1,000 c.c. there are 54·1 grams of solids. And

1,000 c.c. of urine of 1·024 sp. gr. = 1024·0 grams.
The residue from this = 54·1 „
Evaporated water = 969·9 „

But errors arise in this method from the action of the acid phosphate of sodium on the urea, so that in very accurate work the improved methods of Neubauer should be adopted.†

Another better method is to take 5 c.c. of urine and eva-

* Neubauer and Vogel : Loc. cit., p. 216.

† Neubauer and Vogel : Loc. cit., p. 218. Cf. also Salkowski and Leube : "Die Lehre vom Harn.," 1882, S. 13.

porate it in the exhausted receiver of an air-pump in a weighed capsule placed over sulphuric acid, and leave it for twenty-four hours; then weigh, place again in the receiver, and weigh again after it has been twenty-four hours more *in vacuo*.*

Odour of Urine.—The smell of urine is said to be due to small quantities of phenylic, taurylic, and damoluric acids. In diabetes it has a sweetish or hay-like smell. Urine containing cystin smells at first like sweet-briar, but afterwards becomes very offensive. It is said to smell of sweet-briar, also, in oxaluria. Turpentine makes it smell of violets; copaiba, cubebs, and santal oil give it their own odour. The smell of urine after taking asparagus and garlic is well known, and when it contains decomposing blood or pus it has a putrid odour.

Reactions of Normal Urine towards Reagents.—The following reactions give a general idea of the qualitative composition of urine†:—

(1) A little hydrochloric acid added to a large quantity of urine (5 c.c. to 100) causes coloured crystals of uric acid of various shapes to separate out after ten to twelve hours.

A large quantity of hydrochloric acid to a little urine (3 to 1) causes the formation of indigo blue and indigo red, the urine becoming coloured pale red, then brownish red, or violet to deep blue. (Other chromogens may be oxidized at the same time and help in the colouration, *e.g.*, skatol compounds and the reducing substances.)

(2) If some urine is gently poured on to the surface of common red nitric acid, a garnet red zone forms at the surface of contact (Heller's "urophaein ring"), which is better seen against a white back-ground. (In urines rich in uric acid just over the ring there may be a white layer, caused by the separation of urates, which is often mistaken for albumin.)

* Charles: "Elements of Physiol. and Pathol. Chemistry," p. 414.
† Krukenberg: Loc. cit., S. 80.

(3) Caustic soda as well as ammonia precipitates out the phosphates of the alkaline earths, which separate partly in bunches of long needle-shaped crystals, partly in the amorphous form.

(4) Warming with phospho-molybdic acid causes, after acidulation with nitric acid, a lively blue colouration, due to its action on the urates.

(5) Iodized starch is decomposed by urine (presence of H_2O_2).

(6) By adding mercuric nitrate in solution for some time a clouding arises, which disappears on agitation, due to the formation of sodium nitrate and perchloride of mercury ($[NO_3]_2Hg + 2NaCl = 2NaNO_3 + HgCl_2$) soluble in acid urine. After all the chloride of sodium is decomposed, a permanent precipitate forms, due to a combination of the urea with the salt of mercury.* (This is the basis of Liebig's method of estimating urea.)

(7) Chloride of barium precipitates baric sulphate, $BaSO_4$, and phosphate, $Ba_3(PO_4)_2$; when this precipitate is dissolved in hydrochloric acid a cloudiness is left.

(8) Nitrate of silver precipitates silver chloride, AgCl, and phosphate, Ag_3PO_4; the latter first, afterwards all the chlorine in the urine combines with the silver.

(9) Lead acetate precipitates out sulphate of lead, $PbSO_4$, phosphate, $Pb_3(PO_4)_2$, and chloride, $PbCl_2$, besides other substances.

(10) Ferric chloride precipitates, after previously acidulating with acetic acid, phosphate of iron $Fe_2(PO_4)_2$.

(11) An ammoniacal solution of cupric oxide is decomposed by boiling, by the action of the urates.

(12) Tannic acid produces no precipitate with normal urine.†

* The first clouding is due to the urea compound, which is, on shaking, decomposed as long as NaCl is present (Halliburton).

† From Krukenberg : "Grundriss d. med. chem. Analyse," 1884.

CHAPTER III.

CONSTITUENTS OF NORMAL URINE: UREA AND ITS ESTIMATION.

Constituents of Urine.—The best classification of the urinary constituents with which I have met is that given by Hoppe-Seyler in his large work on Physiological Chemistry.* It is as follows† :—

(1) Urea and related bodies: uric acid, allantoin, oxaluric acid, xanthin, guanin, creatin, creatinin, thio-cyanic acid.

(2) Fatty non-nitrogenous bodies: fatty acids of the series $C_nH_{2n}O_2$; oxalic acid, lactic acid, glycerin-phosphoric acid; also inosit.

(3) Aromatic substances: the ether-sulpho-acids of phenol, cresol, pyrocatechin (catechol), indoxyl, scatoxyl; also hippuric acid, cyanuric acid, paraoxyphenylacetic acid, parahydrocumaric acid, &c.

(4) Inorganic salts: chloride of potassium and chloride of sodium, sulphate of potassium, phosphate of sodium, calcium and magnesium phosphate, soluble silicic acid, ammonia combinations, calcium carbonate, &c. (Traces of iron and nitric and nitrous acids may also be present.)

Certain bodies, such as the pigments of normal urine, are too little known to enable their being placed in any of these groups.‡

Of gases the urine contains a little nitrogen and carbonic acid, and it also contains peroxide of hydrogen and sometimes sulphuretted hydrogen.

* Hoppe-Seyler: "Physiologische Chemie," S. 800.

† Slightly modified by myself.

‡ There are also sulphur compounds, pepsin, lævo-rotatory substances, cryptophanic acid, extractives, animal gum, humous substances, &c., present.

In certain diseases there occur, in addition to the above constituents, albumin and other proteids, grape-sugar, milk-sugar, hæmoglobin, methæmoglobin, bilirubin, biliverdin, bile-acids, leucin and tyrosin, oxymandel acid, fats, lecithin, cholesterin, cystin, and abnormal pigments.

From food and medicine, either unchanged or changed materials of different kinds pass over into the urine.

It is quite impossible, in elementary lectures such as these, to consider all the constituents, or, indeed, many of them, in detail; besides, only a few are of interest from a medical point of view, and I shall therefore limit myself to a consideration of the more important, and their detection and significance. I do not intend to describe more than two methods for the quantitative analysis of those which one has at times to estimate, as it would only lead to confusion to describe a number; and some of these methods have not stood the test of time. I will endeavour to select the easiest and most rapid, although I may run the risk of being criticised for so doing.

Amount of some Constituents present in Normal Urine.—According to the most recent analyses—those of Yvon and Berlioz—the following figures represent the mean composition of normal human urine* :—

	Male.	Female.
Volume, per diem	1,360c.c.	1,100c.c.
Specific gravity	1·0225	1·0215
Urea (per liter)	21·5 grams	19·0 grams
„ (per diem)	26·5 „	20·5 „
Uric acid (per liter)	0·5 „	0·55 „
„ „ (per diem)	0·6 „	0·57 „
Phosphoric acid (per liter).	2·5 „	2·4 „
„ „ (per diem)	3·2 „	2·6 „

For comparison with these figures I give here a complete quantitative analysis of urine copied from Foster's

* Yvon and Berlioz: "Rev. Med.," viii., 713—718; "Lancet," ii., 1888, p. 629; and "Journ. Chem. Soc.," Dec., 1888, p. 1320.

"Physiology"—presuming that nothing more accurate has been published up to date.

Amounts of the several urinary constituents passed in twenty-four hours. (After Parkes.)

	By an Average Man of 66 Kilos.	Per One Kilogram of Body Weight.
Water	1,500·000 grams.	23·0000 grams.
Total solids	72·000 „	1·1000 „
Urea	33·180 „	·5000 „
Uric acid	·555 „	·0084 „
Hippuric acid	·400 „	·0060 „
Creatinin	·910 „	·0140 „
Pigment and other substances	10·000 „	·1510 „
Sulphuric acid	2·012 „	·0305 „
Phosphoric acid ...	3·164 „	·0480 „
Chlorine	7·000 (8·21)	·1260 „
Ammonia	·770 „	
Potassium	2·500 „	
Sodium	11·090 „	
Calcium	·260 „	
Magnesium	·207 „	

The various constituents of urine under normal conditions and their variations under diseased states, the methods of detecting them and of estimating quantitatively the more important, may now be referred to.

Urea [$CO(NH_2)_2$].—Urea is of importance to the medical man because its amount is dependent on the amount of the metabolism of the proteid constituents of the food and those of the body. Its sudden diminution in the urine of a case of Bright's disease (the diet being constant) is of far more dangerous import than an increase of albumin. It is as well to become acquainted with its general characters first. It is the diamide of carbonic acid (CO_2) or carbamide.* From urine it

*Although this is now denied, for "although isomeric with carbamide the reactions are not those of an amide" (Allen.)

may be prepared for purposes of study as follows:—The urine is evaporated on the water bath to the consistence of a syrup. On adding to the mass gradually, and with constant stirring, concentrated pure nitric acid to excess, nitrate of urea crystallizes out; this should be then laid aside for twenty-four hours, then decanted, and the precipitate collected on a linen filter and pressed. It should then be spread out on a porous earthenware plate, and after some time dissolved in hot water. To the filtrate baric carbonate is added in excess, then a little

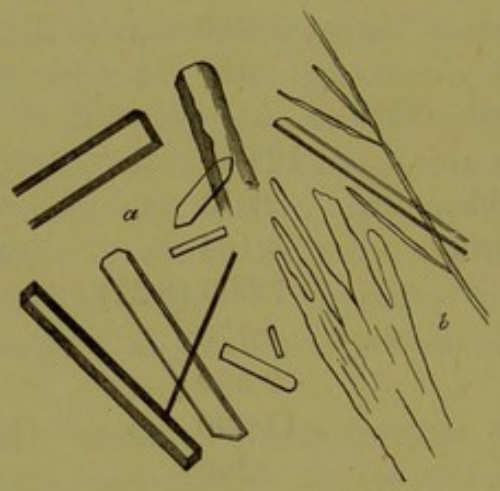


FIG. 7.—CRYSTALS OF UREA.

a, Four-sided pillars. *b*, Indefinite crystals, such as are usually formed in alcohol solutions.—(*Frey.*)

caustic baryta, a stream of CO_2 is now passed through the fluid for a few minutes. The solution is again filtered, the filtrate evaporated, and the impure urea spread on the filter paper to dry, then boiled in absolute alcohol, filtered, and the filtrate allowed to stand, when the urea crystallizes out.*

Pure urea is readily soluble in alcohol and water, but insoluble in ether; it has no action on litmus paper, and has no smell; it has a taste somewhat like saltpetre. It crystallizes in silky four-sided prisms, with oblique ends (Fig. 7, *a*), or when rapidly crystallized it is found in delicate

* Charles: "Elements of Phys. and Path. Chem." p. 423.

white needles (Fig. 7, *b*). It was prepared synthetically in 1828 by Wöhler from ammonium cyanate—$CN.ONH_4$. When this is heated to 100° C. the atoms rearrange themselves and form urea thus : — $CN.ONH_4 = CO(NH_2)_2$. Urea may also be prepared by the action of ammonia on carbonyl chloride, thus :—

$COCl_2 + 4\ NH_3 = CO(NH_2)_2 + 2NH_4Cl$;* and by other methods. By taking up water it is readily changed into carbonate of ammonia, as occurs under the influence of a ferment. Urea is recognized readily by the characteristic

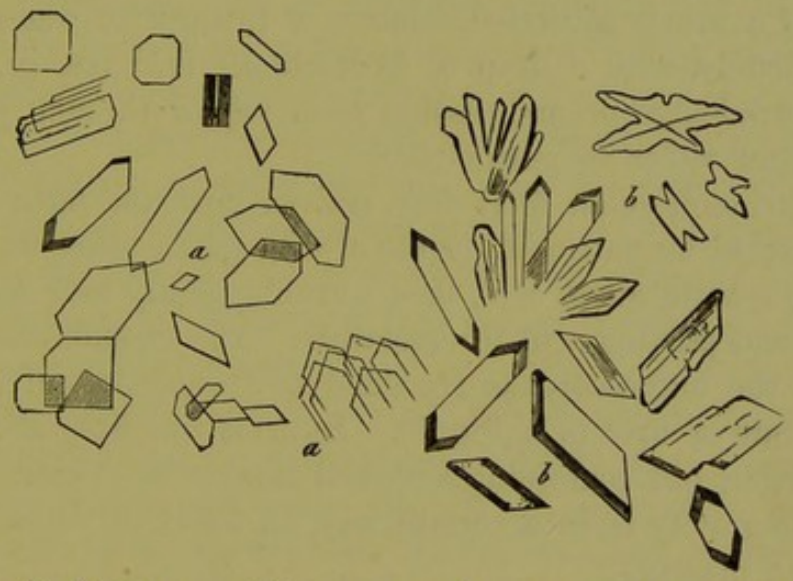


FIG. 8.—CRYSTALS OF NITRATE AND OXALATE OF UREA.—(*Frey.*)
a a, Nitrate of urea. *b b*, Oxalate of urea.

crystals which it gives when treated with nitric and oxalic acid (Fig. 8), as it then forms nitrate (CON_2H_4,NO_3H) and oxalate [$(CON_2H_4)_2C_2H_2O_4 + H_2O$] of urea respectively. It also can be made to give the biuret reaction, a property which it shares with the peptones. Thus, if crystallized urea be kept in a combustion tube over a small flame for a long time in the melted state, and the melted mass, after the tube is cold, be dissolved in water, the solu-

* See Strecker-Wislicenus : "Organic Chemistry," Eng. trans., p. 92 ; or the Appendix to Foster's "Physiology."

tion shows, on treatment with a couple of drops of caustic soda solution and the same of diluted sulphate of copper solution, a purple colour in the cold.* Two molecules of urea furnish in this way one of biuret; or—

$$\begin{matrix} CO\langle^{NH_2}_{NH_2} \\ CO\langle^{NH_2}_{NH_2} \end{matrix} = \begin{matrix} CO\langle^{NH_2}_{NH} \\ CO\langle^{NH_2}_{NH_2} \end{matrix} + NH_3.$$

Another test for urea not generally mentioned in text-books is this—namely, if a crystal of urea be treated with a drop of a nearly saturated solution of furfurol in water, and immediately with a drop of hydrochloric acid (sp. gr. 1·1), a colour-change occurs, passing from yellow through green, blue, and violet to purple-red.†

In urine or other fluid very rich in urea, the latter may be detected by putting a couple of drops on a glass slip and adding a little nitric acid, then warming gently over a spirit lamp, and putting acid to crystallize. The crystals are seen under the microscope as six-sided and quadrilateral plates of nitrate of urea (*a a*, Fig. 8). In urine free from albumin and sugar, and containing a normal amount of chlorides, the specific gravity is, in a certain way, an index to the amount of urea present.

The amount of urea excreted by a healthy adult male‡ in twenty-four hours is from 30 to 40 grams, or from 463 to 617 grains. Children excrete more urea, *in proportion to their size*, than adults, owing to the greater activity of the metabolic processes in childhood. Within three to four hours after a meal, the excretion of urea is at its maximum: it reaches its minimum during the night. Food influences the

* Krukenberg: "Grund. med. chem. Anal.," S. 82; and Foster's "Physiology."

† Krukenberg: Loc. cit.; also Charles: Loc. cit., p. 427.

‡ The following are the estimates given for twenty-four hours (after Charles):—17·5 to 23·5 grams, or 270 to 360 grains (Flügge); 30·0 to 35·0 grams, or 460 to 540 grains (Voit); 25·0 to 40 grams, or 390 to 620 grains (Vogel); and 34·8 grams, or 537 grains (Oppenheim and Meyer).

excretion of urea: it is more abundant after a diet of meat. It is *not* increased by muscular exertion, as was supposed to be the case at one time. "The quantity of urea passed with the urine in a given time does not depend upon the amount of urea produced, but also upon whether the urea formed in the body is completely separated or partially retained in the blood and parenchymatous fluids. Hence the quantity of urea increases temporarily with the increase of the urinary secretion, and diminishes when it lessens."* A long-continued increase of urea in the sick always indicates increased tissue metabolism, but a temporary increase may only be caused by an increase of the urinary secretion, by which the urea *collected in the body* is quickly passed off, and may not be due to an increased production of urea (Neubauer and Vogel).

Diminished excretion of urea may be due either to (1) decreased metabolism of the proteids, or (2) retention of urea in the organism, as in uræmia and dropsy.

In all acute febrile diseases it is generally increased at the commencement of the attack and up to the acme of the fever, and this independently of food; but it does not necessarily fluctuate with the temperature. When the fever diminishes, it may sink below the normal, and return during convalescence to the normal. In intermittent fevers it is increased during the paroxysm of fever, and it may begin to increase even before the cold stage. In most chronic diseases it falls below the normal, but during hectic it increases again. Towards the fatal termination of many it may fall to 5 or 6 grams daily.

In dropsical conditions the urea is often diminished, as a portion may be dissolved in the dropsical fluids, and thus be retained in the body. In anæmia, hydræmia, chronic alcoholism, and syphilis it falls below the normal.

Dr. Andrew Clarke describes a condition under the name of "renal inadequacy," in which too little urea is excreted, although no disease can be otherwise detected, but the

* Neubauer and Vogel: Loc. cit., p. 476.

existence of this condition is considered doubtful. In Dr. Prout's "azoturia," a very large quantity of urea is said to be daily excreted, but there is no such disease. In diabetes, urea is always largely in excess, due, probably, to the great amount of food eaten (at least in part) and to vaso-motor disturbances. And a sudden fall in the excretion in diabetes is an unfavourable sign, and may indicate the onset of diabetic coma; in such cases, the amount of sugar falls also, though after the urea.*

Degenerative changes in the liver may be accompanied by a decrease of urea in the urine. In this connexion, the interesting observations of Dr. Noel Paton on the relationship of urea formation to bile secretion may be profitably studied.† He shows that urea production is increased by destruction of blood-corpuscles (which takes place in the liver), and that bile secretion and urea formation bear a direct relationship to one another. Certain drugs, such as phosphorus in small doses, morphia, codeia, arsenic (Gäthgens), and compounds of antimony, increase the excretion of urea, while quinine diminishes it.‡ It is increased by mineral acids and excess of alkaline chlorides. Urea has been found in the blood, lymph, chyle, liver, lymphatic glands, spleen, lungs, brain, eye, bile, saliva, amniotic fluid, and, pathologically in sweat and in dropsical fluids. §

We do not know, as yet, where it is formed: probably in the liver and lymphatic glands?

Although we know it is formed from proteids, we cannot trace it back through its precursors—the intermediate products of metabolism. At one time it was thought to be formed from uric acid, and that uric acid indicated an imperfect oxidation of urea, but this idea is now abandoned.

* Ralfe: "Clinical Chemistry," p. 120.

† Noel Paton: "Brit. Med. Journ.," July 31, 1886, p. 207.

‡ Oppenhein found that quinine increased it: Pfluger's "Archiv," xxiii., S. 446—504. This is a very important paper.

§ Landois and Stirling: "Physiology," 3rd ed.

"It is the chief end-product of the oxidation of the proteids," and "less oxidized products of such metabolism are uric acid, guanin, xanthin, hypoxanthin, alloxan, allantoin" (Landois and Stirling). "Decomposition of the albumin-molecule may produce leucin, tyrosin, glycocolle (glycocine), and amido-acids, or amides; and these substances, especially the two first, are formed in the alimentary canal, and probably are thence absorbed and carried to the liver. In cases of acute atrophy of the liver, urea is said to disappear from the urine;* and it has been supposed that this occurs because the hepatic cells cannot effect such chemical changes on leucin and tyrosin as normally produce urea. Further, the direct injection of leucin and glycocolle into the bowel increases the amount of urea, and 'all the nitrogen of the leucin and of the glycocolle appears in the urea'? (Beaunis)" . . . . "Thus, whilst it is clear that urea results from decomposition of proteid matters, the exact steps by which this is accomplished are unknown; and the question whether urea may arise from decomposition only, or from the synthesis of the products of decomposition, is still unsettled."†

The urea is, at all events, not formed in the kidneys: it is merely separated from the blood and got rid of by means of them; whether creatin, as at one time was supposed, furnishes a great part of the urea, seems doubtful. But the consideration of that question is discussed by Prof. M. Foster in his masterly work on Physiology, where anyone can read the discussion of this problem for himself.‡

Compounds of Urea.—Besides the nitrate of urea, which occurs in characteristic rhombic crystals, and the oxalate in groups of rhombic tables, which are often irregular in shape, urea forms a combination with phosphoric acid—phosphate of urea—which can be obtained from the urine of pigs fed on dough. A compound of chloride of sodium and urea is

* And to be replaced by tyrosin and leucin.

† McKendrick: "General Physiology," 1888, p. 86.

‡ Foster: "Physiology," 4th ed., p. 436, *et seq.*

sometimes found in human urine when concentrated by evaporation, and occurs in rhombic prisms. Urea also forms a compound with mercuric nitrate, referred to before, and this compound is obtained in Liebig's volumetric process, of which it forms the basis of the reaction. (According to Liebig three separate compounds of urea and mercuric nitrate are formed.) Compound ureas, such as ethyl-, diethyl-, and acetyl-urea, &c., can also be formed.

Uræmia.—The old idea that the train of symptoms included under the name of uræmia, such as drowsiness, coma, convulsions, delirium, sometimes Cheyne-Stokes breathing, vomiting, diarrhœa, and so on, were due to the retention of urea, is now abandoned. The "extractives" and animal alkaloids probably play a much more important part. It is certain that urea injected into the blood of animals whose kidneys are intact, provided that the quantity used is not excessive, is not followed by uræmic symptoms, owing to the rapid excretion of the excess of urea by the kidneys. Feltz and Ritter produced uræmia in dogs by injecting salts of ammonia; but the idea of Frerichs, Stannius, and Treitz that the urea is decomposed in the blood into carbonate of ammonia, and produces "ammoniæmia," cannot now be received.

Ligature of the ureters in animals, in which the urea is replaced by uric acid, as in birds and snakes, produces a comatose condition, as Zalesky has shown, so that urea cannot be the sole cause of the coma. Creatinin injected into the blood of dogs causes feebleness and contractions of the muscles (Meissner). Bernard, Traube, Feltz, and Ritter attributed the effects to an accumulation of the neutral salts of potassium in the blood. The injection of urine itself into the blood, however, produces toxic symptoms, as shown by Bocci and Claude Bernard, in the case of frogs and rabbits. R. Lepine and Aubert* experimented on the effect of injecting normal urine and the salts obtained by evaporating it, respectively, into the femoral vein of dogs, and they found

* Lepine and Aubert: "Compt. Rend.," ci., pp. 90—92.

that 85 per cent. of the toxic effect is due to the *saline* constituents. But they also found that *febrile* urine is much more poisonous in its effect than normal urine, and that *only 55 per cent. of the toxic effect of febrile urine is due to the salts; being due, therefore, to some other poison, which differs in its action from that of normal urine.* Whereas the poison of normal urine produces violent clonic convulsions, that of febrile urine kills by stopping the action of the heart. Teissier and Roque* state that an increase in the toxic effect of urine affords valuable evidence of increased gravity in the condition of the patient; but this does not hold good, especially in cases of nephritis. We might expect this, because in nephritis the poison is retained in the system. They found that albuminous urine secreted during sleep is more poisonous than that secreted when awake, whilst with normal urine the reverse is the case. No definite connexion could, however, be traced between the general composition of the urine and its toxic action.

I think there can be very little doubt that the animal alkaloids known as ptomaines and leucomaines play a most important part in both uræmia and probably in Küssmaul's coma, to be referred to again. "According to M. Gautier we resist auto-infection by two modes or mechanisms: by the elimination of the toxic products, and by their destruction by oxygenation. Elimination is accomplished by the kidneys and by the liver."† Of course, if the kidneys are diseased these are retained in the blood, and poison the nervous centres. We can easily understand, too, how, if the circulation in the liver, in which many poisonous alkaloids are destroyed, undergoes disturbances through vaso-motor influences, they may pass over into the blood of the hepatic vein and into the circulation, and produce their toxic effects.

"Observations on the venous injection of urine tend to . . . . show that the day urine affords a narcotic, sedative

* Teissier and Roque: "Compt. Rend.," cvii., pp. 272—275.

† Brown: "Animal Alkaloids," 1887, p. 147.

principle; the night urine, on the contrary, is marked by a stimulant, convulsive principle."*

It would appear that the condition known as uræmia is, however, due, not to one but to several poisons; but one cannot help thinking that the so-called extractives and ptomaines have much to do with its production.† The presence of a yet unknown acid in the blood has been suggested also as a cause.

Quantitative Estimation of Urea. — The specific gravity of the urine, in the absence of sugar and an excess of chlorides, enables one to form a rough guess as to the amount of urea present. So does the appearance of crystals of urea on evaporating a few drops of the urine on a glass slide. The amount of proteids eaten in the food should be taken into consideration, as these raise the amount of urea in urine more than anything else. The two methods which are generally used for the determination of urea in urine are—(1) Liebig's method or its modifications, and (2) the hypobromite method. Liebig's is preferable, it is said, for accurate work, but is not quite as rapid as the other, and it may require many corrections.

1. *Liebig's Method.*‡

This method depends on the fact that when a dilute solution of urea—about 2 per cent.—is treated with a dilute solution of mercuric nitrate, a precipitate falls, which has this composition, namely:—

$$(CON_2H_4)_2\ Hg(NO_3)_2+3HgO.$$

When to urine a weak solution of mercuric nitrate is added it combines with the phosphates; hence these have to be first removed, and if the urine contains albumin, that has

* Brown: Loc. cit., p. 98. See also an interesting paper on a new theory of sleep, by the same author, in the "Provl. Med. Journ." for Jan., 1889.

† Cf. Carter: Bradshaw Lecture on Uræmia, "Brit. Med. Journ.," Sept. 1, 1888. Cf. Saundby: Lecture on Bright's Disease, pp. 78, 79.

‡ The student is advised to procure Sutton's "Volumetric Analysis," and read therein the methods of urea estimation.

to be removed before estimating the urea. In the case of albuminous urine 100 to 200 c.c. are heated in a closed vessel on a water bath until complete coagulation takes place,

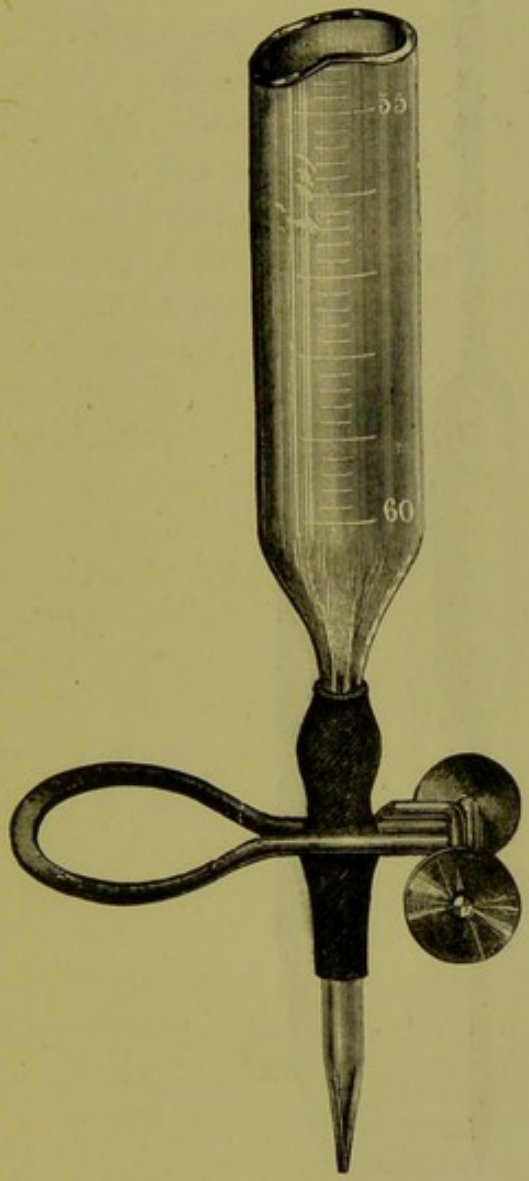


FIG. 9.—LOWER PORTION OF MOHR'S BURETTE, WITH INDIA-RUBBER TUBE AND CLIP.—(*Sutton.*)

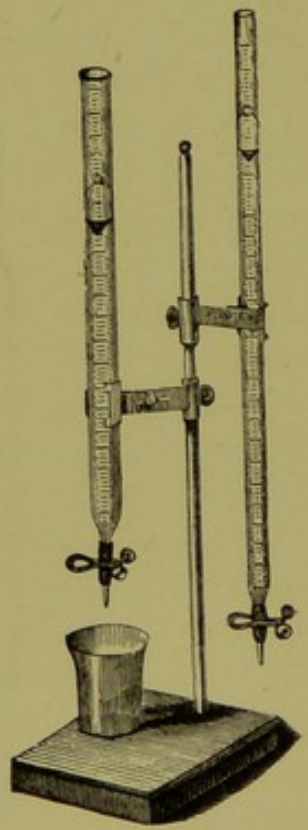


FIG. 10.—STAND FOR TWO BURETTES.—(*Sutton.*)

and thick flocculi have separated. If necessary, a few drops of acetic acid are added to complete the precipitation; but an excess must be avoided, or it will dissolve up some of

the albumin again. Heating for half an hour is sufficient. When the urine is cold it is filtered, and the clear filtrate used for the determination of the urea.

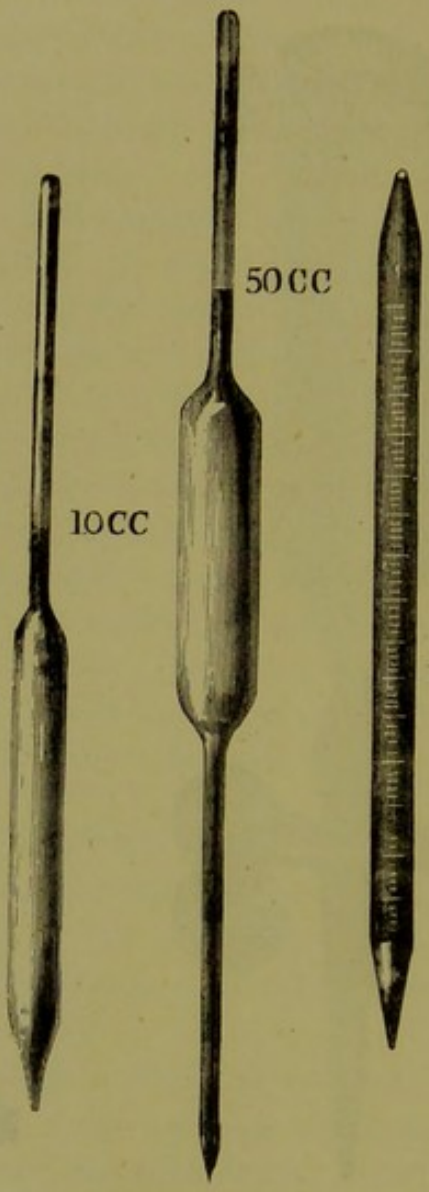


FIG. 11.—GRADUATED PIPETTES.—(*Sutton.*)

The following are required for carrying out the process :—

(1) A solution of mercuric nitrate, of which 1 c.c. is equivalent to ·010 gram or 10 milligrams of urea.

(2) A baryta mixture containing one volume of a cold saturated solution of barium nitrate, and two volumes of a cold saturated solution of barium hydrate, for precipitating the phosphates.
(3) A moderately dilute solution of sodium carbonate or bicarbonate.
(4) A Mohr's burette (Figs. 9 and 10).
(5) Three pipettes (Fig. 11)—one of 10 c.c., another of 15 c.c., and a third of 20 c.c. capacity.
(6) A standard solution of urea, containing 2 per cent.
(7) A glass plate with a bit of black paper underneath it, or a white porcelain plate.



FIG. 12.—A MEASURING FLASK TO HOLD ONE LITER.—(*Sutton.*)

The estimation ought to be done by daylight, as the yellow spot is difficult to detect by gaslight. To prepare the *standard solution of mercuric nitrate*, 71·48 grams of pure mercury are weighed off, placed in a beaker, and dissolved in pure

nitric acid. When dissolved the solution is warmed, and nitric acid frequently added, until no more traces of nitrous vapours appear, *i.e.*, until the mercurous has been changed into mercuric oxide. It is then evaporated to a thick syrup in the same beaker, and then diluted with water up to 1,000 c.c., or a liter, in a liter flask (Fig. 12); if a basic salt separates, it is allowed to subside, the clear fluid is poured off, and the precipitate is redissolved by means of a few drops of nitric acid, and added to the solution. Or, instead of mercury, we may use yellow mercuric oxide (HgO), of which 77·2 grams (dried at 100° C.), are dissolved in weak nitric acid, of which as little as possible is to be used, and dissolved by the aid of a gentle heat in a porcelain dish, and evaporated to the consistence of a thick syrup as before. This is then diluted with water, and made up to 1,000 c.c. or one liter. If on dilution basic salts separate, they should be dissolved in a little nitric acid, and the solution added to the former one.

This solution, prepared in either of the above two ways, has to be standardized by the *standard solution of urea*, which is thus prepared :—

Two grams of pure urea dried at 100° C. are dissolved in 100 c.c. of distilled water, so that 10 cubic centimeters contain 0·2 gram or 200 milligrams of urea. 10 c.c. of this urea solution are now placed in a beaker, and the burette filled up to the 0 mark with the solution of mercuric nitrate, taking care to read by the lower edge of the upper curved surface of the fluid (the lower edge of the meniscus, Fig. 13). The latter solution is then allowed to fall drop by drop into the urea solution, when a dense precipitate is seen to form. When the precipitate does not appear to increase with fresh drops added, a drop of the mixture, after stirring it up with a glass rod, is allowed to fall on a drop of the sodium carbonate solution on the glass plate, which should have several drops of the carbonate solution on it. If no yellow colour appears in the drop, a few more tenths of a cubic centimeter of the mercuric solution are added from the burette, and a drop again taken

from the mixture and added to a fresh drop of sodium carbonate; this procedure is continued until a yellow spot is seen distinctly. When this appears, we know that an excess of mercuric solution has been added, and all the urea is precipitated in combination with the mercuric salt, leaving some of the latter to react with the sodium carbonate, forming sodic nitrate and yellow oxide of mercury. If the mercuric solution has been properly prepared, 20 c.c. should be required to precipitate all the urea out of 10 c.c.

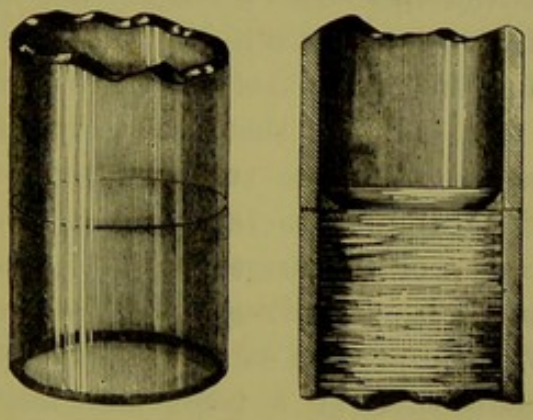


FIG. 13.—SHOWS LOWER CURVED SURFACE OF THE MENISCUS BY WHICH THE READING IS TO BE TAKEN (RIGHT-HAND FIGURE).—(*Sutton.*)

of the urea solution, and leave enough to react with the sodium carbonate.

If instead of 20 c.c., 19 of the mercury solution only were required to produce the end-reaction, we must add more water; we must dilute the solution so that 20 c.c. instead of 19 c.c. = 10 c.c. of urea solution: $19 : 1{,}000 :: 1 : x$

$\therefore x = \frac{1{,}000}{19} = 52{\cdot}6$ c.c. Hence 52·6 c.c. of water are added to 1,000 c.c., and the whole well shaken. If more than 20 c.c. are required the solution is too dilute, and the correct quantity of urea to be added must be calculated.

Application to Urine.* — The urine having been filtered, or, if albumin is present, this having been removed

* In all cases the urea should be estimated in a sample taken from the *mixed* urine of twenty-four hours.

in the manner described, 40 c.c. are mixed with 20 c.c. of baryta mixture; by this means the phosphates, sulphates, and carbonates are removed (and at the same time a little urea is lost). The mixture is then poured on to a *dry* filter, and, if necessary, re-filtered. If the filtrate shows, on adding fresh baryta solution to it, any cloudiness, we must add more baryta solution to the urine. 15 c.c. of this solution containing, of course, 10 c.c. urine, are measured off by means of the 15 c.c. pipette into a beaker. The burette is filled with the mercuric solution, and this solution allowed to fall drop by drop into the beaker. Tyson* gives a good rule for the amount of mercuric solution allowed to run in at first, namely, that a number of cubic centimeters, approaching the last two numbers of the specific gravity, should be allowed to run in first. For example, if the specific gravity of the urine was 1017, then 15 c.c. are allowed to run in before testing with the drop of sodium carbonate. We then proceed cautiously, adding a few tenths of a cubic centimeter at a time, and testing with the sodium carbonate until a yellow colour appears; when that appears the number of cubic centimeters added are read off. This number minus 2, multiplied by ·010 gram, gives the amount of urea in 10 c.c. of the urine. Two cubic centimeters are subtracted, because about that quantity of reagent is used up in converting the chloride of sodium present into the nitrate, and until that takes place the combination with the urea does not commence.

If the chlorides† are present in the urine in a quantity above or below the average, and greater accuracy is aimed at, the amount of the chlorides, calculated as chloride of sodium, in 10 c.c. of the urine must be estimated, and all the chlorides must be removed from a fresh quantity of

* Tyson: "Practical Examination of Urine," p. 147.

† "If the urine contains more than 1 per cent. NaCl, *i.e.*, more than 10 parts per 1000. In this case 2 c.c. must be deducted from the quantity of mercurial solution actually required to produce the yellow colour with 10 c.c. of urine" (Sutton).

urine by means of a standard solution of nitrate of silver. To effect this, a solution of nitrate of silver, made of such a strength that 1 c.c. = 10 milligrams of sodium chloride, is required. It is prepared by dissolving 29·059 grams of fused nitrate of silver in distilled water and diluting to a liter.

The chloride of sodium in 10 c.c. of the urine is then determined by this solution. If, *e.g.*, 17·5 c.c. of the silver solution are required, then there are 175 milligrams of sodium chloride present in the 10 c.c. of urine.

If we now take "30 c.c. (containing 20 c.c. of urine) of the filtrate from the mixture of baryta fluid and urine, add a drop of nitric acid, and then 17·5 × 2 c.c. = 35 c.c. of the nitrate of silver solution; this will precipitate all the chlorides, which should be separated by filtration, and the filtrate may now be estimated for urea. It is important always to bear in mind the exact amount of urine operated with after adding the nitrate of silver solution to a mixture of baryta solution and urine, of which only two-thirds are urine. Thus, if 35 c.c. of the silver solution are added to 30 c.c. of the filtered mixture of urine and baryta fluid, of the resulting 65 c.c. only 20 would be urine minus the chlorine, or out of 32·5 c.c. 10 would be urine minus the chlorine." *

With regard to the correction for chlorides, a titration with the mercuric solution may be made without removing the silver chloride *previously*. Two portions of the urine filtered from the baryta precipitate, amounting to 15 c.c. each, are taken. One is neutralized with nitric acid, and the mercuric solution cautiously added until a distinct cloudiness appears. The number of cubic centimeters used is then read off. The other 15 c.c. is titrated in the usual way, and the number of cubic centimeters necessary to produce the *end*-reaction read off; from this number is deducted the number of cubic centimeters required to produce the cloudiness in the former.

* Tyson: Loc. cit.

If the urine is found to contain more than 2 per cent. of urea, or less than that amount, the process has also to be modified, because it must be remembered that the mercuric solution is standardized by a solution of urea containing 2 per cent.

"If the number of cubic centimeters of mercury solution added to 15 c.c. of the mixture of urine and baryta fluid exceeds 30—that is, if the amount of urea in the unmixed urine exceeds 3 per cent.—we must, for the number of cubic centimeters of the mercurial solution required above 30, add half the number of cubic centimeters of water to the urine mixture and make a second titration.* Thus, suppose 36 c.c. are required on the first titration, the excess is 6 c.c., therefore 3 c.c. of water must be added to the mixture before making the second titration" (Tyson).

It will be noticed that Tyson allows for over 3 per cent. of urea, whereas Neubauer and Vogel, Charles, Foster, and others, make the rule apply to urine containing over 2 per cent.

When the urine contains less than 2 per cent., the amount of urea is found to be apparently increased if the mercuric solution is not diluted. In order to obviate this, "for every 5 c.c. of the mercury solution less than 30 c.c. which are used, 0·1 c.c. must be subtracted from the number of the c.c. of mercury solution used. If to 15 c.c. of urine 25 c.c. of mercury solution are used—that is, 5 less than 30 c.c.—for these 5 c.c 0·1 c.c. is subtracted, and the calculation made for only 24·9 c.c. of the mercury solution" (Neubauer and Vogel). Charles gives this rule:—"Deduct 0·1 c.c. for every 4 c.c. mercuric solution employed less than 20 c.c."†

Unfortunately, other nitrogenized bodies, such as creatinin, allantoin, xanthin, leucin and tyrosin, glycocine, taurin, are precipitated by mercuric nitrate, and it is beyond the power

* If this correction is not made, the true amount of urea would be *diminished*. Cf. Neubauer and Vogel: Loc. cit., p. 236.

† Charles: Loc. cit., p. 438.

of any busy individual to find out how much is to be allowed for these disturbing influences.*

After the use of large doses of salts of potassium, iodine, bromine, and chlorine, it is useless to endeavour to obtain accurate results with Liebig's process. It is then better to calculate the urea by the hypobromite process as well, and then to compare the results.

2. *The Hypobromite Method.*

When urea is acted upon by hypobromite of sodium (or hypochlorite of calcium, as Davy showed in 1854),† it is decomposed into nitrogen, carbonic anhydride, and water; thus with sodium hypobromite the reaction is:—

$$CON_2H_4 + 3NaBrO = N_2 + CO_2 + 2H_2O + 3NaBr.$$

One gram urea contains 0·4666 gram N = 372·7 c.c.; but actually in practice only 354·3 c.c. are obtained, *i.e.*, 8 per cent. less than the total N, but creatinin, urates, &c., also yield some N, which, to a certain extent, compensates for this loss, so that it may be left out of consideration. (M. Depaine deducts 4·5 per cent. from the urea found, to allow for the decomposition of uric acid and creatinin.)‡

In all the forms of apparatus described, there are required a vessel in which the urine and hypobromite can be mixed at the proper time, and a graduated vessel filled with water in which the gas is measured. These vessels being connected in such a way as to allow of the gas to flow from the vessel containing the urine into the graduated one.

* If the urine contains ammonic carbonate, its presence interferes with the correct estimation of the urea. In that case, precipitate with baryta as before, take a quantity corresponding to 10 c.c. of urine, evaporate on the water bath to expel ammonia, dissolve the residue in water and estimate the urea in the usual way. Estimate the ammonia in 50 c.c. of the urine itself by means of normal H_2SO_4 and litmus paper, and calculate the ammonia: 0·017 gram = 0·030 gram urea (Sutton).

† Davy: "Philosoph. Mag.," 1854, p. 345.

‡ Depaine: "Journ. de Pharm.," 1877.

A great number of apparatus have been recommended for estimation of urea by the hypobromite and hypochlorite process.* But one very simple and useful instrument, which gives fairly good results, however, is that of Dr. Doremus, of New York (Fig. 14), made by Southall Bros. & Barclay, of Birmingham. It consists of a bulb and graduated tube, and a small curved pipette (not shown in the figure) to hold one cubic centimeter of urine. The tube is filled with hypobromite solution of the usual strength to the mark on the

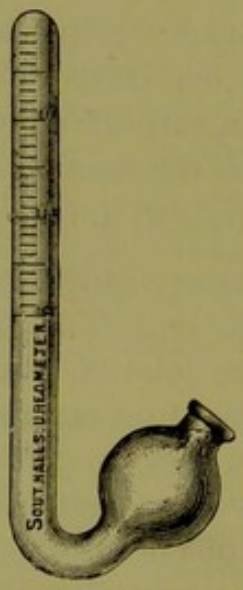


FIG. 14.—UREOMETER OF DOREMUS.

long arm of the apparatus, and then water added to fill the rest of the arm and the lower part of the bulb. The pipette is then filled with urine up to the mark, and its point carefully introduced as far into the bend as it will go, holding the measuring tube perpendicularly. The nipple is then slowly and thoroughly compressed so as to expel all the urine. The tube is graduated so that each of the small divisions = ·001 gram of urea. The percentage can be obtained by multiplying the result by 100.

* In America the solution of chlorinated soda of the U.S.P. is largely used in various apparatus, the simplest of which is Squibb's (figured in Ralfe's "Practical Treatise on Diseases of the Kidneys," p. 73).

Russel and West's apparatus was at one time much used, but it cannot now be recommended. In Germany, Hüfner's apparatus—in America, Greene's and its modified form by Marshall, are used; but for accurate results and for supplying all that is needed the apparatus of Gerrard* is as good as any, and better than most (Fig. 15).

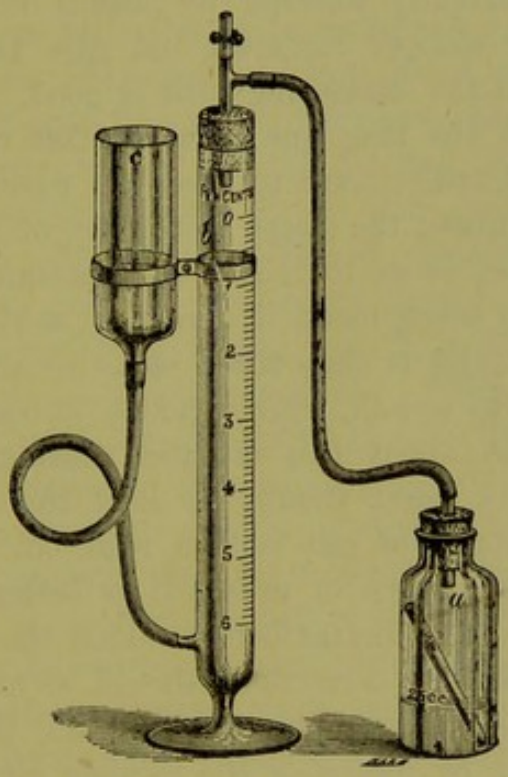


FIG. 15.—GERRARD'S UREA APPARATUS.

It consists of two tubes, supported on a stand, and connected by a piece of india-rubber tubing; the short tube is for filling the larger graduated tube,—in which the nitrogen is collected and measured,—with water, and the latter is connected with a bottle containing the hypobromite and urine, the urine being introduced in a small test tube by means of forceps. The top of the measuring tube is connected with a tube of india-rubber, which can be closed by a clamp. The hypobromite solution is made by dissolving

* Sold and manufactured by Gibbs, Cuxson & Co., of Wednesbury.

100 grams of stick caustic soda in 250 c.c. of water, *and when cold* 25 c.c. of bromine are added (or 3½ oz. of caustic soda, 9 oz. of water, 7 drams of bromine). This is best made in the open air, as the fumes of bromine are very disagreeable. 25 c.c. of hypobromite solution are introduced into the bottle, and a test tube containing 5 c.c. of urine is carefully lowered by means of forceps into the bottle, the stopper replaced, and care taken that the connexion with the measuring tube is good. By means of the short tube the long one is now filled with water up to the mark 0, taking care to keep the water at the same level in both tubes; the clamp at the top of the measuring tube being open so as to relieve the pressure, this is now shut (the water being made, if necessary, to rise to the zero point). The bottle is then tilted so as to allow the urine and hypobromite to mix, when reaction at once begins and is completed in about five minutes (during the reaction the level of the water should be kept the same in both tubes); the volume of gas is then read off. The tube is graduated in per cents. of urea. It is better, however, to wait for about ten minutes before taking the readings. In accurate experiments corrections should be made for atmospheric pressure, for temperature, and for the tension of aqueous vapour, by means of the formula given below. A nitrometer may be used for the hypobromite process, as Lunge proposes, or any other apparatus made on the same principle.

Apjohn's apparatus* is very easily fitted up; it is described in Finlayson's "Clinical Manual." Tyson† figures another, and he also describes Greene's.‡ Graham Steele's§ apparatus is highly spoken of by Graham Brown,‖ and it can easily be fitted up in any laboratory.

* Apjohn: "Chemical News," Jan. 22, 1875.
† Tyson: Loc. cit., p. 152.
‡ Greene: "Philadelphia Medical Times," Jan. 12, 1884, p. 278.
§ Steele: "Edin. Med. Journ.," 1874.
‖ Graham Brown: "Medical Diagnosis," 1883 p. 253.

If a vessel divided into cubic centimeters, such as a burette, be used for measuring the gas it must be raised or lowered—before the operation is begun—until the zero mark exactly coincides with the surface of the water in the containing cylinder, and before reading off the gas the level of the water within the tube and that outside of it must also be made to coincide. We must also remember to raise the measuring tube gradually, or, as in Gerrard's apparatus, lower the side tube as effervescence takes place, so as to relieve the disengaged gas from the hydrostatic pressure.

In some experiments by Pflüger and Bohland* it was found that the *total nitrogen* of the urine (see below) cannot be accurately obtained by Hüfner's method, the results being too low; even for urea alone they found the results too high, the variation ranging from 1 to 10 per cent., and this does not admit of compensation. Pflüger's new method of estimating urea can be applied if the nitrogenous extractives are first removed by acidulating a certain volume of urine with hydrochloric acid (1 of acid to 10 of urine), and then adding enough phospho-tungstic acid to ensure the precipitation of all the extractives, the mixture being then made up to a known volume, and allowed to remain twenty-four hours before filtering. The acid filtrate is then carefully neutralized with powdered lime and again filtered through a dry filter. The mean error of several analyses of urine by this process was + 1·3 per cent. According to Camerer,† however, for every 100 grams of nitrogen found by Hüfner's method, 13·6 must be added to obtain the total nitrogen. And this rule was found to answer well.‡ C. Méhu makes a very useful suggestion for getting rid of the frothing which sometimes prevents one taking the reading

* Pflüger and Bohland: "Pflüger's Archiv," xxxviii., S. 325, and xxxix., S. 1—17, also 143—158. Cf. also Garnier: "Journ. Pharm.," xv., 557—559.

† Camerer: "Zeit. Biol.," xxiv., S. 306—317.

‡ C. Jacobj ("Zeit. anal. Chem.," xxiv., 307-328) defends Hüfner's method, and found that it gave better results than Liebig's.

of the gas given off in albuminous urines in certain forms of apparatus. He introduces a pilule of fat into the tube, and inclines the tube once or twice before putting it in position.* It is advisable, however, in all cases to remove the albumin first, before decomposing the urine with hypobromite.

In accurate experiments corrections must be made for atmospheric pressure, temperature, and the tension of aqueous vapour. These corrections may be made by means of the following formula:—

$$v^\circ = \frac{v(b-w)}{760(1+0{\cdot}00366t)}$$

where v° = required volume.
v = given volume.
b = barometric pressure.
w = tension of aqueous vapour.
t = observed temperature.

Example.—30 c.c. gas were obtained at 10° C. and 750 m.m. pressure, here:—

$$v^\circ = \frac{30(750-9{\cdot}126)}{760(1+0{\cdot}00366\times 10)} = \frac{30\times 740{\cdot}874}{760\times 1{\cdot}0366} = \frac{22226{\cdot}22}{787{\cdot}816}$$

$\therefore v^\circ = 28{\cdot}2$ c.c.; and as 1 gram urea = 354·3 c.c. nitrogen, therefore $(354{\cdot}3 : 1 = 28{\cdot}2 : x)$ $x = 0{\cdot}0792$ gram urea. If the amount of urine taken were 5 c.c., the percentage of urea is $5 : 0{\cdot}0792 = 100 : x$; $\therefore x = 1{\cdot}58$ per cent.†

It must be remembered, in estimating urea by the hypobromite process, that if the urine contains more than 3 per cent. of urea, then 2½ c.c. of urine instead of 5 should be taken and 2½ c.c. of water added, the volume of gas obtained being multiplied by 2. If albumin is present this must be separated by boiling, acidulation, and filtering, and the cooled filtrate used for the estimation. If sugar is present the nitrogen is increased

* Méhu: "Journ. Pharm.," xv., pp. 607—609.

† From Charles, adapted to the Knop-Hüfner apparatus, but the same formula applies to other methods.

7 per cent., so that since the deficiency of nitrogen is about 8 per cent. by the hypobromite process under ordinary circumstances, when sugar is present the theoretical amount is obtained. Ralfe states that the daily average excretion of urea for an adult weighing 10½ stone may be taken as 33·5 grams, about 3 per cent., or if English measures be employed, 535 grains, or rather more than an ounce.*

It would take up too much space to say more here upon urea and its estimation. The reader is therefore referred to Salkowski and Leube—"Die Lehre vom Harn," S. 29 to S. 87.

Estimation of Total Nitrogen.—In order to estimate the nitrogen belonging to other nitrogenous constituents besides urea we must deduct that obtained by means of the hypobromite method (after making due allowance for the errors arising in that process) from the total N. The latter is best calculated by means of Pflüger and Bohland's modification of Kjeldahl's method (" Pflüger's Archiv," xxxv., 454—466):—5 c.c. of urine of average concentration are measured into an Erlenmeyer's flask holding about 300 c.c., and 20 c.c. of concentrated sulphuric acid added. The mixture is then boiled on wire gauze over a large Bunsen flame until all water and gases formed are driven off. The fluid at first becomes black, but afterwards assumes a clear yellow tint, when the heat should be lessened. The heating takes about twenty-five to thirty minutes. The fluid is then allowed to cool, diluted with water to 200 c.c., and placed in a flask; 80 c.c. of caustic soda solution (sp. gr. 1·3) added, the flask corked as quickly as possible, and its contents distilled into *standardized* sulphuric acid. The ammonia is estimated then by titrating the sulphuric acid with standard caustic soda, being calculated from the amount of sulphuric acid that is left

* A nitrometer, as stated above, may be used for estimating urea by the hypobromite process. Lunge describes a modification of this instrument in " Pflüger's Archiv," Bd. xxxvii., S. 45—50. He recommends the " bromum solidificatum " for preparing the hypobromite of soda solution.

uncombined. The whole analysis can be performed in an hour. Kjeldahl's method is described in "Zeits. anal. Chem.," xxii., 366, and very fully in Sutton's "Volumetric Analysis," p. 68, *et seq.*

According to Pflüger and Bohland an *approximate* estimate of the total N may be formed thus:—To 10 c.c. of urine, Liebig's mercuric nitrate solution is added from a burette until the yellow reaction described under that process (*supra*) appears with sodic carbonate. The number of cubic centimeters used multiplied by 0·04 gives the total N.

CHAPTER IV.

URIC ACID AND ALLIED BODIES.

Uric Acid, $C_5H_4N_4O_3$, contains 33·33 per cent. of nitrogen, and, next to urea, is the medium whereby the largest quantity of nitrogen is eliminated from the system. The quantity excreted in twenty-four hours varies from 7 to 10 grains, during hunger about 4 grains, and after a very animal diet from 30—35 grains. According to Parkes it forms from 0·03 to 0·05 per cent. of the urine. The proportion of urea to uric acid is 45 : 1.* It occurs in deposits of urate of soda in the joints in gout.

It is the principal nitrogenous constituent of the urine of birds and reptiles, and it has been found in the green gland of the crayfish by Griffiths,† in the Malpighian tubes of insects and the nephridia of snails by myself,‡ and elsewhere in answering organs of invertebrates by Griffiths and others. It is not correct to say that it is absent in the urine of herbivorous animals and replaced by hippuric acid, as Mittelbach found in the urine of oxen from 8 to 45 milligrams uric acid per 100 c.c. of urine; he also found it in the urine of sheep, horses, and pigs.§ Nor is the statement correct that in carnivorous animals uric acid is always present, as Sanarelli found it absent in the urine of two young foxes.‖ It is increased in amount by animal food,¶ especially

* Landois and Stirling: Loc. cit., vol. ii.

† Griffiths: "Chem. News," li., pp. 121, 122.

‡ MacMunn: "Journ. Physiol.," vii., pp. 128—129.

§ Mittelbach: "Zeits. physiol. Chemie," xii., S. 463—466.

‖ Sanarelli: "Chem. Centralbl.," 1887, S. 804, 805.

¶ Although Garrod says that it is not increased by a nitrogenous diet. Cf. Mares, *infra*.

if sufficient exercise is not taken, and by muscular fatigue (Ranke). It is reduced in amount by active exercise in the open air; by inhalation of oxygen, by large doses of quinine, by caffein, potassic iodide, common salt, sodic and lithic carbonates, by sulphate, carbonate, salicylate, and benzoate of soda. Its excretion is increased by euonymin, perchloride of mercury, and colchicum. For purposes of study it may be prepared from the excrement of serpents, from Peruvian guano, uric acid calculi, or from urine. From the excrement of serpents by boiling with a dilute solution of potassic or sodic hydroxide and filtering hot; the hot filtrate contains the dibasic salt, *e.g.*, $C_5H_2K_2N_4O_3$. On passing carbonic anhydride (CO_2) one-half the metal is displaced and the difficultly soluble monobasic salt precipitated:

$$2C_5H_2K_2N_4O_3+CO_2+H_2O=K_2CO_3+2C_5H_3KN_4O_3,$$

and this, after washing, is decomposed by hydrochloric acid. To prepare it from guano, the latter is boiled with a solution of one part borax in twenty parts of water; the solution, on acidulating, gives a brown, impure precipitate of uric acid, which is then purified, as in the former method.*

To prepare it from urine one-fifth the volume of hydrochloric acid is added to decompose the urates, the urine is allowed to stand in a cool place and decanted after two or three days; the crystals which separate (like cayenne pepper) and are deposited on the sides of the vessel are dissolved in sulphuric acid and precipitated with water. If the urine is of low specific gravity it ought to be evaporated down to half its volume before being treated with the acid.†

Uric acid is a weak dibasic acid, furnishing acid and neutral salts; the neutral salts are more soluble than the acid salts. It crystallizes in a great variety of forms, according to its mode of preparation, but mostly in the

* Strecker and Wislicenus: "Short Text-Book of Organic Chemistry," translated by Hodgkinson and Greenaway (1881), pp. 532, 533.

† Charles: Loc. cit., p. 450.

rhombic form, with the two obtuse angles rounded (Figs. 16 and 17). When the angles are rounded the "whet-stone" forms occur, or it may occur as six-sided tables. If

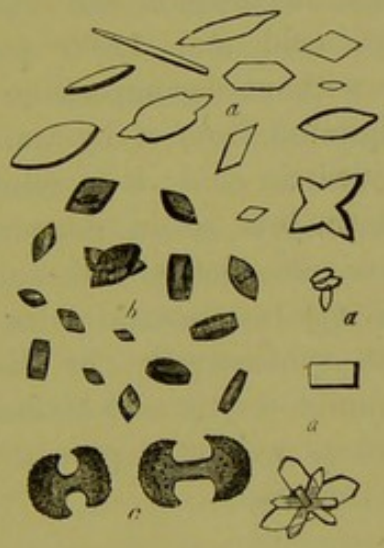


FIG. 16.—VARIOUS CRYSTALLINE FORMS OF URIC ACID.

a a, Crystals such as are met with on the decomposition of urates. *b*, From human urine. *c*, Dumb-bell crystals.

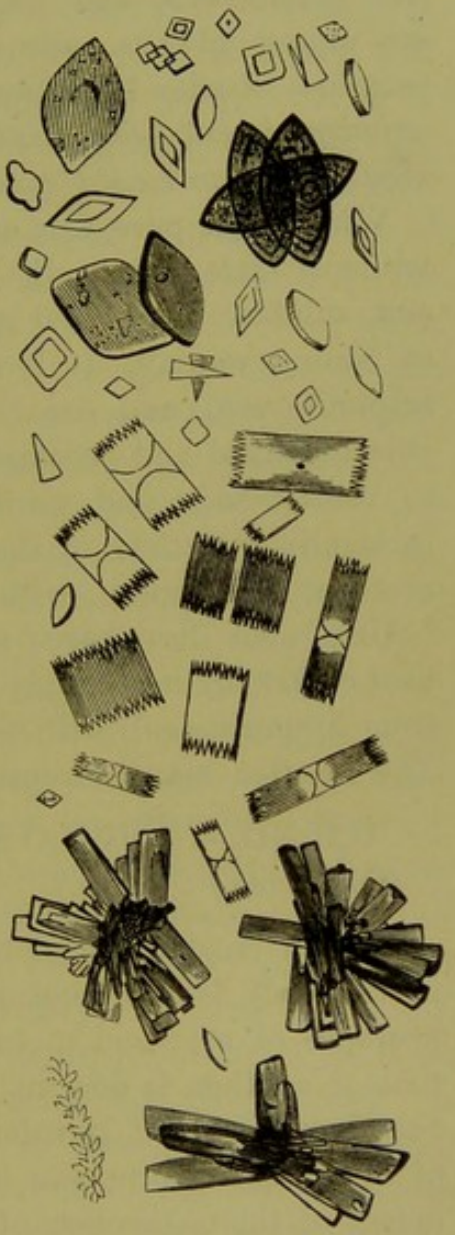


FIG. 17.—OTHER CRYSTALLINE FORMS OF URIC ACID.

precipitated from urine by hydrochloric acid it forms "small transparent rhombic tables, with a few elliptical and oblong plates;" in that case the acid decomposes the urates,

and sets free the uric acid, which is prevented from at once forming crystals by the phosphates in the urine (Brücke). The crystals may also occur in star-like groups, and dumb-bells, and many other forms (Fig. 16), they are almost always coloured, and are soluble in caustic potash. Charles lays down the rule *that every crystalline urinary deposit of a distinct yellow, brown or red colour may be said to consist of uric acid.*

When quite pure, uric acid is a white crystalline powder without taste or smell, insoluble in alcohol and ether, and soluble in 14,000 parts of cold and 1,800 parts of boiling water.* It is soluble without decomposition in sulphuric acid, and can be precipitated from it by water; it is dissolved and decomposed by nitric acid; it is soluble in caustic soda and caustic potash, less so in ammonia, in alkaline solutions of the lactates, phosphates, carbonates, acetates, and borates, with which it forms neutral salts.

Uric acid has been prepared synthetically by Horbaczewski† by heating glycocine (amido-acetic acid obtained from hippuric acid) with ten times its weight of pure urea. The reaction may be represented thus:—

$$C_2H_5NO_2 + 3CH_4N_2O = C_5H_4N_4O_3 + 3NH_3 + 2H_2O$$

(Glycocine.) (Urea.) (Uric acid.)

Latham tried to prove that the antecedent of uric acid in the body is glycocine, and that the reason of the appearance of uric acid in one animal's urine, and of urea in that of another, is due, in the former case, to the fact that the glycocine is not transformed into urea. The appearance of the excess of uric acid in gout he attributes to a similar defect of the metabolism of glycocine into urea in the liver or elsewhere.‡

* Strecker and Wislicenus: Loc. cit., p. 533.

† Horbaczewski: "Monatsh. Chem.," iii., S. 796; and "Journ. Chem. Soc.," 1883, p. 179.

‡ Latham: "Croonian Lectures," March 30, April 1, and 6, 1886 (published 1887).

It was formerly supposed that the appearance of an excess of uric acid in the system or urine was due to suboxidation—that the uric acid was not oxidized in the body into urea; but although we know that uric acid is a less oxidized body than urea, yet there are no grounds for supposing that uric acid is ever transformed into urea within the body, urea being probably derived from a different source. It is known, however, from the experiments of von Wöhler and von Frerichs that when a mammal is fed with uric acid a part of this becomes oxidized into urea, while the oxalic acid in the urine is at the same time increased; on the other hand, when urea was administered to fowls it became reduced to uric acid (H. Mayer, Jaffé), and when fowls were fed with leucin, glycocine, or aspartic acid (von Kniriem), or ammonium carbonate (Schroeder), the uric acid was increased.* We also know that uric acid may be made to yield urea out of the body by oxidation with nitric acid, thus:—

$$C_5H_4N_4O_3 + O + H_2O = C_4H_2N_2O_4 + CON_2H_4$$
(Uric acid.) (Alloxan.) (Urea.)

By reducing uric acid with sodium amalgam, xanthin and then hypoxanthin have been obtained; and its near relationship to other bodies, such as glycocine, allantoin, oxalic acid, oxaluric acid, parabanic acid, and hydantoin, has been proved by other chemical processes. It is also nearly related to such bodies as guanin, $C_5H_5N_5O$, by which it is replaced in the urinary excretion of spiders, and other invertebrates; sarcin or hypoxanthin, $C_5H_4N_4O$; xanthin, $C_5H_4N_4O_2$; hippuric acid, $C_9H_9NO_3$; inosic or inosinic acid, $C_{10}H_{14}N_4O_{11}$, and the bile-acids. It is also nearly related to theobromine (in cacao-beans) and to caffeine and theine.† The latter fact should be kept in mind when prescribing for gouty patients.

In spite, however, of the knowledge of the relationship of uric acid to these bodies, we do not know how or where it is

* Landois and Stirling: Loc. cit., p. 399.

† Strecker and Wislicenus: Loc. cit., pp. 532—543.

produced; we know that it is not a direct decomposition product of albumin, but is probably synthetically built up, in part at least, in the spleen (Ranke). It is intimately related, however, to guanin, sarcin, xanthin, and is the last stage of the oxidation of which these are the products (McKendrick). "It is supposed that a portion of the uric acid formed is eliminated as such, while another portion is transformed by oxidation into other substances. Thus it may readily be changed, first into alloxan, then into parabanic acid, then into oxaluric acid, then into oxalic acid and urea, and ultimately into carbonic acid and water" (Ibid.). Salkowski found that the ingestion of uric acid by dogs is followed by the appearance of allantoin in the urine.

Mares has shown that in a state of starvation a constant quantity of uric acid is excreted in a given time without reference to the quantity of nitrogen excreted within the same period, and that its amount is not influenced by the amount of meat taken by the individual before starvation. From this and other facts he argues that uric acid is a product of living protoplasm, whereas the urea is derived from absorbed albuminoids.*

On the whole, there is reason for supposing that a *portion* of the urea may be derived within the body from uric acid, and, if so, that the latter is a decomposition product preceding the urea, although the results of Mares, just mentioned, do not lead to that conclusion. According to some recent observations of Haig,† the excretion of uric acid is much affected by the digestion of food, being three times as great during the "alkaline tide" as at other times. A large part of this increased secretion (he says) must be regarded as a washing out of the uric acid accumulated in the liver and spleen during the "acid tide" period between meals, or during

* Mares: "Chem. Centr.," 1887, 339, 340; "Journ. Chem. Soc.," 1887, p. 856.

† Haig: "Journ. Physiol.," viii., 211—217; "Med.-Chir. Trans.," lxxi. 125—138, and 283—295.

sleep, and not to increased formation of uric acid during digestion. Haig also found that acids diminish the relative amount of uric acid excreted, and that alkalies increase it, and also its absolute amount. He believes that salicylates act in uric acid diseases by preventing acids from causing retention of uric acid. Some drugs cause retention of uric acid, such as lead, iron and lithia. If lithia has this action it is certainly contrary to what empiricism has taught us to believe. Uric acid is *increased* after attacks of indigestion, in catarrhal and rheumatic fevers, and disturbances of respiration such as pneumonia, emphysema, &c., in leucocythæmia, cirrhosis of the liver, enlarged spleen, paroxysms of intermittent fever; in some skin diseases, such as lepra and eczema, in chorea and diabetes, and after alcoholic excesses.

It is *diminished* in most chronic diseases, except during their active periods, in the later stages of Bright's disease, in gout, in chronic tumours of the spleen, in chlorosis, anæmia, chronic rheumatism, paraplegia, and chronic diseases of the spinal cord. In gout it accumulates in the blood, and is excreted in larger amounts after a paroxysm.

Uric acid does not occur normally in the urine as such; but in combination with bases to form urates, either basic or acid; these dissolve with difficulty in cold water, but are easily dissolved in hot water. They occur generally as yellow, brown, or red-coloured powders, the colour being due to the urinary pigment which they carry down with them. An excess of acid in the urine separates the acid from the base, and then the crystals separate; the same takes place when urates are treated with hydrochloric or acetic acid. The following are the most important combinations of uric acid met with in urine. As uric acid is dibasic, it forms neutral and acid salts; the neutral remain in solution, while the acid salts are precipitated.

(1) *Acid sodium urate* is found forming a great part of the "brick dust" or "lateritious" sediments, which are often coloured by uroerythrin. As a rule, the higher the

specific gravity of the urine, the more deeply the deposit is coloured. It increases in amount as the urine cools. This deposit is generally amorphous, and cannot be distinguished by the microscope from other sediments which form in granules. Sometimes these granules cling to the mucus in such a way as to resemble tube-casts, for which they are mistaken by the unskilled. They disappear, however, on adding a little acetic acid, or by heating the slide. The granules sometimes form little spherules, or hedgehog crystals, with projecting spicules (Fig. 18), although, when these appear, some German observers attribute them to urate of ammonium.

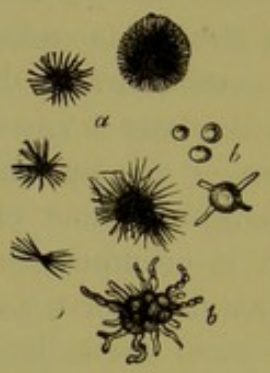


FIG. 18.—ACID URATE OF SODIUM.—(*Frey*).

a, Needles. *b b*, Spheroidal forms, some with projecting spines.

(2) *Acid potassium urate* occurs in similar amorphous granules, which are very soluble, and appear under the same circumstances as the acid sodium urate.

(3) *Acid ammonium urate* is found in urine which has become ammoniacal, either when it is undergoing the ammoniacal fermentation from time, or other cause. It is the only urate found in alkaline urine (Tyson); it may be mixed with free uric acid, and is accompanied by amorphous earthy phosphates and crystals of the triple phosphates of ammonium and magnesium. It is crystalline, and may then appear as shown in Fig. 19. It is said sometimes to appear as imperfect dumb-bells. These crystals readily dissolve in

hot water, and form uric acid crystals when treated with acids. With potassic hydrate they give off ammonia, and can be made to give the murexide reaction.

(4) *Acid calcium urate* is rare; it occurs as a white



Fig. 19.—Acid Urate of Ammonium.

amorphous powder, mixed with other urates. It dissolves with difficulty in water. When heated on platinum foil it leaves an ash of calcium carbonate. All these urates dissolve when heated, and also by adding caustic soda or caustic potash, and yield uric acid crystals when treated with acids.

Corresponding to each of these acid urates are the normal urates: thus we have normal urates of ammonium, sodium, potassium, and calcium; also of lithium. These are much more soluble than the acid urates, and remain in solution when the latter are precipitated. The change from neutral to acid urate may take place in urine from the formation of acid, or from the formation of carbonic acid, thus:—

$$2\,C_5H_2Na_2N_4O_3 + H_2O + CO_2 = 2\,C_5H_3NaN_4O_3 + CO_3Na_2.$$

(Normal sodium urate.) (Acid sodium urate.)

This is due to the fact that uric acid is dibasic, and therefore capable of entering into combination with bases to form neutral and acid salts.

The following table, from Ralfe, gives the chemical formulæ, the solubility, and the character of the deposits assumed by the various urates:—*

* Ralfe: "Practical Treatise on Diseases of the Kidneys," 1885, p. 81.

Urates.	Formula.	Solubility in Water.	Deposited as
Acid Ammonium ..	$C_5H_3N_4O_3(NH_4)$	1 – 1600	Amorphous or spiked globular masses.
Neutral Sodium ...	$C_5H_2N_4O_3Na_2$	1 – 77	Nodular masses.
Acid Sodium ...	$C_5H_3N_4O_3Na$	1 – 1200	Amorphous—rarely crystallized.
Neutral Potassium	$C_5H_2N_4O_3K_2$	1 – 44	Amorphous, or in fine needles.
Acid Potassium ...	$C_5H_3N_4O_3K$	1 – 800	
Neutral Calcium ...	$C_5H_2N_4O_3Ca$	1 – 1500	Fine granules.
Acid Calcium ...	$(C_5H_3N_4O_3)_2Ca$	1 – 600	Amorphous, or in fine needles.
Acid Lithium ...	$C_5H_3N_4O_3Li$	1 – 60	Ditto.

Tests for Uric Acid.—(1) The *Peculiarity of the Crystals,* and their formation on adding an acid in the case of urates.

(2) The *Murexide Test.*—A urate or uric acid in small quantity is heated gently with a drop of common red nitric acid, on a white porcelain dish, over the free flame. It soon becomes reddish-yellow, or yellow if only a small quantity of uric acid be present; nitrogen and carbon dioxide are given off, while urea and alloxan remain as a yellow deposit. When quite cold, a drop of ammonia, diluted with water, is brought on a glass rod, and allowed to touch the yellow deposit, when the latter becomes purple-red, owing to the formation of murexide, which contains purpurate of ammonia $[C_8H_4(NH_4)N_5O_6]$. Sometimes a better result is obtained if the vapour of ammonia is blown from the drop over the yellow spot. On adding caustic potash, after the ammonia, the spot becomes purplish-blue.

If caustic potash or caustic soda alone is used, instead of the ammonia, a violet colour appears. On heating this disappears, which distinguishes uric acid from guanin.

(3) *Schiff's Test.*—A little uric acid is dissolved in as small a quantity of sodic carbonate as possible; a piece of filtering

paper being moistened with some solution of nitrate of silver, a drop of the uric acid solution in the sodic carbonate carried on a glass rod is made to touch the paper, when a greyish stain of metallic silver appears.

(4) If a solution of uric acid, or a urate, be boiled with Fehling's solution (*infra*), a white precipitate, afterwards becoming reddish, forms, due to reduction of the copper solution.* But the murexide test alone is characteristic.

Quantitative Estimation of Uric Acid. — A good method for the quantitative estimation of uric acid in urine, and which is sufficiently easy of application for clinical purposes, is still wanted. A rough estimation of the amount present may be made by adding to 100 c.c. of filtered urine 5 c.c. of hydrochloric acid, and letting the mixture stand for twenty-four hours. A deposit of uric acid generally occurs then, in cayenne pepper-like crystals, which can be separated, dried, and weighed, but it is not all precipitated out.

Heintz adds 10 c.c. of hydrochloric acid to 200 c.c. of urine, and leaves in a cool place for forty-eight hours. The crystals are then collected on a dried and weighed filter, and thoroughly washed with distilled water ; the whole dried at 100° C. until it ceases to lose weight, and weighed again. The weight of the filter alone, deducted from this, gives the weight of uric acid. Some uric acid is retained in the acid and washings, and to allow for this Neubauer adds 0·0038 gram of uric acid for every 100 c.c. of liquid employed.

Haycraft† has devised a method which somewhat resembles that of E. Salkowski.‡ It is highly spoken of by Herrmann.§

Haycraft's method is based on the fact that uric acid

* Hence urates may, when in excess in urine, be mistaken for grape-sugar.

† Haycraft : "Zeits. anal. Chem.," xxv., 165—169 ; "Brit. Med. Journ.," Dec., 1885. Cf. Gossage : "Proc. Roy. Soc.," xliv., 284—285.

‡ Salkowski and Leube : "Die Lehre vom Harn.," S. 96—98.

§ Herrmann : "Zeits. f. physiol. Chemie," Bd. xii., Heft 6.

combines with silver as silver urate, which is practically insoluble in water, ammonia, or acetic acid, but perfectly soluble in nitric acid. The chief drawback to the method is the peculiar nature of the precipitate of silver urate, which is slimy and difficult to wash; this, however, is overcome by collecting the precipitate on an asbestos filter attached to a filter pump. The filter is easily made by half filling a small funnel with broken glass, upon which small asbestos fibres, suspended in water, are poured to the depth of $\frac{1}{4}$ inch, and evenly distributed. Such a filter may be used repeatedly for the same operation. The estimation of the uric acid depends upon the titration of the silver with which it is combined, by Volhard's method.*

The necessary solutions are :—

A centinormal solution ($\frac{N}{100}$) of *Ammonic Thiocyanate* standardized by a silver solution of known strength. 1 c.c. of this = 0·00168 gram uric acid.

Ammoniacal Silver Solution.—5 grams silver nitrate in about 100 c.c. of water, precipitated and redissolved in ammonia to a clear solution.

Ferric Indicator and Pure Nitric Acid.—The ferric indicator may be a saturated solution of iron alum; or may be made by oxidizing ferrous sulphate with nitric acid, evaporating with excess of sulphuric acid to dissipate nitrous fumes, and dissolving the residue in water, so that the strength is about 10 per cent.

The Analysis.—25 c.c. of urine are placed in a small beaker, together with about 1 gram of sodic bicarbonate and 2 or 3 drops of strong ammonia. This precipitates ammonio-magnesic phosphate, and prevents reduction of silver: 1—2 c.c. of ammoniacal silver solution are then added, which at once precipitates the silver as urate. The mixture is now placed on the filter, and washed until the washings show no trace of silver by testing with salt (NaCl.)

* Volhard: "Journ. Chem. Soc.," 1878, p. 743, and Liebig's "Ann. d. Chem.," cxc. 1.

The precipitate is then dissolved in a few cubic centimeters of nitric acid, washed into a flask, and the titration carried out as in Volhard's process. The number of cubic centimeters of thiocyanate used multiplied by 0·00168, gives the uric acid. If the urine contains albumin, it must first be removed by acidifying slightly with acetic acid, heating, and filtering.*

As it is not easy to get hold of Volhard's method, used for estimating silver, it may be here briefly described. There are required for its performance :—

(1) *Decinormal Ammonic Thiocyanate.*—Dissolve 8 grams of the purified crystals in a liter of water, and afterwards correct the strength by a correct decinormal silver solution.

(2) *Decinormal Silver Solution.*—10·766 grams of silver dissolved in pure nitric acid and made up to 1 liter; or 16·966 grams of pure silver nitrate dissolved in a liter of distilled water.

(3) *Ferric Indicator.*—Described above.

5 c.c. of either of these solutions are used for each titration, which always must take place at the ordinary temperature.

(4) *Pure Nitric Acid.*—Dilute the *pure* acid with a fourth part of distilled water, boil till colourless, and keep in the dark.

Process for Silver.—50 c.c. of decinormal ($\frac{N}{10}$) silver solution are placed in a flask, diluted somewhat with water, and 5 c.c. of ferric indicator added, together with about 10 c.c. of nitric acid. If the iron solution should cause a yellow colour, the nitric acid will remove it. The thiocyanate is then added from a burette; at first a white precipitate is produced, rendering the fluid milky, and as each drop falls in, it produces a reddish-brown cloud, which quickly disappears on shaking. As the point of saturation approaches, the precipitate becomes flocculent and settles easily; finally, a drop or two of thiocyanate

* From Sutton's "Volumetric Analysis," 5th ed., 1886, pp. 324, 325.

produces a faint brown colour, which no longer disappears on shaking. If the solutions are correctly balanced, exactly 50 c.c. of thiocyanate solution is required to produce this effect. The colour is best seen by holding the flask so as to catch the reflected light of a white wall.—(From Sutton's "Volumetric Analysis," p. 116.)

Herrmann found that the presence of sugar and albumin interferes very slightly with Haycraft's process, and they need not, according to him, be separated before estimation. He also found that the method is more accurate than Ludwig's,* although in both there is a loss of 2 per cent.: in Haycraft's method some bodies of the xanthin group being precipitated with the uric acid.

Czapek† proposes a modification in Haycraft's method, but in the modified method the average error is from 10—15 per cent., which renders the method useless.

Fokker's‡ method, modified by E. Salkowski §, is as follows:—200 c.c. of urine are made strongly alkaline with sodic carbonate, and after an hour 20 c.c. of a concentrated solution of chloride of ammonium added; the solution allowed to stand at a low temperature for forty-eight hours, then filtered through a weighed filter, and washed three times. The filter is filled with dilute hydrochloric acid (1 to 10), and the filtrate collected. The dilute acid is added again on the filter, and this operation repeated until all the acid urate is dissolved. The filtrate is allowed to stand six hours, and the uric acid which separates collected on the same filter, washed twice with water, then with alcohol until all acid reaction disappears, and dried at 110° C. To the weight obtained add 0·030 gram. If the urine is of low specific gravity, it should be evaporated till its specific gravity is 1017 to 1020.

In "Die Lehre vom Harn" Salkowski and Leube show (S. 93)

* Ludwig: "Chem. Centralblatt," 1885, S. 523.
† Czapek: "Zeit. physiol. Chem.," xii., S. 502—511.
‡ Fokker: "Pflüger's Archiv," Bd. x., S. 153.
§ Salkowski: "Virchow's Archiv," Bd. lxviii., S. 401.

that we may not be able to get uric acid crystals from urine by adding hydrochloric acid and allowing to stand, and yet uric acid may be present. The acidulated urine should in that case be made *strongly* alkaline with ammonia, and some ammoniacal magnesia mixture added (see Appendix), the precipitate filtered off, and the filtrate decomposed with a solution of oxide of silver in nitric acid. A gelatinous precipitate forms, being a combination of uric acid with silver and bases. The uric acid can easily be obtained from the precipitate.

As *derivatives* of uric acid I may mention: alloxantin, alloxan, uramil, purpuric acid, murexide, parabanic acid, and allantoin.

Allantoin, $C_4H_6N_4O_3$, can be prepared from uric acid by oxidation with permanganate of potassium, and occurs in small traces in normal human urine when the diet consists of flesh.

It is more abundant during the first few weeks of life, and during pregnancy. It occurs in the amniotic and allantoic fluids of the embryo, in the urine of sucking calves, in that of the dog and cat, and in the former after feeding with uric acid. It may be obtained by evaporating allantoic fluid; the crystals, after washing with water, should be dissolved in hot water, and the solution decolourized with animal charcoal. It may be separated from urine by precipitating with lead acetate, filtering, passing sulphuretted hydrogen through the filtrate, evaporating the filtrate to a syrup, and letting stand for several days. It then crystallizes out. The crystals are to be washed in cold and then dissolved in hot water, from which the allantoin separates. Köhler and Schottin have found allantoin in the urine of patients taking tannic acid.* It is soluble in 160 parts of cold and 60 parts of hot water, insoluble in cold alcohol or ether. It forms

* Köhler "De allantoini in urina impedita respiratione praesentia," Diss. Halens, 1857. Schottin: "Lehmann's Handb. d. physiol. Chem.," 1859, S. 93.

colourless shining prisms (Fig. 20), but is of no clinical importance. It is easily changed into uric and allantoic acid ($C_7H_{10}N_6O_6$?).

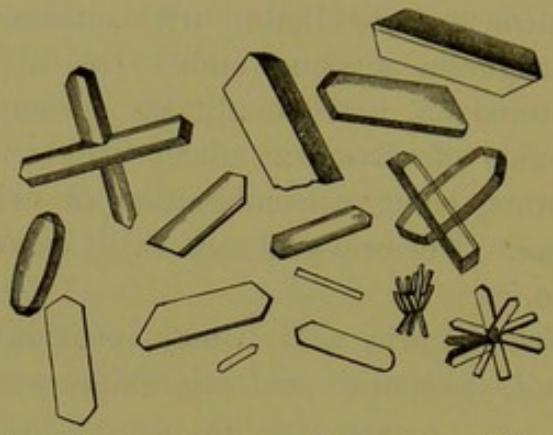


FIG. 20.—CRYSTALS OF ALLANTOIN.—(*Frey.*)

Creatinin, $C_4H_7N_3O$.—Creatinin is derived in the body from creatin (Fig. 21), which is a constant constituent of muscle juice. It is a normal constituent of urine, in which it amounts daily to from 0·6 to 1·3 grams, or from 8 to 18 grains. By heating creatin, water is given off, and

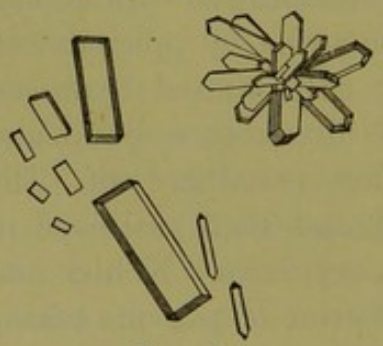


FIG. 21.
CRYSTALS OF CREATIN.—(*Frey.*)

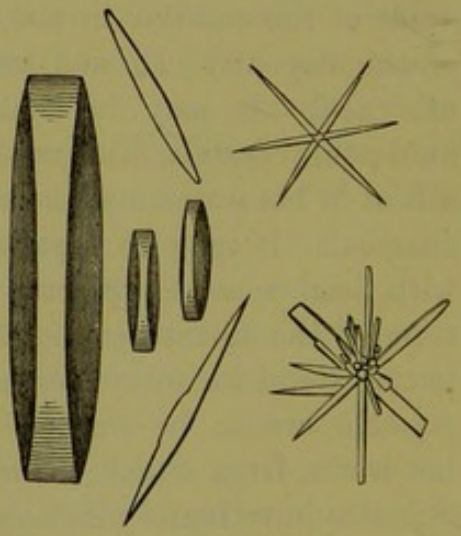


FIG. 22.
CRYSTALS OF CREATININ.—(*Frey.*)

creatinin formed, the latter being thus merely a dehydrated form of creatin. Its amount is increased by a flesh diet. It is alkaline in reaction, soluble in cold water (1 in 11·5), also

in alcohol, but scarcely soluble in ether. It forms compounds with acids and salts, which can be crystallized. It is generally recognized by its compound with zinc chloride, which occurs in characteristic balls with radiating striæ or needles, whereas creatinin itself crystallizes in oblique rhombic prisms and other forms (Fig. 22). Its amount in the urine is increased in typhus, pneumonia, intermittent fever* and tetanus, and is diminished in convalescence from acute diseases, in progressive muscular atrophy, anæmia, marasmus, chlorosis, phthisis, and paralysis. When given with the food it appears again in the urine (Voit). It is not diminished by fasting. There is no proof that creatinin is transformed into urea in the body (M'Kendrick). A long and troublesome process is required for its preparation from urine.† It can be estimated quantitatively by the method of Neubauer.‡

Creatinin may be detected in urine by Weyl's test. A few drops of a very watery solution of sodium nitroprusside are added, and then weak caustic soda solution drop by drop to the urine ; a fine ruby-red colour appears, which soon disappears. When heated with glacial acetic acid this colour changes to green, then after a time blue (Salkowski), owing to the formation of Berlin blue. If this reaction does not succeed, the urine should first be boiled with dilute sulphuric acid. Creatinin, when warmed with Fehling's solution, reduces it. It gives a yellow crystalline precipitate, if the urine is heated with a dilute solution of phosphomolybdic acid after previous acidification of the urine with nitric acid.

Xanthin, $C_5H_4N_4O_2$, is now considered to be a normal ingredient of urine. It has long been known as comprising some rare forms of urinary calculi, and Bence

* Munk : "Deutsche Klinik," 1862, S. 299.

† See Ralfe : "Diseases of the Kidneys," loc. cit., p. 89. Charles : Loc. cit., p. 405 ; Krukenberg : Loc. cit., S. 82 ; and Salkowski and Leube, S. 107.

‡ Neubauer and Vogel : Loc. cit., p. 291 ; also Charles, p. 466.

Jones found it as a crystalline sediment* in urine.† In 300 liters of urine only 1 gram of xanthin is present (Neubauer). It is intermediate between hypoxanthin and uric acid, and can be obtained from hypoxanthin and guanin; it can easily be changed into uric acid. It is, when pure, an amorphous yellow-white powder partially soluble in boiling water, insoluble in cold water, and in alcohol and ether, soluble in alkaline solutions and in strong mineral acids. When evaporated to dryness with fuming nitric acid, a

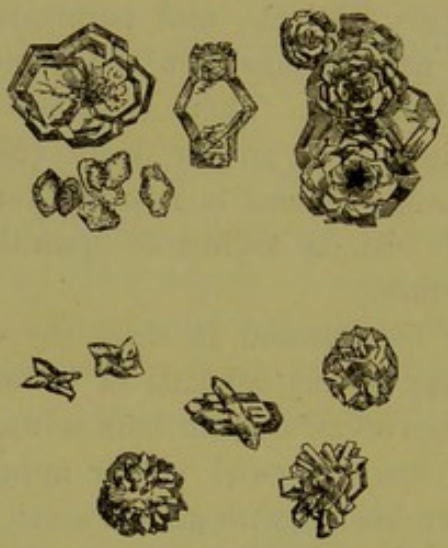


FIG. 23.—CRYSTALS OF NITRATE AND CHLORATE OF XANTHIN. NITRATE ABOVE, CHLORATE BELOW.—(*Frey.*)

yellow residue is left, which becomes an intense red on adding caustic soda, and does not disappear as quickly on heating as in the case of the uric acid reaction. According to Sturr and Stromeyer, xanthin is increased in amount by the use of sulphur baths.

Hypoxanthin, or Sarcin, $C_5H_4N_4O$, has not certainly been recognized as a normal constituent of urine, although Salkowski has found a body nearly related to it in normal urine. It has been found in the urine of cases of leucocythæmia (Jakubasch). It occurs in traces in muscle,

* Its crystalline forms, when in the condition of chlorate and nitrate, are characteristic (Fig. 23).

† Bence Jones: "Journ. Chem. Soc.," 1862, p. 68, *et seq.*

spleen, thymus, brain, bone, liver, and kidney (Landois and Stirling). Hypoxanthin can be changed into xanthin by

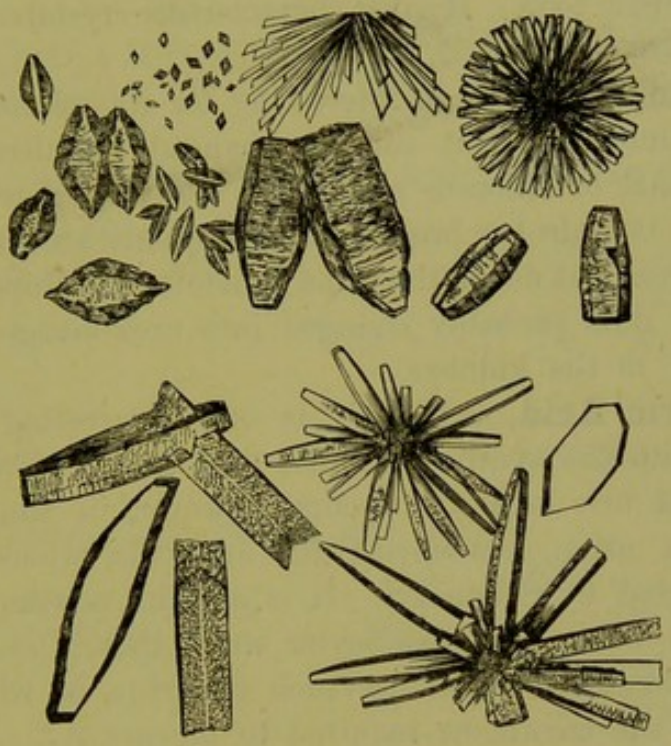


FIG. 24.—CRYSTALS OF NITRATE OF HYPOXANTHIN, UPPER HALF, AND OF CHLORATE LOWER HALF.—(*Frey.*)

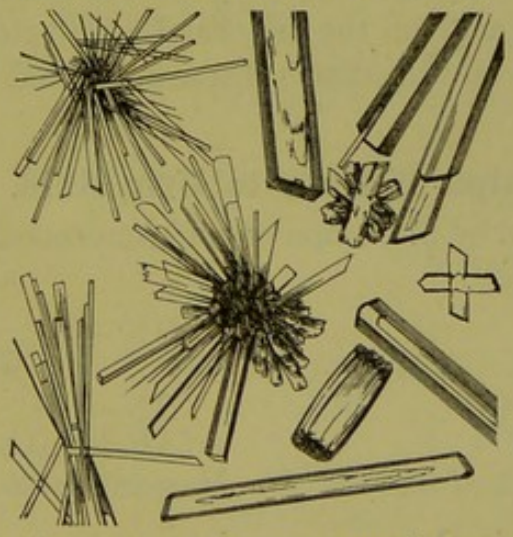


FIG. 25.—CRYSTALS OF CHLORATE OF GUANIN.—(*Frey.*)

oxidation. Uric acid, also, by the action of nascent hydrogen, can be changed into xanthin and hypoxanthin. When

evaporated with nitric acid, hypoxanthin gives a light yellow stain, which becomes deeper, but *not* reddish-yellow, on adding caustic soda. It gives characteristic crystals of nitrate and chlorate (Fig. 24).

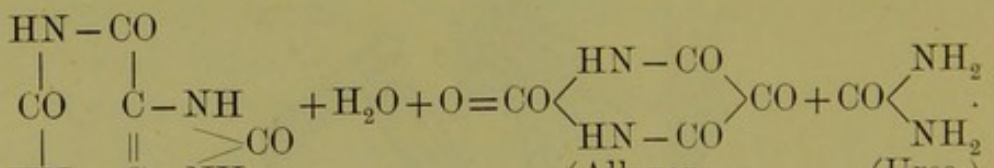
Guanin, $C_5H_5N_5O$, is interesting for its relationship to xanthin, into which it can be changed by nitrous acid. The crystals of chlorate of guanin are very characteristic (Fig. 25). Guanin has been found in the muscles, lungs, liver, and pancreas, but not in the urine; therefore, as Hoppe-Seyler remarks,* it is probably changed into urea either in those organs or in the kidneys.

Oxaluric Acid, $C_3H_4N_2O_4$, is only interesting in connexion with the synthesis of uric acid, and is an oxidation product of uric acid. It occurs, however, in small traces in human urine, combined with ammonia, where it was first detected by Schunck.† It is a white powder, slightly soluble in water. Hoppe-Seyler states that it is yet uncertain whether it exists as such in urine, or whether it arises by the treatment required to prepare it.‡

I give here a condensed summary of various syntheses and reactions, by which one can easily see the connexion between urea and uric acid on the one hand, and uric acid and the xanthin bodies on the other. These are copied from Krukenberg.§

Relationships of Urea to Uric Acid.

(1) Uric acid by the action of cold concentrated HNO_3:—



(Uric acid, after *Medicus.*) (Alloxan = Mesoxalylurea.) (Urea.)

* Hoppe-Seyler: "Physiol. Chem.," S. 820.
† Schunck: "Proc. Roy. Soc.," vol. xvi., p. 140.
‡ Hoppe-Seyler: "Physiol. Chem.," S. 819.
§ Krukenberg: Loc. cit., S. 94 and 95.

(2) Alloxan gently heated with baryta water, and the barium salt decomposed by H_2SO_4:—

$$\underset{\text{(Alloxan.)}}{C_4H_2N_2O_4} + H_2O = CO\begin{cases}NH-CO-CO-CO-OH.\\ NH_2\end{cases}\ \text{(Alloxanic acid.)}$$

(3) Barium alloxanate boiled with much water for ten minutes:—

$$\underset{\text{(Barium alloxanate.)}}{\begin{matrix}CO-N-CO-NH_2\\ |\quad\ |\\ CO\quad Ba\\ |\quad\ |\\ CO-O\end{matrix}} + H_2O = \underset{\text{(Barium mesoxalate.)}}{\begin{matrix}CO-O\\ |\quad\ |\\ CO\quad Ba\\ |\quad\ |\\ CO-O\end{matrix}} + \underset{\text{(Urea.)}}{\begin{matrix}NH_2\\ |\\ CO\\ |\\ NH_2\end{matrix}}.$$

(4) Alloxanic acid heated with HI:—

$$\underset{\text{(Alloxanic acid.)}}{C_4H_4N_2O_5} = CO\begin{cases}NH-CH_2\\ \qquad\ |\\ NH-CO\end{cases} + CO_2.$$

(= Hydantoin or glycolyl urea.)

(5) Alloxan treated with HNO_3:—

$$\underset{\text{(Alloxan.)}}{C_4H_2N_2O_4} + O = CO\begin{cases}NH-CO\\ \qquad\ |\\ NH-CO\end{cases} + CO_2.$$

(Parabanic acid = oxalyl urea.)

(6) Parabanic acid treated with alkalies:—

$$\underset{\text{(Oxalyl urea.)}}{C_3H_2N_2O_3} + H_2O = CO\begin{cases}NH-CO-CO-OH\\ NH_2\end{cases}\ \text{(Oxaluric acid.)}$$

(7) Oxaluric acid boiled with water:—

$$\underset{\text{(Oxaluric acid.)}}{C_3H_4N_2O_4} + H_2O = \underset{\text{(Oxalic acid.)}}{\begin{matrix}COOH\\ |\\ COOH\end{matrix}} + \underset{\text{(Urea.)}}{CON_2H_4}.$$

(8) 1. Bromacetyl urea by boiling with alcoholic NH_3:—

$$CO\begin{cases}NH_2\\ NH-CO-CH_2Br\end{cases} = CO\begin{cases}NH-CH_2\\ \qquad\ |\\ NH-CO\end{cases} + HBr.$$

(Bromacetyl urea.)

2. Urea by heating with oxalic acid and phosphorus oxychloride :—

$$\begin{array}{l} \text{COOH} \\ | \\ \text{COOH} \end{array} + \text{CO}\begin{array}{l}\diagup \text{NH}_2 \\ \diagdown \text{NH}_2\end{array} = \begin{array}{l} \text{CO}-\text{NH} \\ | \quad \diagup \\ \text{CO}-\text{NH} \end{array} \text{CO} + \text{H}_2\text{O}.$$

(Oxalic acid.) (Urea.) (Parabanic acid.)

Relationships of Uric Acid and of Urea to the Xanthin Bodies and to Guanidin.

(1) Uric acid in alkaline solution is changed through very weak sodium amalgam into xanthin and hypoxanthin (Strecker and Reineck).

(2) Uric acid furnishes parabanic acid, by boiling with dilute HNO_3.

(3) Guanin gives, by treatment with potassic chlorate and hydrochloric acid, parabanic acid, guanidin, oxaluric acid, oxalic acid, and urea.

(4) From theobromine and a chromic acid mixture monomethyl-parabanic acid is obtained, and from caffeine, by oxidation with potassic chromate and sulphuric acid, dimethyl-parabanic acid.

(5) Xanthin yields, by warming with HCl and potassic chlorate to 50°—60°C., urea and alloxan.

(6) From biuret, by heating with hydrochloric acid gas to 100°C., guanidin is formed :—

$$\underset{\text{(Biuret.)}}{\text{C}_2\text{O}_2\text{N}_3\text{H}_5} + \text{HCl} = \underset{\text{(Hydrochlorate of guanidin.)}}{\text{CN}_3\text{H}_5 \,.\, \text{HCl}} + \text{CO}_2.$$

(7) Guanidin, boiled with baryta water, yields urea :—

$$\begin{array}{l} \text{NH}_2 \\ | \\ \text{C}=\text{NH} \\ | \\ \text{NH}_2 \end{array} + \text{H}_2\text{O} = \begin{array}{l} \text{NH}_2 \\ | \\ \text{CO} \\ | \\ \text{NH}_2 \end{array} + \text{NH}_3.$$

(Guanidin.) (Urea.)

(8) Carbodiimide furnishes, by heating with NH_4Cl in alcohol solution, guanidin :—

$$\text{C}\begin{array}{l}\diagup\!\!\diagup \text{NH} \\ \diagdown\!\!\diagdown \text{NH}\end{array} + \text{NH}_4\text{Cl} = \text{C}\begin{array}{l}\diagup \text{NH}_2 \\ - \text{NH}_2 \\ \diagdown \text{NH}\end{array}.$$

(Carbodiimide.) (Guanidin.)

Hippuric Acid, $C_9H_9NO_3$ (Benzoyl-amido-acetic acid or benzoyl glycin = benzoyl glycocine). — Hippuric acid is not of so much clinical significance as uric or oxalic acid, yet it is interesting as being an example of a body which is synthetically built up within the organism, after benzoic acid, or some substance containing it, is given with the food. It is really one of the aromatic series, which are mainly derived from the action of the putrefactive and digestive ferments on certain food products, but for convenience' sake it is considered now.

It is a monobasic acid, with a bitter taste and no smell,

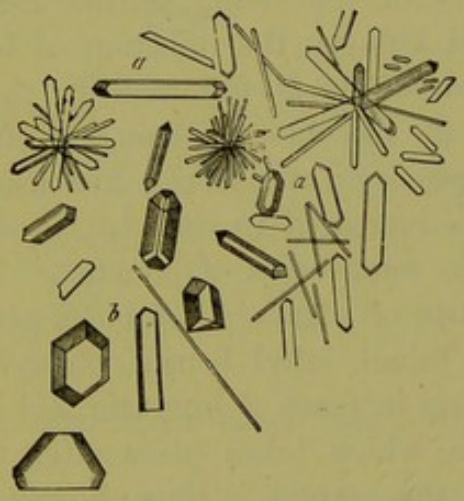


FIG. 26.—CRYSTALS OF HIPPURIC ACID.—(*Frey.*)

a a, prisms. *b*, crystals formed by slow evaporation, and resembling those of ammonio-magnesium phosphate.

and crystallizes in transparent, colourless, four-sided prisms (Fig. 26). The amount in human urine is small, from 0·3 to 3·8 grams, or from 5 to 50 grains, in twenty-four hours. In the urine of herbivora, such as the horse, cow, and sheep, it replaces uric acid to a great extent, but not altogether, as I have stated before. It dissolves easily in hot alcohol or ether, but is only sparingly soluble in water. Hydrochloric acid dissolves it, but on boiling the solution the hippuric acid is changed into benzoic acid and glycocine. It is a conjugated acid, and arises mainly in the body from benzoic acid or some

substance related to it, or containing it; but in herbivora, it is now said not to be derived from benzoic acid.* Even on flesh diet it may appear in the urine, and is then derived from the decomposition of proteids. The change of benzoic into hippuric acid appears to take place, to a great extent, in the kidney, but Salomon found that after excision of the kidneys in rabbits and injection of benzoic acid into the blood, hippuric acid could be detected in the blood, muscles, and liver. In disease of the kidneys, however, it has been found that benzoic acid is no longer capable of being changed into hippuric acid; this, of course, shows that the renal epithelium has the property of converting benzoic into hippuric acid. Even in excised kidneys, the injection of benzoic acid into the renal blood-vessels is followed by the appearance of hippuric acid in the blood given off from the organ. Eating plums, pears, or cranberries increases its amount in urine, and it is increased in diabetes, in some diseases of the liver, and in some cases of jaundice. The ingestion of the cuticular parts of plants, oil of bitter almonds, cinnamic or quinic acid, toluol, ethyl benzol, phenyl propionic acid, &c., also causes an increase of hippuric acid in the urine.

The synthesis which takes place after the ingestion of benzoic acid in the system is thus shown:—

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

Conversely, the change of hippuric acid into glycocine and benzoic acid under the action of mineral acids aided by heat, or alkalies, or certain ferments as in decomposing urine, is thus shown:—

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

Hippuric acid can be readily obtained from the urine of the horse or cow, where it occurs mainly as hippurate of sodium. On merely evaporating down such urine to a small volume, and adding strong hydrochloric acid to the cold syrupy

* Garrod and others maintain that it is. See "Lancet," 1883, vol. i., p. 673.

fluid, the whole sets into a mass of brown crystals, which on dissolving in hot water and cooling, separate out in an impure, coloured condition. But to obtain it pure, it is necessary to boil the urine with milk of lime, filter the fluid hot, concentrate on the water bath to one-sixth its volume, and then add hydrochloric acid in excess. After twenty-four hours, the fluid is decanted from the crystals, the latter dissolved in hot water, filtered hot through animal charcoal to remove the colour, and the fluid allowed to cool, when the crystals separate, and can be purified by further recrystallization.*

If a dose of benzoic acid (Fig. 27) be given over-night to a

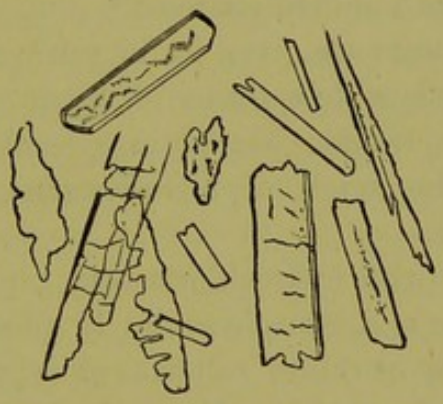


FIG. 27.—CRYSTALS OF BENZOIC ACID.—(*Frey.*)

patient, and the urine collected next morning, hippuric acid may easily be obtained. The urine is evaporated down, and treated when cold with hydrochloric acid, and then laid aside in a cool place. After some hours, the deposit is collected on a filter, washed on the filter with cold alcohol and water. The crystals may then be purified as before. If the hippuric acid, however, is present in small amounts, as in human urine, it may be obtained by the method of Bunge and Schmiedeberg, as follows:—2 liters urine are made slightly alkaline by means of soda solution, evaporated almost to dryness, and extracted thoroughly with absolute alcohol. If the extraction with alcohol is quite complete, as shown by fresh portions of alcohol

* The crystals are prismatic, and when slowly evaporated, resemble those of triple phosphate (Fig. 26).

not removing any more solid matter from the residue, the united alcohol extracts are filtered, the alcohol distilled off, and the residue warmed on the water bath, while small quantities of water are added to it, until all traces of alcohol are removed. The concentrated watery solution is strongly acidified with hydrochloric acid, and then repeatedly shaken with acetic ether. The acetic ether, when separated and evaporated, leaves the hippuric acid, which can be separated from the benzoic acid mixed with it, by treatment with light petroleum (in which benzoic acid and the fats dissolve), and from colouring matters by recrystallization from boiling water and decolourization with animal charcoal.*

The following tests are given for hippuric acid :—

(1) Boiled with a few drops of nitric acid, heated to dryness, and the residue heated in a small tube, hippuric acid gives off the smell of nitro-benzene. Benzoic acid behaves similarly.

(2) On boiling with hydrochloric acid, hippuric acid splits up into glycocine and benzoic acid. If an excess of caustic potash and a drop of dilute sulphate of copper solution are added, the glycocine gives a blue colour not destroyed by boiling.

(3) When crystals of hippuric acid are heated in a test tube they become decomposed: a mixture of benzoic acid and ammonium benzoate condenses in the cool part of the tube, there is a smell of new hay, and oily drops remain in the tube (Stirling).

According to Tappenier there is reason for concluding, with Salkowski, that besides being furnished from (1) the synthesis of benzoic acid and glycocine in the organism, and (2) from the metabolism of the animal tissues, hippuric acid is derived from phenyl propionic acid, which is one of the putrefactive products of proteids arising in the intestinal canal.†

* Krukenberg : Loc. cit.

† Tappenier : "Zeits. f. Biol.," xxii., S. 236—240.

Baas has recently shown that there is no increase of hippuric acid after giving tyrosin by mouth, and, therefore, that it does not arise from tyrosin in the intestines.*

Hippuric acid may be estimated approximately by the method of Schmiedeberg and Bunge mentioned above, or better by Völcker's method,† which is as follows:—Evaporate 200—300 c.c. urine to one-third in a thin glass basin; add 4 grams of sodium phosphate; concentrate to a syrup. Add excess of plaster of Paris, and dry till the mass can be powdered. Introduce the powder, together with the crushed basin into a Soxhlet's apparatus, and extract with freshly-distilled light petroleum. After four to six hours, change the flask, extract for six to ten hours with absolute ether, and distil off the ether. Dissolve the residue in hot water; decolourize with animal charcoal; wash the charcoal well; concentrate at 50°—60° C. to 1 or 2 c.c., and leave to crystallize. Rinse with the mother liquor into a weighed filter; wash with a few drops of water and ether; dry and weigh. Add for every cubic centimeter of filtrate 0·0015 gram.

* Baas: "Zeits. f. physiol. Chem.," xi., S. 485—491.

† Völcker: "Chem. Centr.," 1887, S. 124, 125; "Journ. Chem. Soc.," 1887, p. 535; also "Zeits. anal. Chem.," xxvi., S. 402, and "Journ. Chem. Soc.," 1887, p. 1001.

CHAPTER V.

THE NON-NITROGENOUS ORGANIC ACIDS.

Oxalic Acid, $H_2C_2O_4 + 2H_2O$.—Of the non-nitrogenous organic acids of urine, perhaps the most important is oxalic acid.* This acid never occurs free in the urine, but is united with calcium, in which combination it forms the greater part of the "mulberry" calculus. It is a normal constituent of urine, and its daily amount is about 20 milligrams. When the urine is alkaline its detection is not difficult, but normally it is dissolved in the urine, being held in solution, according to Neubauer, by the acid phosphate of sodium. Its presence is not indicative of any special disease, although its excess may be due to different pathological causes, generally, however, to disturbances of digestion. "When we find oxalate of lime crystals in the urine we must not at once conclude the patient is suffering from oxaluria, any more than we must conclude that he is suffering from Bright's disease, because we find sugar or albumin in the urine."† Oxalate of lime may be derived from the food, and frequently is, after eating rhubarb and cabbage, although, according to Esbach, cabbages are very free from oxalate of lime. "It is not the occasional occurrence, but the more or less persistent presence of crystals of oxalate of lime in the urine that is associated with a peculiar group of symptoms, of which the most prominent is, perhaps, mental depression" (Brunton).

* The urine is the only secretion in which oxalic acid has been found.

† Lauder Brunton: "Disorders of Digestion," p. 38.

The crystals of calcium oxalate are very characteristic (Fig. 28): they occur either as the envelope-like crystals, or as dumb-bells; the latter are, according to Ralfe, probably formed in the urinary passages.* Or they may occur as hour-glass shaped crystals (rarely). They are soluble in mineral acids, but not in acetic or oxalic acid, which helps to distinguish them from crystals of the earthy phosphates; they are also insoluble in alcohol and water. The origin of oxalic acid in the body is unknown. Von Frerichs and Wohler found that dogs fed with uric acid excreted oxalic

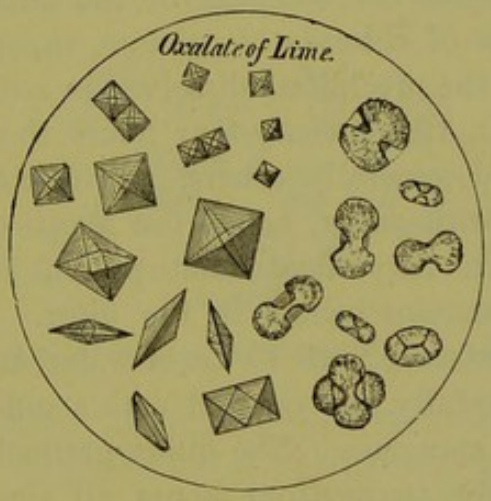


FIG. 28.—OXALATE OF LIME CRYSTALS, IN OCTAHEDRA AND DUMB-BELLS.

acid in their urine. Some think it may arise from the imperfect oxidation of starch and fat in the food, or of the non-nitrogenous fatty matters in the body. Esbach denies the formation of oxalic acid in the body. He showed that if sulphuretted hydrogen is made to act on a solution of urates, crystals of oxalate of lime are produced.†

The use of vegetable food, sparkling wines and beer, the internal use of alkaline bicarbonates and salts with the organic acids, and of free uric acid and urates, often increases the amount of calcic oxalate in the urine (Neubauer).

In spermatorrhœa oxalate of calcium crystals are often

* Ralfe: "Clinical Chemistry," p. 131.

† Esbach: "Journal des Connaissances Med.," 1883, p. 155.

found, having in that case possibly, as Ralfe suggests, a local origin. Ralfe bases his conclusion on the theory of Meckel, who assumes that the formation of oxalate of lime in mucus may be due to a catarrh in the urinary passages, the mucus undergoing an acid fermentation, attended by the deposition of oxalate of calcium crystals.

Neubauer states that he has been able to prove the presence of tolerable amounts of oxalate of calcium in solution in the urine by using a method, when otherwise it could not be discovered in the sediment. This method is as follows:*—400 to 600 c.c. of the urine are treated with a solution of chloride of calcium, supersaturated with ammonia, and the precipitate dissolved in acetic acid, avoiding an excess. After twenty-four hours the precipitate, in which uric acid generally occurs, is collected on a small filter, washed with water, and a few drops of hydrochloric acid poured on it. Any calcic oxalate present is dissolved, and the uric acid remains behind in the filter. The filtrate is diluted in a test-tube with 15 c.c. of water, and by means of a pipette is carefully just covered with a sufficient quantity of very dilute ammonia. The fluids gradually mix if left at rest, and after twenty-four hours all the calcic oxalate present will have collected on the bottom, and under the microscope appears in the form of the most beautiful octahedra. If it is now poured on a weighed filter, washed and dried, and then incinerated in a platinum crucible, the amount of caustic lime found, gives, by multiplying by 1·6071, the amount of oxalic acid.

Beneke has proposed a method for the approximate estimation of calcic oxalate,† and Vogel for that of oxalic acid,‡ but neither can be relied upon, as has recently been shown by Nickel.§ Methods have also been devised by Schultzen

* Neubauer and Vogel : Loc. cit., p. 168 ; Cf. Salkowski : "Zeits. physiol. Chem.," x., 120—122.

† Beneke : Quoted by Vogel in Neubauer and Vogel, loc. cit., p. 346.

‡ Vogel : Loc. cit., p. 321.

§ Nickel : "Zeit. physiol. Chemie," xi., S. 186—200.

and Bucheim (see Salkowski and Leube: "Die Lehre vom Harn," S. 118).

Succinic Acid, $H_2C_4H_4O_4$ — this third acid of the oxalic series, separated from oxalic acid by the intermediate malonic acid, has been found in urine by Meissner,* who maintained that it is a normal constituent of urine. Salkowski denies this,† and he is supported by von Speyer. Since urine and fermented liquors contain this acid, and since it can pass unchanged into the urine, it may occasionally be present in urine (Sheridan Lea). It crystallizes in monoclinometric prisms (Fig. 29) soluble in

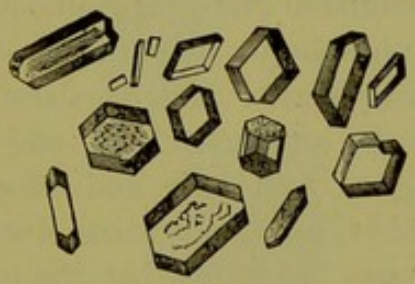


FIG. 29.—CRYSTALS OF SUCCINIC ACID.—(*Frey.*)

water and alcohol. Baumann found no succinic acid in the urine of a dog which he fed with succinate of sodium. It is said by some, and denied by others, that asparagus causes this acid to appear in the urine.‡

Lactic Acid, $C_3H_6O_3$. — Of the four (Strecker and Wislicenus) lactic acids which are all isomeric with each other, three occur in the body, namely—(1) Ethylidene, or ordinary lactic acid, which is the characteristic acid of the lactic fermentation; it is found in the stomach and intestines and in muscle, and is excreted in the urine after phosphorus poisoning as well as in many diseases (Sheridan Lea); (2) Ethylene-lactic acid, found in the watery extract of muscles; and (3) Sarcolactic or paralactic acid,

* Meissner and Shepard: "Unters. ü. d. Entsteh. der Hippursaure," &c., Hannover, 1866.

† Salkowski: "Pflüger's Archiv," Bd. ii., S. 367; Bd. iv., S. 95.

‡ Hoppe-Seyler: "Physiol. Chemie," S. 826.

also found in the muscles, and in large quantities in Liebig's extract of meat; it differs from the others by its having the power of rotating the plane of polarized light. All these acids possess a sour taste and a strong acid reaction; they are syrupy, colourless liquids, soluble in alcohol, ether, and water.

It seems doubtful which of these acids is the one* which goes over into the urine, so I shall merely call the latter lactic acid. Lactic acid can appear in the urine, according to Spiro, after strong activity of the muscles;† Simon and Wibel found it in the urine of cases of trichinosis,‡ Bouchardat in diabetic urine,§ Schultzen and Riess‖ in acute yellow atrophy of the liver and phosphorus poisoning, and Gorup-Besanez¶ in rickety children, Körner and Jacubasch in leukæmia,** Moers and Myk in osteomalacia,†† and Wibel in trichinosis. According to Colasanti and Moscatelli, paralactic (=sarcolactic) acid occurs in the urine of soldiers after forced marches.‡‡ Marcuse maintains that the urine of mammalia during activity is free from lactic acid.§§

The occurrence of lactic acid in normal urine is doubtful. "There are no characteristic tests for lactic acid, but the microscopic appearance of some of its salts is distinctive and very important for its recognition" (Neubauer). These salts are the lactate of calcium (Fig. 30) and lactate of zinc. Neubauer and Scherer have devised methods for its separation from urine.‖‖

* According to some it is sarcolactic acid.

† Spiro: "Zeits. f. physiol. Chemie," Bd. i., S. 117.

‡ Simon and Wibel: "Ber. d. deutsch. chem. Gesell.," 1871, S. 139.

§ Bouchardat: "Jahresb. d. Thierchemie," 1876, S. 155.

‖ Schultzen and Riess: "Ann. des Charité Krank.," Bd. xv., S. 1.

¶ Gorup-Besanez: "Lehrb. d. physiol. Chem.," 4 Aufl., 1878, S. 606.

** Körner and Jacubasch: "Arch. f. path. Anat.," Bd. xliii., S. 196.

†† Moers and Myk: "Zeits. anal. Chem.," 1869, S. 520; "Deutsch. Arch. f. klin. Med.," 1869, Bd. v., S. 486.

‡‡ "Gazetta Ital.," xvii., 548—557.

§§ Marcuse: "Bied. Centralbll.," 1887, 92—94.

‖‖ Neubauer and Scherer: Neubauer and Vogel, loc. cit., pp. 132, 133.

According to v. Jaksch, *fatty acids* occur in traces in normal urine,* the amount not exceeding 0·008 gram daily. By oxidizing normal urine formic (CH_2O_2), acetic ($C_2H_4O_2$), and probably butyric ($C_4H_8O_2$) acids are formed to the extent of 0·9—1·5 grams daily. Under certain pathological conditions, the proportion of free acid is increased, but not that obtained by oxidation. In cases of fever and liver

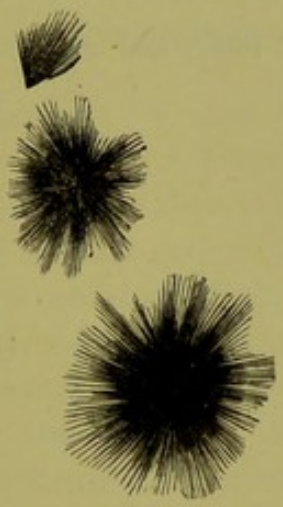


FIG. 30.—CRYSTALS OF LACTATE OF LIME IN GROUPS OF FINE NEEDLES.—(*Frey.*)

disease, from 0·6—1 gram of free acid, chiefly acetic, was found in the day's urine. The same observer used the name "*lipaciduria*," to denote the excess of volatile fatty acids in the urine (p. 17).

Glycerin-Phosphoric Acid, $C_3H_9PO_6$, occurs, according to Klüpfel and Fehling, in small traces in normal human urine, and it has been recognized therein also by Sotnitschewsky.† It is found in leukæmic urine, in transudations, muscles, brain, nerves, egg-yolk, pus, &c., and arises as a decomposition product of lecithin in the blood.‡ It

* Von Jaksch: "Chem. Centr.," 1885, S. 905; and "Zeits. physiol. Chemie," x., S. 536—560.

† Sotnitschewsky: "Zeits. physiol. Chem.," Bd. iv., S. 214.

‡ Hoppe-Seyler: "Handbuch phys. und path. chem. Anal.," 1875, S. 119.

is only known as a syrupy liquid, not in the solid state; it is decomposed by heating, into glycerin and phosphoric acid, from which it has been synthetically prepared. Hoppe-Seyler gives a method for its preparation.*

Closely connected with these acids are muscle sugar or inosit, which has been recognized as a component of normal human urine by von Strauss and Kultz, milk sugar, and grape sugar. These will be considered further on.

* Ibid. : Loc. cit., S. 120.

CHAPTER VI.

THE AROMATIC BODIES IN URINE.

THE term "aromatic bodies" is used by chemists merely in a technical sense, to denote a large class of substances containing at least 6 atoms of carbon. Benzene, C_6H_6, is the fundamental substance of the group; it is obtained from bituminous coal, and is contained therefore in coal tar, and with other members of the series, such as toluene, xylene, mesitylene, pseudocumene, and so on, belongs to the C_nH_{2n-6} group. The reason why these substances are named "*aromatic*" is that many members of the series occur in bodies possessing an aromatic smell, such as gum-resins, essential oils, and so on.

Phenol or carbolic acid belongs to this series, and its relationship to benzene is thus shown:—

Benzene, C_6H_5H.

Phenol, $C_6H_5(HO)$, in which one atom of hydrogen is replaced by hydroxyl.

We may have the following classes * derived from the C_nH_{2n-6} series: (1) Halogen derivatives, (2) nitro-compounds, (3) amido-compounds, (4) diazo-compounds, (5) sulphuric acids, (6) phenols, (7) alcohols, (8) aldehydes, (9) acids, (10) ketones and quinones, and (11) hydroxy acids. The occurrence of these aromatic substances in urine is in great part, but not altogether, dependent on the food, as in the fasting condition the urine is not altogether free from them.† When

* Remsen: "Organic Chemistry," p. 253.

† Hoppe-Seyler: "Physiol. Chem.," S. 830.

introduced with the food or subcutaneously injected, or injected into the blood, or merely applied to the skin, as in the case of carbolic acid, they suffer, as a rule, only slight change after absorption, although they may be easily capable of oxidation. And they pass over into the urine unchanged or partially oxidized, in combination with other substances, or uncombined. In this they show a distinction from the fatty bodies, as these, such as fat and sugar, undergo oxidation in the organism. De Jonge, for instance, found that after giving a dog only 4 milligrams of pyrocatechin (catechol), he was able to detect it in the urine unchanged.*

The aromatic substances occurring in urine belong to four classes (Hoppe-Seyler): — (1) Ether-sulpho-acids, of which phenol-sulphuric acid is the simplest example; (2) Glycocine combinations of aromatic substances, of which hippuric acid is an example; (3) Combinations with glycuronic acid, of which only a few are yet known, and which do not exclusively belong to the aromatic series; and (4) Uncombined aromatic substances, such as cumarin, hydroparacumaric acid, and paroxyphenyl-acetic acid.

To Baumann is due the credit of working out the behaviour of the aromatic substances in the animal body, and the changes they undergo on passing over into the urine. He discovered, in 1876, the conjugated sulpho-acids of the aromatic hydroxyl combinations in urine,† and he prepared them synthetically the same year. Since then a number of bodies which, until recently, were entirely unknown, have been discovered rapidly one after another. And it is only necessary to look over Hoppe-Seyler's "Zeitschrift," or the translations from it in our English "Journal of the Chemical Society," to be convinced of the great progress of knowledge in this direction. I cannot give here a full account of these substances, as it would occupy too much space, so

* De Jonge: "Zeits. f. physiol. Chem.," Bd. iii., S. 177.

† Baumann: "Pflüger's Archiv," Bd. xii., S. 69.

that I am limited merely to a short reference to the most important.

In 1851 Städeler* discovered in the urine of cattle and of men substances which, on distillation with sulphuric acid, yielded phenol (carbolic acid). Hoppe-Seyler and Bugalinski showed later that the phenol was not present in appreciable quantities in the uncombined state in urine, but could be found after distillation with diluted acid.†

Baumann then showed that phenol and substances like it are contained in the urine in an ethereal combination with sulphuric acid, and from horses' urine he obtained phenol- and cresol-sulphate of potassium, also phenol-sulphate of potassium from the urine of men to whom phenol had been administered, and he prepared it synthetically by the action of pyro-sulphate of potassium on potassium phenol.‡ Baumann found afterwards that the most different hydroxyl combinations of benzol are changed into this ethereal combination.

Regular amounts of phenol- and cresol-sulpho-acids occur in combination in the urine of men and dogs, but their presence is more difficult of explanation than in the case of the herbivora, where they can be traced to the aromatic substances taken with the food. But even on a purely animal diet, these ether-sulpho-acids occur in urine. Senator found traces of them in newly-born children,§ and even in the liquor amnii; Christiani in the urine of dogs after an exclusively flesh diet;‖ and von den Velden, Baumann, and Herter found the conjugated sulpho-acids in men's urine. Brieger¶ examined the variations of phenol in the urine in different diseases, and found it increased in septic conditions, and he

* Städeler: "Ann. chem. Pharm.," Bd. lxxvii., S. 17.

† Hoppe-Seyler: "Physiol. Chem.," S. 836.

‡ Baumann: "Ber. d. deutsch. chem. Gesell.," Bd. ix., S. 1715; "Zeits. physiol. Chem.," Bd. ii., S. 335.

§ Senator: "Zeits. physiol. Chem.," Bd. iv., S. 1.

‖ Christiani: Ibid., Bd. ii., S. 273.

¶ Brieger: "Centralbll. f. d. med. Wiss.," 1878, No. 30; "Zeits. f. physiol. Chem.," Bd. ii. S. 241.

also found that after giving tyrosin by mouth it became increased. Hence phenol must be derived from something else besides phenol in the food. Baumann afterwards found that phenol was formed by the putrefaction of proteids in the presence of pancreatic ferment,* and Brieger† found it in the intestine. But Baumann showed that the phenol formed in the intestine consists mainly of paracresol, and Weyl‡ found that paracresol results from the putrefaction of tyrosin. It has been stated by Baumann that the phenol which occurs in the urine as alkaline phenol sulphate is probably derived from the further putrefaction of the tyrosin formed in the intestine, and passes through the stages of paracresol and paroxybenzoic acid.§

Pyrocatechin (catechol) and hydrochinon have also been found in the urine after the administration of benzol; these also occur as ether-sulpho-acid salts (Baumann and Herter), and pyrocatechin-sulpho-acid has been found in traces in human urine, also in horses' urine constantly, in combination with potassium.

The dark colour of the urine after carbolic acid has been absorbed into the blood has been ascribed by Baumann and Preusse to hydrochinon, and the dark colour of the urine of herbivora, as well as that of their blood serum, is probably due to decomposition products of aromatic substances such as pyrocatechin (catechol) and pyrogallol (W. G. Smith, Hoppe-Seyler).

The method of examining urine for phenol consists in adding sulphuric or hydrochloric acid to excess, and distilling. Bromine water is added to the distillate as long as a precipitate or turbidity forms. If phenol be present, a yellowish-white crystalline precipitate of tribromide of phenol forms, which may be collected and weighed, and from this

* Baumann: "Zeitschrift physiol. Chem.," Bd. i., S. 60; Bd. iii., S. 250.

† Brieger: Ibid., Bd. iii., S. 149.

‡ Weyl: Ibid., Bd. iii., S. 312.

§ Purser: "Dub. Journ. Med. Soc.," Jan., 1880, p. 57.

the quantity of phenol excreted may be calculated. Phenol and cresol are excreted in excess in cases of strangulated hernia, artificial stoppage of the small intestine (Salkowski), and peritonitis (Brieger)—that is, in those cases where there is increased putrefaction going on in the intestine. In putrid empyema, putrid bronchitis, &c., the amount of these substances is also increased (Brieger), so that they do not arise altogether from the putrefactive products formed in the intestine. Brieger* stated that in scarlet fever, diphtheria, erysipelas, and pyæmia there is an increased excretion of phenol, and he accordingly grouped these diseases together as putrefactive diseases (Fäulniss-Krankheiten); but Haldane,† on repeating these experiments, found Brieger's method faulty, and he used Salkowski's method of analysis instead,‡ which consists in precipitating the urine with an equal volume of a mixture of one part of saturated barium chloride solution and two parts of baryta water. By this method the sulphates are precipitated with the phosphates: 100 c.c. of the filtrate are then acidified with 5 c.c. of hydrochloric acid, boiled, and digested on a water bath until the additional barium sulphate, which comes down, has settled. This is then filtered off, washed, ignited, and weighed. In another quantity of urine the sulphates and ether-sulphates are estimated together in a similar way. Taking the normal proportion of the sulphuric acid combined as ethereal hydrogen sulphate, to that combined as ordinary sulphates, as 1 to 10, it was found in sixteen cases of scarlet fever, in which the urine was passed during the fever, to be 1 to 17, and in thirteen cases, in which the urine was passed during the first three days of convalescence, to be 1 to 20·9, so that, according to Haldane, there is no ground for including scarlet fever among the "putrefactive" diseases.

We may now consider, in detail, some of the aromatic

* Brieger: "Zeits. f. klin. Med.," 1881., iii. S. 465.
† Haldane: Journ. Physiol.," vol. ix., No. 4, pp. 213–219.
‡ Salkowski: "Die Lehre vom Harn," p. 176.

bodies present in urine; but first of all, let us see how they are produced.*

Two actions on the food take place in the digestive tract: (1) Digestive processes brought about by the action of unorganized ferments on the food, and (2) putrefactive processes brought about by the action of organized ferments which are swallowed with the food.† The putrefactive processes occur only in the intestine, not in the stomach, under ordinary conditions, since the reaction of the gastric juice is acid, and they can only occur when the reaction is alkaline. Hence they occur in the small and upper parts of the large intestine. In these the pancreatic juice plays a prominent part, its ferments splitting up starch into dextrin and sugar, fats into glycerin and fatty acids, and proteids into albumose or propeptone and peptones, from which there may be formed leucin, tyrosin, hypoxanthin, aspartic acid, and so on. But if putrefactive micro-organisms are present as well, either in digestion with pancreatic juice within or without the body, a number of other substances are formed, of which indol, skatol, phenol, and cresol are the most important. This is the mode, then, in which these aromatic bodies arise, and we may now consider some of them.

Indican, $C_8H_6NSO_4K$, is a normal constituent of urine, and arises primarily from indol—one of the putrefactive products just mentioned, which has the formula C_8H_7N. It is a conjugated sulpho-acid salt of potassium, or indoxyl-sulphate of potassium, as it occurs in urine. Indol, the radical, can be obtained by the ordinary putrefaction of albumin, by heating it to 200° C., or by fusing it with caustic alkali. This substance, indol, when formed in the intestine, or introduced with the food, or injected under the

* It is usual now to say that some of these aromatic bodies occur as sulphonates, not sulphates, and in the above description these terms may be regarded as synonymous. Sulphuric may also be read as sulphonic.

† I am indebted to an able article by Prof. J. M. Purser, of the Dublin University, on the "indigo-producing substances in urine," which will be found in the "Dublin Journal of Medical Science" for January, 1880, p. 55, for part of the above account.

skin, reappears in the urine as indican or indoxyl-sulphate of potassium. Schunck supposed* that it was identical with plant indican, but Hoppe-Seyler found that plant indican is more decomposable than that present in urine. By the action of hydrochloric acid in the presence of water it splits up into indoxyl and acid potassium sulphate :—

$$C_8H_6NSO_4K + H_2O = C_8H_6NOH + KHSO_4.$$

By oxidation the indoxyl is converted into indigo blue :—

$$2(C_8H_6NOH) + O_2 = C_{16}H_{10}N_2O_2 + 2H_2O.$$
(Indoxyl.) (Indigo blue.)

Indican is increased in the urine† in obstruction of the intestine, incarcerated hernia, peritonitis, and paralytic conditions of the intestine, in typhus, lead colic, trichinosis, catarrh of the stomach, cholera, carcinoma of the liver, long-standing suppuration, paraplegia, Addison's disease, and after taking certain drugs, such as turpentine, oil of bitter almonds, nux vomica, creosote, &c. It is absent from the urine of new-born children (Senator).

It forms white glistening tablets and plates (when prepared chemically pure), easily soluble in water, less soluble in alcohol; but when obtained from urine it is a clear brown syrup, soluble in water, alcohol, and ether. It is probably identical with Heller's uroxanthin. When normal urine is boiled with common hydrochloric acid (about equal parts of each), cooled, and agitated with chloroform, the chloroform is generally violet and shows an absorption band before D, due to indigo blue, and another after D, probably due to indigo red. The bluer the solution the darker is the band before D, and the redder the darker that after D.‡ At the same time it may be stated that it is not acknow-

* Schunck : "Philos. Magaz.," (4) vol. xiv., p. 288.

† In 1,500 c.c. of normal human urine Jaffé found from 4·5—19·5 milligrams of indigo blue. Horses' urine contains twenty-three times as much.

‡ MacMunn : "Proc. Roy. Soc." No. 226, 1883.

ledged by chemists that indoxyl-sulphate of potassium, like plant indican, yields indigo red. There are certainly reasons for concluding that the mother substance in urine does not yield these red and blue pigments in equal amounts in different cases, as sometimes the band before D is darker, sometimes that after D; but this test always can be made to prove the presence of indican in normal urine. I found that this test reveals the presence of indican when Jaffé's test fails. Sometimes the addition of two or three drops of nitric acid to the hydrochloric may be required.

Jaffé mixes equal parts of urine and hydrochloric acid, and then adds cautiously, drop by drop, a saturated solution of the so-called chloride of lime ("bleaching salt"), which contains, in addition, hypochlorite of calcium, until the maximum of blue colour appears. If the urine is then agitated with chloroform the blue colouring matter goes into the chloroform, and is left after its evaporation, when it may be weighed, and a rough approximation made of the amount. *But this method does not give accurate results:* besides, the bleaching salt destroys faint traces of indican, as I have proved.* Albumin, if present, must be separated before performing Jaffé's test, as it develops a blue colour with hydrochloric acid.

Ludwig obtained indican by heating hæmatin or urobilin with a caustic alkali and zinc dust (Landois).

Sometimes urine shows, when decomposing, a bluish-red pellicle of microscopic crystals of indigo blue, owing to the decomposition of the indican (Hill Hassal). Ord has described a calculus composed of indigo. And indican has been found in the sweat by Bizio.

Phenol, C_6H_6O, or carbolic acid, has been referred to before. It occurs in the urine as phenol-sulphonate of potassium, $C_6H_5O—SO_2—OK$. It may be recognized as follows:—The urine is treated with sulphuric acid until

* It is necessary to dilute the solution of "bleaching salt" in cases where only traces of indican are present.

it contains 5 per cent. of the acid, and is then distilled in a glass vessel as long as the distillate becomes cloudy with bromine water. The distillate shows these reactions :—*

(1) Bromine water causes a precipitate, soon becoming crystalline, of tribromo-phenol ($C_6H_2Br_3OH$). (331 parts represent 94 parts phenol.)

(2) The ammoniacal liquid is coloured blue by gentle warming with dilute hypochlorite of sodium solution (1 to 20).

(3) A strip of pine-wood saturated with HCl is coloured in the liquid a dark blue after some time.

(4) The neutralized liquid is coloured violet with a neutral solution of perchloride of iron.

(5) A dilute solution of phenol heated with Millon's reagent gives a red colour.

Both phenol-sulphate of potassium and para-cresol-sulphate of potassium give the first reaction.

Cresol-Sulphuric Acid,† $C_6H_4(CH_3)SO_4H$, is abundant in the urine of herbivora, but scanty in human urine. Its potassium salt—in which combination it occurs—may be obtained by evaporating horses' urine (3—4 liters) on the water bath to a syrup, extracting the residue with absolute alcohol, filtering, and, when cold, precipitating with an alcoholic solution of oxalic acid, after a quarter of an hour removing the precipitate by filtering, making the filtrate slightly alkaline with caustic potash, filtering off the precipitate, and evaporating to a thin syrup, and then keeping the filtrate at a temperature of 0° C. The leafy crystals are purified by recrystallization out of boiling alcohol (Krukenberg).

A solution of cresol-sulphate of potassium gives no reaction with Fe_2Cl_6. It gives a red colour with Millon's reagent.

* Krukenberg : Loc. cit., S. 87.

† Or sulphonic acid.

Cresol occurs in human urine as a cresol-sulphate or sulphonate of potassium.*

Pyrocatechin-mono-ether-sulphate of potassium and pyrocatechin-disulphate of potassium may also occur in urine.

Pyrocatechin (catechol or brenzcatechin), $C_6H_4\begin{cases}OH\\OH'\end{cases}$ has been frequently confounded with protocatechuic acid; but, as Dr. Walter G. Smith and Preusse have shown, they are easily distinguished from each other.†

Protocatechuic Acid, $C_6H_3\begin{cases}(OH)_2\\CO_2H\end{cases}$ (dioxy-benzoic acid), sometimes occurs in urine; the urine containing it *gets darker on exposure to the air, the dark colour proceeding from above downwards.* This substance has been recognized in the urine by Dr. Walter G. Smith,‡ of Dublin, and was described in 1861 under the name of alkapton by Bödeker.§ In Dr. W. G. Smith's case the urine became reddish-brown on adding an alkali, and showed no indigo reaction. It slightly reduced cupric hydrate and gave a distinct green colour with Fe_2Cl_6. It also reacted slightly with Löwe's bismuth test. Dr. Smith shows that the name alkapton conveys no real information, having been given at a time when the physiological chemistry of urine was very imperfectly understood.

Dr. R. Kirk has described the occurrence of Bödeker's alkapton in urine,‖ and thinks he has proved that the body giving the characteristic reaction consists of two distinct substances (uroleucic and uroxanthic acids).¶

* In the form of paracresol and its isomers ortho- and meta-cresol, as sulphonic combinations.

† Smith, W. G.: "Dub. Journ. Med. Sc.," Jan., 1882; and Preusse: "Zeitsch. f. physiol. Chem.," ii., S. 324. This reaction has been attributed by Epstein and Muller (Virch. Archiv., Bd. lxii., S. 554), also by Salkowski and Leube, and others, to pyrocatechin.

‡ Smith, W. G.: Ibid.

§ Bödeker: "Ann. Chem. Pharm.," cxvii., Jan., 1861.

‖ Kirk: "Brit. Med. Journ.," ii., 1888, pp. 232, 233.

¶ Dr. Maguire ("Brit. Med. Journ.," October 25th, 1884) thinks that

Skatol (C_9H_9N, Nencki and Brieger) is another body of the aromatic series, arising from the process of putrefaction in the intestine, and is derived from the splitting up of proteids under that influence. It is said to be present in the urine as skatoxyl-sulphate of potassium. Skatol compounds give a violet colour with dilute nitric acid, and are precipitated in red flakes by fuming nitric acid (Nencki).

Brieger* and Nencki† first obtained skatol from human fæces, then Baumann‡ obtained it by the putrefaction of a mixture of nitrogenous acids from urine, and Baeyer§ lastly obtained it from commercial indigo as a bye-product. He found that it had no smell when pure, whereas Brieger and Nencki said it had a decidedly fæcal odour. Salkowski‖ obtained by putrefaction a skatol-carbonic acid, from which, by heating, skatol was separated. Brieger, on feeding dogs with skatol, found skatoxyl-sulphuric acid in their urine combined with potassium ;¶ and he showed that such urine gave, with hydrochloric acid containing chlorine,** not blue indigo, but a more violet colouring-matter, soluble in water and ether. Human urine contains less indoxyl- than skatoxyl-sulphuric acid. These conjugated sulpho-acid salts arise, for the most part then, from the putrefaction of proteids in the intestines, but, as Hoppe-Seyler remarks,†† "it would be incorrect to conclude that all aromatic substances occurring in urine, either free, or conjugated with sulphuric acid, which are known to arise as decomposition

pyrocatechin is identical with Bödeker's alkapton, and attributes the darkening of urine, as described above, to pyrocatechin. The tests for pyrocatechin are given in Salkowski and Leube, loc. cit., S. 145.

* Brieger : "Ber. d. deutsch. chem. Ges.," Bd. viii., S. 722 ; Bd. x., S. 1027 ; Bd. xii., S. 1985 ; "Journ. f. pract. Chem.," N.F., Bd. xvii., S. 124.

† Nencki : "Centralb. f. d. med. Wiss.," 1878, No. 47 ; "Journ. f. pract. Chem.," N.F., Bd. xx. ; "Zeit. phys. Chem.," Bd. iv., S. 371.

‡ Baumann : "Ber. d. deutsch. chem. Ges.," Bd. xiii., S. 284.

§ Baeyer : "Ber. d. deutsch. chem. Ges.," Bd. xiii., S. 2339.

‖ Salkowski : Ibid., Bd. xiii., S. 2217.

¶ Brieger : "Zeits. f. physiol. Chem.," Bd. iv., S. 414.

** Hydrochloric acid and bleaching salt.

†† Hoppe-Seyler : "Physiol. Chem.," S. 845.

products of the albuminoids, must arise in the intestine. Baumann* has obtained hydroparacumaric acid from pus obtained from purulent peritonitis." J. Otto obtained a red pigment from the urine of a patient suffering from diabetes, which was considered to be skatoxyl-sulphate of potassium,† as it gave the reactions of that body. Bruno Mester‡ has lately endeavoured to investigate the relationship between skatol and this colouring matter by feeding a dog with skatol, and then looking for the salts of skatoxyl-sulphuric acid in the urine by G. Hoppe-Seyler's method;§ but he either failed to find any, or but the slightest trace, so that the pigment belonging to skatol, which occurs in urine on acidulation, and which Mester found in abundance in dogs' urine after feeding with skatol, is not, according to this observer, a compound of skatoxyl-sulphuric acid, but a chromogen of an unknown nature. The urine of the dog experimented upon gave these reactions:—Freshly passed, it was reddish-yellow; by long standing in the air, especially in the upper layer, it became reddish. By applying Jaffé's test (*v. supra*) for indigo, and even on adding concentrated hydrochloric acid, it became dark red; by warming the colour became more intense, and by further heating it became violet. It reduced an alkaline copper solution, and was lævorotatory. Mester considers that the pigment is an oxidation product of skatoxyl. It was found to be amorphous. A solution of the chromogen, at first colourless, became dark violet, and later brown on exposure to the air. It dissolved in hydrochloric or sulphuric acid with a red, and in alkalies with a yellow colour. It was soluble in alcohol, amyl alcohol, ether, and chloroform, but not in water. It appeared to be unaltered by ammoniacal fermentation. Mester suggests that his pigment is probably identical with those

* Baumann: "Zeits. f. physiol. Chem.," Bd. iv., S. 307.
† Otto: "Pflüger's Archiv," xxiii., 614.
‡ Bruno Mester: "Zeit. physiol. Chemie," xii., S. 130—144.
§ Hoppe-Seyler G.: "Zeit. physiol. Chem.," vii., S. 423.

described under the names of urorubin, uroroseïn, uroerythrin, purpuric acid, &c. I cannot agree with Mester in this statement, as I shall show further on when discussing the urinary pigments. But although he does not, unfortunately, give the spectroscopic characters of the pigment, yet his data are useful for purposes of comparison.

Every medical man has come upon such chromogens in urine which assumes a red or red-violet colour on adding a mineral acid, and in cases of anæmia this is not at all uncommon, as I have frequently observed. This would tend to support Sir Andrew Clark's theory, that in chlorosis and allied conditions there are fæcal matters absorbed from the intestine into the blood which have a rapidly destructive effect upon the red corpuscles.

Other bodies of the aromatic series occur in urine, but I have not space to discuss them here, and must refer those who want further information to Hoppe-Seyler's "Physiologische Chemie," Salkowski and Leube's "Die Lehre vom Harn," Hoppe-Seyler's "Zeitschrift für Physiologische Chemie," and the "Journal of the Chemical Society," where the pith of recent German physiological chemistry is given, especially since Dr. Halliburton has been added to the staff of abstractors.

CHAPTER VII.

THE COLOURING MATTERS OF URINE.

I SHALL here describe mainly the intrinsic colouring matters of urine—that is, those which are not due to pigments belonging to drugs taken by mouth, or of substances intentionally added to the urine in order to deceive the practitioner, or to the presence of blood or of bile. It is inconvenient to separate the normal pigments from those which arise in disease ; accordingly, morbid pigments will be mentioned.

I shall only describe the result of recent work, leaving matters of historical interest for the curious to look up for themselves; but I may say that there is great danger of being led astray by reading a good many English and American text-books on the urine, the authors of which seem, some of them at least, unable to divest themselves of preconceived ideas.

The principal colouring matter of normal urine is—

Normal Urobilin.*—It is a nitrogenous, amorphous, yellowish-brown pigment, soluble in alcohol, chloroform, acidulated water, acids, partially in ether and benzol.† It gives, when in acid solution, a band close to and enclosing Fraunhofer's line F; when alkalies are added to the urine until it is neutral, this band disappears. It exists in urine partly

* Vierordt ("Zeits. f. Biologie," Bd. ix., S. 160) points out that urine shows other appearances when examined by spectrophotometric methods than do solutions of urobilin.

† MacMunn : "Proc. Roy. Soc.," No. 208, 1880. "Journ. Physiol.," vol. x., Nos. 1 and 2.

as a chromogen, as can be found by passing a little chlorine gas into normal urine, or by adding some dilute permanganate of potassium solution to it, as the band then appears darker.* It appears to be identical with choletelin, or with a substance which can be procured by treating hæmatin in acid solution with peroxide of hydrogen and isolating the oxidation product. Sometimes subsequent brief reduction with sodium amalgam may be necessary, as I have shown. When isolated from urine and treated in alcohol solution with zinc chloride and ammonia, the band at F is replaced by another narrower one nearer the red end of the spectrum, the solution showing at the same time a green fluorescence, which is not nearly as well marked as in the case of pathological urobilin. It may be obtained from urine by complete precipitation of the colouring matter with neutral and basic lead acetate, filtering, treating the precipitate with rectified spirit acidulated with sulphuric acid, filtering, diluting the filtrate with water, and agitating in a separating funnel with chloroform; on evaporating the chloroform it is left behind, and possesses the characters described above.

Normal urobilin is therefore an *oxidation* product of effete hæmatin and bile pigments, not a reduction product, and it is not identical with hydrobilirubin.

The old theory was that hydrobilirubin was produced in the intestine by the action of the nascent hydrogen generated by the putrefactive processes on bilirubin, and that it was then absorbed into the blood and excreted as the urobilin of urine†; but I have shown, and in this my

* MacMunn: Loc. cit., p. 210.

† The earlier observations of Jaffé[1] and Maly[2] are known to readers of modern text-books of physiology; the former found urobilin first in the bile, then in urine, and described its green fluorescence with zinc chloride and ammonia, he also described its chromogen in urine. Maly then showed how to prepare a like substance, which he considered identical with Jaffé's urobilin from bilirubin, and he named this hydrobilirubin, and subsequently said it was

[1] "Centralb. f. d. med. Wiss.," 1863, S. 241. "Archiv. f. path. Anat.," Bd. xlvii., S. 405. "Zeit. f. anal. Chem.," Bd. ix., S. 105 and Bd. iii., S. 245.

[2] "Ann. Chem. Pharm.," Bd. clxi., S. 368 and Bd. clxiii., S. 77.

statement has been confirmed by Le Nobel of Leyden,* that hydrobilirubin gives certain bands when its solutions are treated with zinc chloride and ammonia, which are never seen in similarly treated solutions of urobilin. Moreover, the black, broad absorption band at F of hydrobilirubin differs completely from that of normal urobilin, as can easily be seen by comparing an alcohol or other solution of the respective pigments.

Choletelin, according to Maly,† may be represented probably by the formula $C_{16}H_{18}N_2O_6$: it is the end-product of the oxidation of bilirubin or biliverdin.

Hydrobilirubin, according to the same observer,‡ may be

* Le Nobel: "Pflüger's Archiv," Band xl., 1887.

† Maly: "Sitzb. d. Wien. Akad.," Bd. lvii., 1868, 2 Abth. Feb., also Band lix., 1869, 2 Abth., April.

‡ Maly: "Centralb. f. d. med. Wiss.," No. 54, 1871; also, "Annal. d. Chem.," Bd. clxiii., 1872, S. 77.

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identical with the stercobilin of Vaulair and Masius.[1] He supposed that the bilirubin of the bile is changed into urobilin by reduction in the intestine, and is then absorbed in part and appears in the urine. Hoppe-Seyler[2] showed that a body with the reactions of urobilin could be obtained by the action of tin and hydrochloric acid on hæmoglobin and hæmatin, and he could not find it in blood-serum. He says that normal urine contains no traces of urobilin; on the contrary, a chromogen which gradually forms urobilin by a spontaneously occurring oxidation—an observation first made by Jaffé. Hoppe-Seyler, in his book on "Physiological and Pathological Chemical Analysis," refers to Baumstark's[3] urorubrohæmatin and urofuscohæmatin, and says that both of these pigments stand in near relationship to hæmatin. Among later contributions to this subject, I may mention those of Esoff[4] and Disque.[5] The latter extended the knowledge of urobilin and hydrobilirubin considerably, and showed that the hydrobilirubin of Maly cannot be considered a pure body, a statement with which I agree. He could only find the band of Jaffé's urobilin in urine after long exposure to the air, but he evidently considered hydrobilirubin and urobilin identical substances, and he found the latter most abundantly in all diseases where the urine is diminished in quantity, also in copious perspiration, in heart disease and lung diseases, especially pneumonia.[6]

1 "Centralb. f. d. med. Wiss.," 1871, No. 24.

2 "Archiv. f. d. ges. Physiol.," Bd. x., S. 208. "Berich. d. deutsch. Chem. Ges.," Bd. vii., S. 1065.

3 "Berichte d. deutsch. chem. Gesell.," 1874, vii., S. 1170.

4 "Archiv. f. d. ges. Physiol.," xii., S. 50.

5 "Zeitsch. f. physiol. Chemie," Bd. ii., S. 259.

6 From the author's paper in "Journ. Physio.," vol. x, Nos. 1 and 2 p. 72.

represented by the formula $C_{32}H_{44}N_4O_7$, and was obtained by him from bilirubin by the action of sodium amalgam. Hence it was supposed to be due to reduction. I have lately found that there are various intermediate substances formed in this reaction, which differ in no essential respect from intermediate oxidation products obtainable from bilirubin, so that we cannot assume that hydrobilirubin is produced by reduction; on the contrary, it appears to be an intermediate product of *oxidation*. This can be explained by Hoppe-Seyler's hypothesis that it is nascent oxygen which plays the most important part in these so-called reduction processes*—an hypothesis which explains many paradoxes in physiological chemistry. At one stage of the reaction we can isolate a substance which, on treatment with zinc chloride and ammonia, possesses not a *green* but a *red* fluorescence, and this, as I have recently shown, is very like, if not identical with, a similar substance found in bile. I formerly named this "*biliary*" urobilin,† and, strange to say, both the substance prepared artificially from bilirubin, and that occurring naturally in bile, change into a substance which gives a *green* fluorescence with zinc chloride and ammonia. So that in bile we have also such intermediate products of oxidation. But I may again say that neither of these ever appears in urine except when the patient is recovering from jaundice, although I am not sure that they occur even then.‡

Febrile Urobilin, or, as I have lately named it, *pathological urobilin*, is a different substance, although obtainable in the same way, and soluble in the same media, as normal urobilin; the band at F, which characterizes the urobilin series, being much broader and much darker in its various

* Hoppe-Seyler: "Berichte d. deutsch. chem. Ges.," xvi., 117—123; also Ibid., S. 1917—1924; "Zeits. physiol. Chem.," x., S. 36—39. See "Journ. Chem. Soc.," 1880.

† MacMunn: "Proc. Roy. Soc.," No. 208, 1880, p. 221.

‡ Le Nobel: Loc. cit.; and MacMunn: "Journ. Physiol.," vol. x., Nos. 1 and 2.

solutions than that of normal urobilin, and it shades off towards violet. Besides, other bands are visible in the red half of the spectra of its solutions, and with zinc chloride alone, with that and ammonia, and with other reagents, characteristic differences are seen between pathological and normal urobilin. We know that by reducing the artificially prepared pigment, when it is obtained either from hæmatin or from bile pigments (and which corresponds to normal urobilin), with sodium amalgam, or otherwise, we obtain a substance more closely resembling pathological urobilin ; *hence we may safely infer that this substance represents a less oxidized stage than does normal urobilin.* But there is another pigment found in the body which is so like pathological urobilin that it can hardly be distinguished from it, and that substance is stercobilin — the colouring matter of fæces — so named by Vaulair and Masius,* as they did not consider it to be identical with hydrobilirubin, although Maly afterwards endeavoured to prove the identity. I have found, however, that, while stercobilin is not identical with hydrobilirubin, it is indistinguishable from pathological urobilin.† If this be so, then the presence of pathological urobilin in urine is, to a certain extent, an indication of the absorption of fæcal matters from the intestine, and with them of poisonous alkaloidal bodies—ptomaines, which have escaped the destructive action of the liver.‡ This is a most important matter, and by such an assumption we can explain many of the symptoms which accompany its presence in urine. In attributing the origin of stercobilin solely to the action of nascent hydrogen in the intestine on the bile pigments, physiological chemists have forgotten another source—namely, the products resulting from the action of

* Vaulair and Masius : "Centralb. f. d. med. Wiss.," 1871, No. 24.

† MacMunn : "Journ. of Physiology," vol. vi., Nos. 1 and 2, and vol. x., Nos. 1 and 2.

‡ It would appear that in those cases where pathological urobilin occurs in urine, there is either some vaso-motor disturbance of the hepatic circulation, or some mechanical interference with it as in passive congestion.

the digestive and putrefactive processes on the hæmoglobin of meat. Everyone is familiar with the colour of fæces when a milk diet only is taken, and I have no doubt that the colour in that case is due to the absence or scarcity of stercobilin.

Another pigment, closely connected with these, is that which I have named **urohæmatoporphyrin.*** This pigment owes its origin entirely to hæmatin, not to bile pigments, and can be prepared artificially by the action of zinc and sulphuric acid, sodium amalgam, and other reducing agents, on hæmatin. In *acid* solutions the spectrum of this pigment is very characteristic, consisting of a narrow band before and touching D, another darker between D and E, and frequently a feeble shading midway between these bands; there is also a band at F indistinguishable at first sight from that of urobilin, but careful examination shows differences. If the pigment be dissolved in alcohol, after isolation, and ammonia added, a five-banded spectrum is obtained, closely resembling that of hæmatoporphyrin. The presence of the band at F has been attributed by Le Nobel to urobilin, even in the case of the pigment prepared from hæmatin; but it can be easily shown that this is not so. Moreover, the band nearest violet is moved towards the red by ammonia, whereas that of urobilin disappears. Treated with zinc chloride and ammonia solutions of urohæmatoporphyrin show a *faint* green fluorescence, not nearly as well marked as in the case of urobilin, stercobilin, and hydrobilirubin. This pigment can be isolated from urine in the same way as urobilin. It has been found, so far, in the urine of Addison's disease, acute rheumatism, cirrhosis of the liver, croupous pneumonia, so-called idiopathic pericarditis, peritonitis, measles, meningitis, Hodgkin's disease, and typhoid fever. Urohæmatoporphyrin is very probably closely related to Baumstark's urorubrohæmatin and uro-

* MacMunn: "Proc. Roy. Soc.," No. 208, 1880; "Journ. Physiol.," loc. cit.; also vol. x., Nos. 1 and 2; "Brit. Med. Journ.," Feb. 4th, 1888 Ibid.: July 21st, 1888.

fuscohæmatin,* which he found in the urine of a patient suffering from leprosy. To the former Baumstark assigns the formula $C_{68}H_{94}N_8Fe_2O_{26}$, and to the latter $C_{63}H_{106}N_8O_{26}$. Hoppe-Seyler† points out the likeness of the former to hæmatoporphyrin. The above three colouring matters have hitherto been included under the name of urobilin by physiologists, but, although they are all very closely connected, yet they are quite distinct colouring matters. While both normal and pathological urobilin may be derived either from bile pigments or from hæmatin, *urohæmatoporphyrin is derived entirely from hæmatin.*

Hence, then, *normal* urine contains a colouring matter, or its chromogen, which has been produced within the organism by *oxidation* of bile pigment and of hæmatin.

Under *pathological* conditions, the urine may contain *pathological urobilin*, also, probably, an *intermediate oxidation product* of bile pigment, and perhaps of biliary urobilin, and this may be derived from stercobilin in certain cases. In some diseases these may be replaced, or accompanied by, urohæmatoporphyrin, which is a *reduction* product of hæmatin, and has been produced in the organism by reduction of effete hæmoglobin, or effete histo-hæmatin.

Other Pigments.—When urine is boiled with hydrochloric or other mineral acid, it becomes very much darker in colour: this *is due to the oxidation of more than one chromogen.* It the first place, urobilin is formed from its own chromogen; in the second, indican is split up into indigo-blue, and, probably, indigo-red; and, if the skatol chromogen is present, it, too, yields the skatol pigment. The so-called "urohæmatin" and allied bodies are due to the presence of these chromogens and also to others, which, on heating with mineral acids, yield uromelanin. The bodies yielding the latter belong, according to Udránszky,‡ to the

* Baumstark: "Pflüger's Archiv," Bd. ix., S. 568.

† Hoppe-Seyler: "Physiol. Chemie," S. 875.

‡ Udránszky: "Zeits. physiol. Chem.," Bd. xi., S. 537—560; Bd. xii., 33—63; cf. Hoppe-Seyler: "Zeits. physiol. Chemie," Bd. xiii., S. 66—121.

humous substances—bodies of carbohydrate origin, which also occur in plants. Thudichum maintained that uromelanin was derived from urochrome, which latter, according to him, is the principal colouring matter of urine,* and I have myself prepared urochrome according to Thudichum's direction, and found it in normal urine; but I could not find any characters which might enable me to connect it with urobilin, nor did I find it contaminated with urobilin. It is now stated, however, that this urochrome is not altogether the source of uromelanin; that the urochrome is derived from the "reducing substance" of urine, whatever that may be, and that uromelanin itself is a mixture of the decomposition products of the humous substances (Udránszky).

The red colour which some urines assume on adding a mineral acid in the cold is probably due to a skatol pigment, but it would lead too far to discuss this subject. I may, however, state here that the urorosein of Nencki and Sieber,† and the red pigment found in the urine by Plosz,‡ and that found by Giacosa,§ are considered by Mester to be skatol pigments.‖ Mester, however, goes too far in saying that **uroerythrin** is such a derivative, as it becomes green when treated with caustic alkalies, especially when the pigment is in the solid state, and gives a peculiar absorption spectrum, which distinguishes it from Mester's or Brieger's skatol pigments.¶ Quincke** has shown that the urine of patients taking copaiba may contain a chromogen, which, on adding

* Thudichum: "Hasting's Prize Essay," 1864; also, "Pathology of the Urine," 1877, pp. 217—246.

† Nencki and Sieber: "Journ. prak. Chem.," [2], xxvi., S. 333—336.

‡ Plosz: "Zeits. physiol. Chem.," Bd. vi., S. 504, and Bd. viii., S. 85.

§ Giacosa: "Ann. di chimica e di farmacol.," Serie iv., 3, p. 201; "Berichte d. deutsch. chem. Gesell.," Bd. xx., S. 393 (Ref.).

‖ Mester: Loc. cit.

¶ Uroerythrin, the colouring matter of *pink* urates, may be extracted from these by boiling with alcohol, and then gives two ill-defined bands before F. In the solid state it becomes green with caustic soda or potash. At present we know nothing of its origin in the body.

** Quincke: "Archiv f. exper. Pathol. u. Pharm.," xvii., S. 273.

a little mineral acid to the urine and gently heating, gives a fine red colour. If a few drops of nitric acid are added to such urine, and a gentle heat applied, it becomes a fine lake-red colour. Any turbidity should be cleared up with spirit, and, if the urine be then examined with the spectroscope, it shows a band between C and D, another between D and E, while the whole violet end is strongly absorbed. In a specimen of urine, which Dr. Walter G. Smith kindly sent me in 1884, I found this pigment, and, strange to say, it persisted for nine days after the drug was discontinued. In cases of melanotic cancer, a pigment allied to melanin* may be present in urine, and a brown pigment containing iron may be carried down with uric acid (Kunkel).

Other pigments have been described, but the above are those which are of greatest interest from a clinical point of view.

The spectra of some of the above colouring matters are represented in the accompanying Chart of Spectra (Frontispiece).

* See von Jaksch: "Zeits. f. physiol. Chem.," 1889, Bd. xiii., S. 385—394.

CHAPTER VIII.

THE INORGANIC CONSTITUENTS OF URINE.

THE urine contains many inorganic substances, and some of these, in their quantitative relationships, afford important help in enabling us to appreciate the conditions of the metabolic processes that are going on in the organism, not only in health but in disease.

Sodium, potassium, ammonium, calcium, and magnesium, and, according to recent researches, iron, are always present in health; sometimes silicic acid and fluorine. These are mostly combined with acids to form salts: the acids are mainly sulphuric, hydrochloric, and phosphoric. The only free gases present are carbonic acid, nitrogen, and traces of oxygen. Peroxide of hydrogen is also said to be present (Schönbein).

The amount of salts excreted in twenty-four hours varies from $\frac{1}{4}$ to $\frac{3}{4}$ of an ounce, or from 9 to 25 grams, but the whole amount of non-volatile salts differs considerably in different people and under various pathological conditions.

These inorganic constituents are either taken in with the food and pass unchanged into the urine, or they may be formed in the organism by the oxidation of the sulphur and phosphorus of the food, the products of oxidation uniting with the bases to form salts.

Chlorides.—Chloride of sodium (NaCl) is one of the most important inorganic salts found in the urine. About 12 grams, or 180 grains, is the daily amount excreted.* It is contained in all the tissues and secretions of the body, and by its presence metabolism is increased, secretion stimu-

* Vogel places the mean at 10 to 13 grams NaCl daily.

lated, and it is required for the preparation of some of the secretions such as the gastric juice. It crystallizes mostly in cubes and octahedra (Fig. 31), and sometimes forms a combination with urea in the urine, which occurs in rhombic plates.

Barral has shown that the whole of the chloride of sodium in the food is not excreted as such, about a fifth of it being decomposed by the acid phosphate of sodium to form potassic chloride and acid phosphate of sodium.

The amount of sodium chloride is increased after a meal, by copious draughts of water, by muscular exercise, by

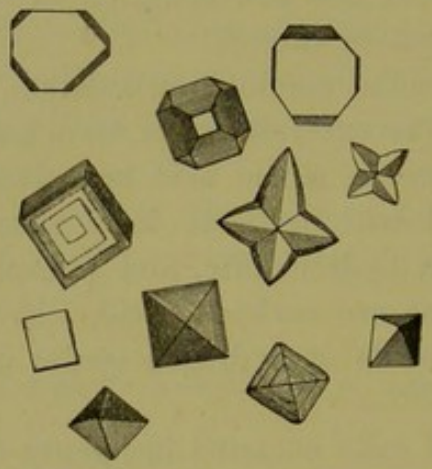


Fig. 31.—Various Crystalline Forms of Chloride of Sodium.

taking large quantities of common salt, while it is diminished by the opposite conditions. It is most abundantly excreted in the afternoon; it diminishes at night, and increases again in the morning. Slight disturbances of health diminish the quantity rapidly (Kaupp). Beer diminishes the amount considerably.

No good explanation has yet been given of the change which its excretion undergoes in acute febrile diseases. Vogel* states that the diminished excretion in all acute diseases is due to the loss of appetite and the meagre diet, poor in salt, taken by such patients, or it may be due to watery diarrhœa or serous exudation robbing the blood of a

* Neubauer and Vogel : Loc. cit., p. 494.

great part of it. In many continued febrile diseases, such as pneumonia, pleurisy, typhus, &c., the diminution of the excretion runs parallel to the height of the fever, and even for some time hardly any chlorides are excreted in the urine. With the onset of improvement the sodium choride begins to appear again, and during convalescence it may reach the normal amount. In diarrhœa, and especially in Asiatic cholera, its excretion is much diminished, and the bulk of it occurs in the dejections. Its amount is increased in diabetes and in polyuria, perhaps owing to greater consumption of food, in ague and in Bright's disease; and it is, of course, much influenced by the amount of urine secreted—thus, in profuse sweating it is diminished, also in long-standing albuminuria and in dropsies. The return of chloride of sodium to the urine in a case of pneumonia, where it is diminished, is considered a favourable sign. It is diminished in chorea and pemphigus, and in febrile diseases accompanied by exudation. In chronic diseases its aomunt runs almost parallel with that of the urine passed. According to Zuelzer, the amount is diminished in conditions of excitement, while the potassic chloride is increased; but in conditions of depression the reverse is the case (Landois).

According to G. Sticker,* a rich secretion of gastric juice induces a transient decrease of the chlorides in urine. The amount increases gradually after the chief meal, and slowly diminishes again after from six to seven hours. Towards night it decreases.

Kast† shows that the chlorides increase considerably after the administration of chloroform, whereas ether has no effect. Chloral, on the contrary, produces no increase, as it leaves the body, according to von Mering, as urochlorlalic acid, and does not part with its chlorine. Carbon tetrachloride, methyl chloride, ethyl dichloracetate also gave

* Sticker: "Chem. Centralbll.," 1887, S. 1561, 1562.
† Kast: "Zeit. physiol. Chemie," xi., 277—285.

negative results, but ethyl trichloracetate produced a decided increase. Zeller also found the chlorides increased after chloroform narcosis.* The chlorides, being soluble, never appear in the urine as a deposit.

Tests for Chlorides in Urine.—(1) On evaporating a couple of drops of urine containing the normal amount of chlorides on a glass slip, characteristic crystals, occurring in octahedra and rhombic plates, appear: these belong to a combination of urea and chlorine.

(2) The addition of nitrate of silver in solution, after previously acidifying the urine with nitric acid in order to hold the phosphates in solution, precipitates out the chlorides as opaque white chloride of silver. If the urine contains much albumin, this must first be removed. According to Tyson,† from normal urine containing from $\frac{1}{2}$ to 1 per cent., the chlorides are precipitated by a *single* drop of a solution of nitrate of silver, 1 part to 8, in cheesy lumps, which do not further divide themselves, or make the urine more milky on moving the glass about. *If, however, the chlorides are diminished* to $\frac{1}{10}$th per cent. or less, the addition of a single drop of the silver solution no longer produces the white cheesy lumps, but a simple cloudiness, and the entire fluid appears equally milky. If, finally, there should be no precipitate whatever, then the chlorides are totally absent. This white precipitate is soluble in ammonia, and blackens on exposure to light.

(3) Mercurous nitrate gives a white precipitate of calomel, insoluble in water, and blackening with ammonia.

Quantitative Estimation of Chlorides.—One of the best methods for estimating chlorides in urine is that of Mohr, according to Neubauer,‡ who slightly modified it, as Liebig's method is liable to error. For this there are required:—

(1) A solution of nitrate of silver, of which 1 c.c. =

* Zeller: Ibid., viii., S. 70—78. † Tyson: Loc. cit., p. 162.

‡ Neubauer and Vogel: Loc. cit., p. 246.

10 milligrams NaCl, or 6·065 milligrams of chlorine; made by dissolving 29·075 grams of pure fused silver nitrate in distilled water, and diluting to a liter.

(2) A cold saturated solution of neutral potassium chromate.

10 c.c. of the urine are placed in a platinum crucible, and 1 or 2 grams of potassium nitrate,* *free from chlorides*, are added, the solution being then evaporated to dryness on the water bath. The residue is then heated gently over a free flame, afterwards strongly, until all the carbon is burnt off, and the residue appears as a white, fused, saline mass. This is then dissolved in a little water, the solution poured into a beaker, and the platinum crucible washed with distilled water from a wash-bottle until all trace of the salt is removed, and this solution added to that in the beaker. Dilute pure nitric acid is then added drop by drop until the solution is faintly acid, and then enough calcic carbonate added to make it neutral. The excess of calcic carbonate may be then filtered off, but Neubauer says this is not necessary. Two or three drops of the neutral potassic chromate solution are added to the mixture, and then the silver solution is allowed to flow in under constant stirring, until a distinct reddish tinge is produced, which remains permanent after stirring. The fluid is, at first, of a canary-yellow colour, and shows, when the silver solution falls, red spots, which disappear on stirring as long as any chloride of sodium remains free. As soon as this is all decomposed, the next drop shows a permanent reddish tinge, due to the formation of silver chromate. The number of cubic centimeters of silver solution used multiplied by ·010 gram gives the amount of chlorides present in the 10 c.c. of urine, estimated as sodium chloride, from which the whole amount in the twenty-four hours' urine can be calculated. If to 5 c.c. of urine 5 c.c. of silver

* Sutton ("Volumetric Analysis," p. 309) uses ammonic nitrate, owing to the solvent effect which potassic nitrate produces on silver chromate.

solution have been used, 50 milligrams NaCl are present; and 1,000 c.c. of urine contain, therefore, 10·0 grams NaCl, or 6·065 grams chlorine.

Pribram* uses permanganate of potassium at a boiling temperature to destroy all the organic matter, but Neubauer did not get good results by this method.

When iodine and bromine are present, Salkowski's modification should be adopted. 10 c.c. of the urine are evaporated with nitrate of potassium as before, and ignited; the residue dissolved in water, acidified with sulphuric acid; and the iodine removed by shaking with carbon bisulphide. If the nitrite, which forms on fusing, is not sufficient to set free all the iodine present, a few drops of a solution of nitrite of potassium are added to the acidulated solution before shaking with the bisulphide. The aqueous solution is finally neutralized with sodium carbonate, evaporated, dissolved in water, and titrated with the silver solution as before (Neubauer).

Charles† recommends Volhard's method,‡ modified by Salkowski. Habel and Fernholz also give a method, which they say yielded good results;§ and Salkowski‖ has used Volhard's method of direct estimation with silver and ammonium thiocyanate, and speaks well of it;¶ while Zuelzer** has modified Mohr's method, but this method cannot be recommended.

Phosphates in Urine.—Phosphoric acid†† is met with

* Pribram: "Zeits. f. anal. Chemie," Bd. ix., S. 428.

† Charles: "Physiol. and Pathol. Chem.," p. 485.

‡ For Volhard's method see Sutton's "Volumetric Analysis," 5th ed., 1886, p. 310.

§ Habel and Fernholz: "Pflüger's Archiv," xxiii., S. 85—126; also Habel: Ibid., Bd. xxiv., S. 2.

‖ Salkowski: "Centralbll. f. d. med. Wiss.," 1880, S. 177, 178.

¶ See also Arnold in "Pflüger's Archiv," xxxv., S. 541—557. Volhard's method is described in "Journ. Chem. Soc.," 1878, p. 746, and in Sutton's "Volumetric Analysis," loc. cit.

** Zuelzer: "Berichte der deutsch. chem. Ges.," xviii, S. 320, 321.

†† Phosphorus occurs also in urine in an incompletely oxidized form as glycerin-phosphoric acid (p. 89), which amounts to 15 milligrams in a liter of urine (Landois and Stirling).

in urine in combination, as sodic and perhaps potassic phosphate, and calcic and magnesic phosphates: the latter are known as the *earthy* phosphates, and the former as the *alkaline* phosphates. They are more abundant after a flesh than after a vegetable diet. The composition of the phosphates in urine is liable to variation. In acid urine the acid salts NaH_2PO_4 and $Ca(H_2PO_4)_2$ are generally present; in urine of neutral reaction the neutral salts Na_2HPO_4, $CaHPO_4$, $MgHPO_4$; and in alkaline urine the combinations Na_3PO_4, $Ca_3(PO_4)_2$, $Mg_3(PO_4)_2$ may also be present (Charles, Hoppe-Seyler). The phosphoric acid in urine is partly derived from the earthy and alkaline phosphates of the food, and is partly a decomposition product of lecithin and nuclein. It may, therefore, be relatively increased owing to increased metabolism of the nervous substance (Landois). About one-third to one-fourth of the phosphates taken with the food is excreted through the intestine. The amount of phosphates formed in nervous tissue is relatively small, and supplies *a very limited quantity* of those found in the urine. From 2·5 to 3·5 grams of phosphoric acid are excreted by the kidneys in twenty-four hours: the mean may be taken at 2·8 grams. The earthy phosphates excreted in urine amount, in twenty-four hours, to about from 1 to 1·5 grams (15·43 to 23·14 grains). The proportion of calcium to magnesium phosphate is as 33 to 67 (Tyson). The alkaline phosphates excreted in twenty-four hours amount, according to Breed, to 4 grams (61·72 grains), though according to Neubauer only to 2 grams (30·86 grains).

The *alkaline* phosphates are *never* precipitated in the urine, even on the addition of ammonia or alkalies, *so that they may be present in excess and yet invisible;* the *earthy* phosphates, on the other hand, being insoluble in water alone, require for their solution weak acids, such as carbonic and acetic or acid salts. Albuminous bodies and mucus are also said to have the property of dissolving them to a certain extent. Uric acid has the power of decomposing the alkaline

phosphates, forming acid phosphates and a urate. Besides these phosphates, ammonio-magnesium or triple phosphate is met with when the urine undergoes decomposition ; it has the composition $NH_4MgPO_4+6H_2O$, and occurs mostly in

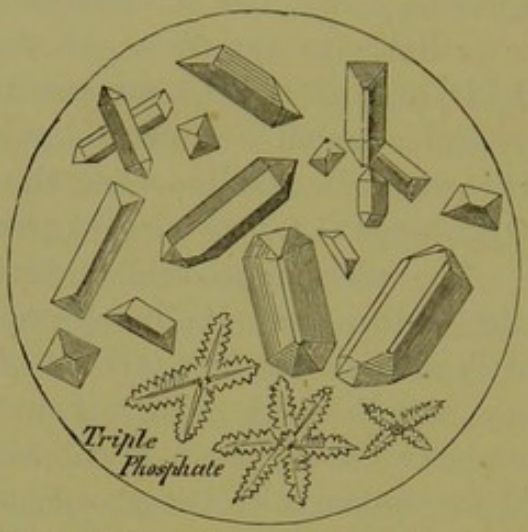


FIG. 32.—AMMONIO-MAGNESIUM OR TRIPLE PHOSPHATE CRYSTALS.
A "coffin-lid" crystal is seen near the centre of the figure.

triangular prisms (Fig. 32), if precipitated slowly out of urine, whereas when precipitated quickly it occurs in feathery crystals, four or five of these feathery rays forming

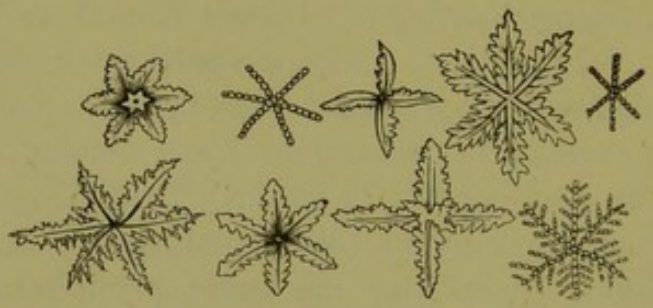


FIG. 33.—STELLATE CRYSTALS OF TRIPLE PHOSPHATE.

a star-shaped body (Figs. 33 and 34). The thin iridescent pellicle which forms sometimes on the surface of decomposing urine contains crystals of triple phosphate. These crystals are soluble in weak acid, but although usually found in alkaline urine, they may also be found in slightly acid

urine. The crystallized phosphate of lime is shown in Fig. 35.

According to Hill-Hassal,* a crystalline calcium phosphate separates out of concentrated acid urine, which can also be obtained from acid urine by adding to it calcic chloride, and especially after the internal use of lime water or of potassic carbonate; this has the composition $CaHPO_4 + 2H_2O$ according to Stein.†

Neutral or even tolerably acid urine often, when boiled, gives a precipitate which is frequently mistaken for albumin;



Fig. 34.
Feather-like Crystals of Triple Phosphate.

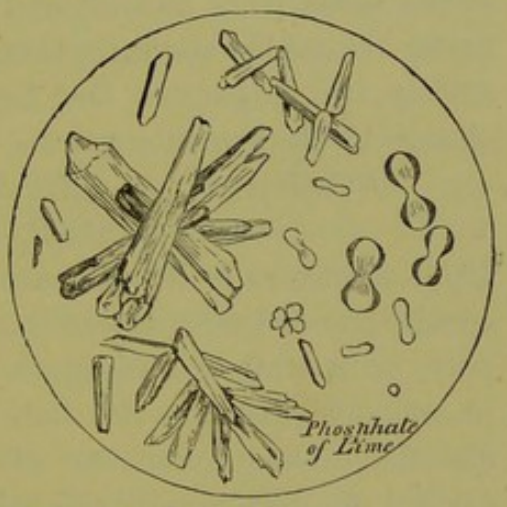


Fig. 35.—Crystals of Phosphate of Lime.

this precipitate is amorphous phosphate of lime, and is easily soluble in acids, which distinguishes it from albumin. The phosphatic precipitate, which forms on boiling urine, has been investigated by Dr. Walter G. Smith,‡ Stokvis,§ and Salkowski.‖ Salkowski shows that urine which became cloudy by heating, often became clear again on cooling—a reaction which the twenty-four hours' urine frequently exhibits:—"When the calcium phosphate is precipitated, in a floccu-

* Hill-Hassal: "Proc. Roy. Soc.," 1860, vol. x., p. 281.
† Stein: Liebig's "Ann.," Bd. clxxxvii., S. 90.
‡ Smith, W. G.: "Dub. Journ. Med. Sc.," July, 1883.
§ Stokvis: "Chem. Centralb.," 1884, S. 42.
‖ Salkowski: "Zeits. f. physiol. Chemie," 1883, Bd. vii., S. 119.

lent form, *i.e.*, corresponding to a relatively large separation, the precipitate may not clear up on heating." This precipitation was formerly attributed by some to the evolution of carbonic acid (CO_2), but Salkowski attributes it to the decomposition of a combination of calcium phosphate and alkaline phosphate. Smith believes the precipitate is due to a delicate adjustment in the proportions and basicity of the phosphatic salts existing in urine; he says: "Now, we do not know exactly in what state or states calcium phosphates occur in urine, but let us assume that we have in solution at the same time dicalcic and monocalcic phosphate; then, under suitable conditions of relative proportion and of acidity, we might, as Dr. Reynolds suggests, express the change by heat in this way:—

$$2(Ca_2H_2P_2O_8) + CaH_4P_2O_8 = Ca_3P_2O_8 \text{ (insoluble)} + 2CaH_4P_2O_8 \text{ (soluble)}.$$

Upon reduction of the temperature the inverse change would occur, attended with resolution of the tricalcic phosphate in part, or in full, according to the relative amount of acid phosphates present. So that, in a word, the phenomenon in question seems to be one of unstable equilibrium among certain phosphatic salts, the balance of solubility being easily disturbed by changes of temperature, modified possibly by the kind and amount of other salts in the solution. These considerations tend to throw some light upon the deposition of calcium phosphate within the bladder, and on the formation of urinary calculi, and show how such precipitation may take place in a feebly acid condition of the urine." Stokvis came to much the same conclusion* as Dr. W. G. Smith.

The quantity of the collective phosphates separated by the urine varies at different times. It increases in the afternoon after dinner, reaches its maximum in the evening, falls during the night, and reaches its minimum in the forenoon (Hoppe-Seyler).

* Cf. A. Ott: "Zeit. f. physiol. Chem.," x., S. 1—10. Also P. Carles "Journ. Pharm.," xiii., pp. 49—50.

With regard to the variation of the phosphates in disease, there are many different statements made, some of them contradicting each other. Stokvis* found them diminished in gout, in one case a daily mean of only 0·688 gram was excreted. They are increased by administering phosphoric acid, not so much by giving hydrochloric acid, also after administering soluble phosphates, such as sodic phosphate. The quantity excreted is affected by the amount of phosphoric acid, or of substances capable of being transformed into phosphoric acid taken with the food, thus during fasting the excretion is diminished, although even in complete abstinence from food they are regularly separated by the urine (Bidder and Schmidt). So that the phosphates do not altogether come directly from the food-products in the intestine, but occur in organic combinations in the body, and are separated from such combinations by other processes. They are more abundant on a flesh than on a vegetable diet. Copious draughts of water increase their excretion, and in this case they are not derived from phosphates dissolved in the water, so that the increase is due either to increase of general metabolism, or by the increased activity of the kidneys, or to both causes combined (Vogel). It follows certainly from this and other facts, "that the organism, under certain conditions, may contain an increased amount of phosphates on account of the retention of those ingested, or a diminished amount on account of their increased elimination" (Vogel). Riesell found the quantity of phosphoric acid decreased in urine after giving large doses of chalk; in that case a considerable portion of it combined with the calcium passed with the fæces. This result was only temporary, lasting two days, as the phosphate of calcium formed in the intestine, was, later on, absorbed and eliminated with the urine.

According to Vogel,† the elimination is very irregular in

* Stokvis: "Centralb. f. d. med. Wiss.," 1875, No. 47; "Neederland Tijdschrift voor Geneesk.," 1875 and 1876, No. 36.

† Neubauer and Vogel: Loc. cit., p. 509.

chronic disease; in acute, it diminishes during the first few days, probably on account of the scanty diet, then it increases gradually as the patient eats more. Sometimes, even in severe fever, when it is only of short duration, the decrease is very slight. According to Brattler,* it is diminished in diseases and functional disturbances of the kidneys, attended by a diminished secretion of urine, as in some forms of Bright's disease, and in heart diseases, also in disorders of digestion attended by a diminished absorption of food. In fever it is increased, owing probably to the increased decomposition of the tissues containing phosphorus. Haxthausen† found it diminished in intermittent fever during the interval; Mendel‡ found it *diminished* in chronic disease of the brain,§ still more in maniacs, but in them it increases with recovery again, and it was increased after epileptic and apoplectic seizures. In some cases, after sleep was produced by bromide of potassium or chloral hydrate, he found the phosphoric acid very much increased. It is said to be increased in inflammation of the brain, in osteomalacia, in chorea, in acute yellow atrophy of the liver, in phthisis, in leukæmia, in diabetes, and in oxaluria, and to be diminished during pregnancy owing to formation of the fœtal bones, also after the use of ether and alcohol, and in inflammation of the kidneys (Landois).

In fact, phosphoric acid is increased by *increased metabolism in the tissues* containing its combinations, or by food rich in such combinations; and diminished in the opposite conditions.

Phosphaturia.—A condition known as "*phosphaturia*" has been described, but we must carefully distinguish an *actual* increase in the amount of phosphates, from an *apparent* increase, due to purely local or transient causes. The appearance of insoluble earthy phosphates in the urine may

* Brattler: "Ein Beitrag zur Urologie," München, 1858.

† Haxthausen: "Acidum phosphoricum urinæ, &c.," Diss. Halle, 1860.

‡ Mendel: "Arch. f. Psychiatrie," 1872, iii., S. 636, &c.

§ Cf. A. Lailler: "Compt. rend.," xcix. pp. 572—573.

be merely due to a difference of balance between the alkaline and earthy phosphates in the urine, or to absence of the acid salts which keep the latter in solution, or to the presence of an excess of alkali due to an unhealthy condition of the mucous membrane of the bladder or urethra, or other cause. If a patient happens to pass urine containing a copious deposit of earthy phosphates, which may make the urine quite milky, or if, at the end of micturition, a creamy fluid is discharged, accompanied by irritation of the neck of the bladder, we are not at once to rush to the conclusion that this is due to true phosphaturia, *until we have estimated quantitatively the phosphoric acid in the twenty-four hours' urine.* Patients often think that this creamy fluid is due to spermatorrhœa, and others that their nervous tissues are wasting, a belief often fostered by quacks. The addition of a few drops of hydrochloric acid will convince a man that the discharge is not seminal, and relieve his mind.

If the cause of the deposit be an excess of *fixed* alkali in the urine, it is probable that the patient is debilitated or dyspeptic, but it does not follow that his nervous tissues are wasting, for there may not really be an *excess of phosphates*, but an *excess of alkali* leading to their precipitation. The alkaline reaction, if due to fixed alkali, is caused by the presence in the urine of the carbonate of soda or of potash, and this condition, according to Ralfe,* arises from (1) general debility and feebleness of the respiratory process leading to the accumulation of carbonic acid in the system, as in convalescence from acute diseases; (2) diminished secretion of bile, leading to the retention of alkaline salts in the blood, and consequently to their increased elimination by the urine; and (3) the acids formed by fermentative changes, belonging to the fatty acid series, on entering the system are oxidized into carbonic acid; and then, uniting with the bases of the alkaline oxides, form carbonates of those bodies, which by increasing the alkalinity of the blood,

* Ralfe: "Diseases of the Kidneys," loc. cit., p. 62.

diminish the natural acidity of the urine, and thus lead to the deposition of a phosphatic precipitate. "This 'last' form of alkaline urine is chiefly met with in debilitated persons, and those suffering from flatulent dyspepsia, especially that affecting the small intestines. It is associated with tolerably distinct features, such as loss of weight, weariness, irregularity of bowels, flatulence, frequent micturition, more or less sallowness of complexion, great despondency, urine alkaline or else neutral or faintly acid, depositing phosphates on boiling, and effervescing on the addition of dilute acid" (Ralfe).

In the true phosphaturia the phosphoric acid excreted in twenty-four hours may amount to as much as 7 or 9 grams, instead of the normal 2·5 to 3·5 grams. The urine in these cases is generally alkaline, of medium specific gravity, and may deposit a dense mealy precipitate of phosphate of lime, which may become deposited in the bladder. Sometimes the urine may be acid, and no deposit be present in it, and then a quantitative examination of the twenty-four hours' urine is necessary before one becomes aware that he is dealing with phosphaturia. In such cases a peculiar train of symptoms is present, such as nervous irritability, digestive disturbances, great wasting, aching pains in the back and loins. As the disease advances the urine increases in quantity, so much so, as to lead to the suspicion that either saccharine diabetes or that known as diabetes insipidus is present, and Ralfe observes that the disease seems to merge into that condition. Tessier, indeed, calls this condition "phosphatic diabetes,"* but its existence can only be proved by an accurate *quantitative* estimation of the phosphoric acid passed in the twenty-four hours' urine.

With regard to the supposed increase of phosphates in the urine of patients suffering from brain exhaustion, it may be mentioned that Vanni and Pons† are led to conclude

* Tessier: "Du Diabète Phosphatique," Lyons, 1877.

† Vanni and Pons: "Chem. Centr.," 1887, S. 1526, from "Ann. Chim. Farm.," lxxxvii., pp. 259—268.

—though with all reserve—that, as a general rule, the amount of phosphates excreted in the urine *diminishes* in diseases of the brain and spinal cord, and frequently diminishes in cases of neurosis. Then, again, Mairet* finds that the effect of intellectual exertion, like that of muscular exertion, is closely connected with the *sufficiency or insufficiency of the diet of the individual.* The general result being a *decrease* in the quantity of *alkaline* phosphates excreted in the urine, the amount of diminution depending on the duration of the intellectual effort. When the diet is insufficient, relatively to the amount of work done, an additional effect is produced, as the phosphates of the alkaline earths are increased in the urine. Hence, according to Mairet, it follows that phosphoric acid is intimately connected with the nutrition and activity of the brain, and that when it works the brain absorbs alkaline phosphates, and gives up phosphates of the alkaline earths. But as he shows that brain work retards general nutrition, it comes simply to this, *that there is a disturbance in the normal metabolism,* leading to a disturbance of the balance between the various phosphates formed and eliminated in the urine.

The *earthy* phosphates are precipitated from urine by adding any alkali, such as ammonia or caustic potash, to the urine. Their quantity may be estimated *approximately* by the method of Hoffman and Ultzmann:—"A test-tube, 16 centimeters (6·2992 inches) long and 2 centimeters (·787 inch) wide, is filled *one-third* with clear or filtered urine, to which a few drops of caustic ammonia or caustic potash solution are added, and warmed gently over a spirit-lamp until the earthy phosphates begin to separate in flakes. It is then placed aside for ten or fifteen minutes for them to subside. If the layer of sediment is 1 centimeter (·3937 inch) high, the earthy phosphates are present in normal amount; if they occupy 2 to 3 centimeters (·787 to 1·181 inch), they are increased: if, on the other hand, only a few

* Mairet: "Compt. Rend.," xcix., pp. 282—285.

flakes are visible, the earthy phosphates are diminished" (Tyson). They are sometimes coloured by the presence of abnormal colouring matter in the urine.

To estimate accurately the *alkaline* phosphates, it is first necessary to remove the earthy phosphates, by precipitating with ammonia and filtering them off; but for a tolerably approximate idea of their amount this is not required, and we proceed as follows:—Some urine is placed in a beaker, and about one-third of a solution containing 1 part each of magnesium sulphate and ammonium chloride, distilled water 8 parts, and pure liquor ammoniæ 1 part. All the phosphates are precipitated out of the urine by this mixture as a snow-white deposit composed chiefly of ammonio-magnesian phosphate and amorphous calcic phosphate. If the entire fluid presents *a milk-like cloudy appearance*, the alkaline phosphates may be present in normal amount; if it is denser, more cream-like, there is an increase. If, on the other hand, the fluid is but slightly cloudy, transmitting light distinctly, the phosphates are diminished (Tyson).

Quantitative Estimation of the Phosphoric Acid present in Urine.—When a phosphate in solution, after acidulating with acetic acid, is treated with a solution of acetate or nitrate of uranium, a precipitate consisting of phosphate of uranium falls. Potassium ferrocyanide gives a reddish-brown precipitate, with a soluble salt of uranium. On these two reactions the volumetric process for estimating the phosphoric acid in urine is based.

The following solutions are required:—

(1) A saturated solution of ferrocyanide of potassium.

(2) A standard solution of sodium phosphate, prepared by dissolving 10·085 grams of crystallized sodium phosphate ($Na_2HPO_4 + 12H_2O$) in distilled water, and diluting to make up one liter; of this solution 50 c.c. contain ·1 gram of P_2O_5 (anhydrous phosphoric acid).

(3) A solution of acetate of uranium, of which 1 c.c. = ·005 gram phosphoric acid.

(4) A solution of sodium acetate, prepared by dissolving 100 grams of this salt in 100 c.c. of pure acetic acid, and adding water to one liter.

The solution of uranium acetate is thus prepared:—20·3 grams of yellow uranium oxide are dissolved in dilute acetic acid, prepared by adding to 25 c.c. of pure glacial acetic acid enough water to make a liter. The strength of the solution must then be determined by placing 50 c.c. of the standard solution of sodium phosphate in a beaker with 5 c.c. of the solution of sodium acetate, and heating on a water bath to from 90° to 100° C (194° to 212° F). A burette is then filled with the uranium solution, and the latter allowed to run into the warm mixture until a precipitate ceases to form. Then a drop of the mixture is taken up on a glass rod, and mixed with a drop of the ferrocyanide solution on a white plate, or on a bit of filtering paper saturated with the ferrocyanide solution. If no reddish-brown colour appears, some more fluid from the burette is allowed to run into the beaker, and a drop from the mixture again tested with the ferrocyanide. This is cautiously repeated until the brownish-red colour appears. When this is seen the quantity of uranium acetate solution run in is read off. It is the amount required to decompose the sodium phosphate corresponding to ·1 gram of P_2O_5; from this we calculate the amount of distilled water to be added to the uranium solution in order that 1 c.c. shall correspond to ·005 gram of phosphoric acid.

To estimate the phosphoric acid in urine.—50 c.c. of urine are taken, then 5 c.c. of the sodium acetate solution added, and the mixture is heated on the water bath to 80° C. The burette being filled with the uranium solution, the latter is allowed to run into the hot mixture, which is tested from time to time with the ferrocyanide. When the colour appears, the number of cubic centimeters of uranium solution used is read off. This number multiplied by ·005 gives the amount of phosphoric acid in the 50 c.c. of urine taken, and from this the amount in the twenty-four hours' urine is calculated.

This gives the amount of the total phosphoric acid, but we can also easily calculate the amount of phosphoric acid present in combination with the alkaline earths, and that combined with the alkalies, respectively.*

Phosphoric acid also exists in urine in an incompletely oxidized form, as in glycerin-phosphoric acid ($C_3H_9PO_6$); which Klüpfel and Fehling found in normal urine, where it amounts to 15 milligrams in a liter of urine (p. 89). It is probably derived in the organism from the decomposition of lecithin. According to Lepine it is increased in nervous diseases, and according to Zuelzer after chloroform narcosis.

Sulphates in Urine.—Sulphuric acid occurs in urine in combination with alkalies such as sodium and potassium, ("preformed sulphuric acid"), and also with indol, phenol, skatol, and other aromatic substances, forming the aromatic ether-sulphonic compounds or ethereal hydrogen sulphates ("combined sulphuric acid"). The urine also contains sulphur in a state of incomplete oxidation, and in the urine of patients suffering from diseases of the liver, it may amount to 40 per cent. of the total sulphur excreted. The total sulphuric acid excreted in the urine in twenty-four hours amounts to from 2·5 to 3·5 grams (37—52 grains). If the amount of sulphuric acid in combination with the alkalies be put down as 1, then that united to form the ethereal hydrogen sulphates is 0·1045 gram (v. d. Velden). The sulphuric acid in urine is chiefly derived from the decomposition of proteids in the organism, and the amount excreted runs parallel with that

* The principle is simple. 100 or 200 c.c. of urine are measured into a beaker and made freely alkaline with ammonia, then set aside for twelve hours for the precipitate to subside; this precipitate is the earthy phosphates. The clear fluid is then decanted through a filter, the precipitate added to that on the filter, and washed with ammoniacal water; a hole is then made in the filter and the precipitate washed through; the paper moistened with a little acetic acid, and washed into the vessel holding the precipitate. Acetic acid is added to dissolve this, then some sodic acetate, the mixture diluted to 50 c.c., and estimated as above. The amount of phosphoric acid thus found, deducted from the total obtained as above, is that in combination with the alkalies (Sutton).

of the urea excreted. It is difficult to determine how much arises from decomposition of the albumin of the food, and how much from that of the tissues. When the albumin of the food is diminished the sulphuric acid excreted falls in amount. It increases generally for some hours after a meal, especially after a meat meal, and is diminished by a vegetable diet; it is increased after prolonged exercise, after the ingestion of sulphur, or sulphates, sulphuric acid, or sulphur-containing substances. According to Bence Jones it is increased in delirium tremens and other forms of delirium; it is increased in eczema, in acute inflammatory diseases of the brain and spinal cord, in pneumonia and acute rheumatism, and in some cases of diabetes insipidus. An increase in fever indicates an increased metabolism of the tissues (Landois); in the convalescence from fever it is diminished (Zuelzer). In chronic affections, as a rule, it is diminished; also in inflammation of the kidneys, in leucocythæmia and chlorosis.

The ethereal hydrogen sulphates, which, as I have said before, are the combinations of sulphuric acid with the aromatic substances, have been estimated by G. Hoppe-Seyler* in various diseases, and he has drawn up carefully-prepared tables showing the amount of sulphuric acid excreted as ethereal hydrogen sulphates and in combination with alkalies, respectively. He shows that deficient digestion or increased absorption of the normal products of digestion, as in peritonitis, tubercular disease of the intestines, and so on, leads to an increase of the ethereal hydrogen sulphates in the urine, owing to the normal products of digestion undergoing putrefactive changes and the products being absorbed from the intestines. In typhoid fever there is no increase, nor after simple constipation; but in diseases of the stomach, in which the food lies in that organ a long time, and then undergoes putrefactive change, there is an increase. Putrefactive changes

* Hoppe-Seyler, G.: "Zeit. physiol. Chem.," xii., S. 1—32.

outside the alimentary canal, putrid cystitis, putrid abscesses, putrid peritonitis, &c., have the same result. In the case of abscesses the ethereal hydrogen sulphates in the urine diminish after the pus is let out (see p. 95 and *infra*).

Sulphuric acid forms a heavy white precipitate of barium sulphate with any of the barium compounds, which is insoluble in nitric acid, and this test suffices for its detection.* Tyson† gives this method for the approximate estimation of the total sulphates :—"If to a small quantity of urine in a beaker-glass, one-third as much of the acidulated solution of barium chloride (1 part to 8, plus $\frac{1}{2}$ a part hydrochloric acid) is added, and there occurs an *opaque* milky cloudiness, the proportion of sulphates is normal; if the opacity is intense, and the white mixture has the appearance and consistence of cream, the sulphates are increased; if, on the other hand, there is only a slight cloudiness, so that light is still transmitted, the sulphates are diminished." The *total* sulphuric acid may be estimated either gravimetrically or volumetrically.

Volumetric Estimation of the Sulphuric Acid in Urine.—Chloride of barium in solution will continue to throw down a precipitate of barium sulphate from urine, as long as any sulphuric acid is present.

The solutions required for the process are :—

(1) A solution of barium chloride, made by taking some crystallized baric chloride, powdering it, drying between folds of filtering paper, and weighing off 30·5 grams, dissolving this in distilled water, and making up to one liter : 1 c.c. of this solution will precipitate exactly 12·25 milligrams H_2SO_4, or 10 milligrams SO_3.

(2) A solution of potassium sulphate, of such a strength that 1 c.c. = 12·25 milligrams H_2SO_4, or 10 milligrams SO_3. It is prepared by dissolving 21·775 grams chemically pure

* A little HCl should be added to the urine *first* to prevent the precipitation of baric phosphate.

† Tyson : Loc. cit., p. 169.

potassium sulphate in powder, and dried at 100° C., in distilled water, and diluting to 1 liter.

100 c.c. of the urine acidified with 20 to 30 drops of hydrochloric acid, are placed in a beaker and heated on the water bath or sand bath. When boiling, 5 to 8 c.c. of the barium solution are allowed to run in from a burette. The heat is then discontinued, and the precipitate allowed to subside.* If the fluid becomes rapidly clear, another cubic centimeter of the barium solution is allowed to run in, the solution again heated, and 10 or 12 drops of the solution filtered into a test-tube; some of the barium solution is added to this, and if a precipitate does not form, a few drops of the potassium sulphate solution are added to another portion, when, if a precipitate forms, we know too much baric chloride has been added, and the process must be repeated. If the barium solution still produces a precipitate in the fluid filtered into the test-tube, the latter is returned to the beaker, and the barium solution again added to the urine in the beaker from the burette. The filtering process being again repeated as before, until no precipitation takes place in the filtrate with the barium, and until a *slight* cloudiness only takes place on adding the potassium sulphate to the filtrate. If more than a slight cloudiness appears, too much barium chloride has been used. If, for instance, up to that point 10 c.c. have been added we repeat the process, adding only 9 c.c. from the burette to a fresh 100 c.c. of the acidulated urine, and then tenths of a cubic centimeter, until the right point is reached.

Suppose 10 c.c. are required: then 0·10 gram of SO_3 was present in the 100 c.c. of urine, from which the total for twenty-four hours may be calculated.

In accurate estimations, the amount of sulphuric acid present *in combination as ethereal hydrogen sulphates* ought to be calculated. For this process we should estimate the *total* sulphates, and the *combined* (*i.e.*, ethereal) sulphates

* Much time is saved by using Beale's filter.

separately,* and the amount of "preformed" sulphates is then calculated from these data.

Gravimetric Estimation of the Preformed and the Combined Sulphates (Baumann).—100 c.c. of clear filtered urine are acidulated with acetic acid, and heated nearly to boiling, chloride of barium added in excess, and warmed on the water bath until the supernatant fluid is clear. The fluid is then passed through a Swedish filter paper, the precipitate washed with water, the filtrate and wash-water collected, which contain the *combined* sulphuric acid. The precipitate on the filter must now be freed from phosphate of barium. For this purpose it is treated with dilute hydrochloric acid and washed with water. The filtrate and wash-water are then acidulated with hydrochloric acid and warmed, until in a few minutes the precipitate which forms, consisting of sulphate of barium, leaves the supernatant fluid clear. But owing to the difficulties attending this method, Salkowski has proposed another, by which in one portion of urine the *total* sulphuric acid is estimated, in another the *combined;* the difference gives the *"preformed"* sulphuric acid.

Quantitative Estimation of the Total Sulphuric Acid (Salkowski).—100 c.c. clear filtered urine are mixed with 5 c.c. hydrochloric acid (sp. gr. 1·12) boiled, and baric chloride added to complete precipitation. The heating is continued until the supernatant fluid remains clear, the precipitate collected on a small Swedish filter paper (6 to 7 centimeters in diameter), previously washed with dilute hydrochloric acid, all the precipitate being collected on this by means of a glass rod tipped with caoutchouc. A little of the filtrate, with a drop of sulphuric acid, should give a precipitate of baric sulphate showing an excess of barium

* The combined sulphates are decomposed by warming with strong acids, *e.g.*, HCl, and then, like ordinary sulphuric acid or sulphates, give a precipitate with chloride of barium. The methods here given are copied from Salkowski and Leube's "Die Lehre vom Harn."

salt. If this be the case, the precipitate is thoroughly washed with hot water, then with hot alcohol, and afterwards with ether. The filter is then dried with its precipitate, placed in a weighed platinum capsule, covered up, heated to redness till quite white, cooled, and weighed. This gives the amount of barium sulphate (= ordinary and combined sulphates).

Salkowski's Method of Estimating the Combined* Sulphuric Acid.—100 c.c. urine and 100 c.c. alkaline baric chloride solution (a mixture of 2 vols. cold saturated solution of caustic baryta and 1 vol. cold saturated solution of baric chloride) are mixed in a previously dry beaker, well stirred, and after a few minutes filtered through a dry filter into a dry cylinder. Of the quite clear filtrate 100 c.c. (=50 c.c. urine) are strongly acidulated with hydrochloric acid (10 c.c. to 100 of filtrate), heated to boiling, kept at 100° C. on the water bath for an hour, and then allowed to stand until the precipitate has completely settled. The further treatment of the latter precipitate (= combined sulphates) is then carried out as in the last case.

Calculation.—233 parts baric sulphate correspond to 98 parts H_2SO_4 or 80 parts SO_3, or 32 parts S. To calculate the H_2SO_4 multiply the weight of baric sulphate found by $\frac{98}{233}=0{\cdot}4206$; to calculate the SO_3 multiply by $\frac{80}{233}=0{\cdot}34335$; to calculate the sulphur multiply by $\frac{32}{233}=0{\cdot}13734$. *Example:* 100 c.c. urine have given 0·487 baric sulphate then the amount of $H_2SO_4=0{\cdot}487\times0{\cdot}4206=0{\cdot}2048$ per cent., that of sulphur (S) $=0{\cdot}487\times0{\cdot}13734=0{\cdot}0669$ per cent. All old estimations of sulphuric acid in urine made before Baumann's discovery,† in 1876, of the conjugated sulphoacids in urine, were vitiated by confusing them with the ordinary sulphates (Hoppe-Seyler). Besides the sulphates the urine contains sulphur in other combinations, such as

* "Virchow's Archiv," Bd. lxxix., S. 551. See also "Zeits. physiol. Chemie," Bd. x., 346—360.

† Baumann: "Pflüger's Archiv," Bd. xii., S. 69.

sulphocyanic acid or thiocyanic acid, 0·08 gram in 1,000 c.c. (Munk),* or 0·0225 gram in 1,000 parts (Gscheidlen),† or it may be present as sulphurous acid, or in combination with cystin, and sulphur-carrying compounds derived from the bile (Kunkel, von Voit, &c.) Hyposulphurous acid is said to occur in typhus as an alkaline salt, and sulphuretted hydrogen may be present rarely, being due, according to Müller, to the action of micro-organisms on some constituents of the urine.‡

Other Inorganic Constituents.—Minute traces of nitric and silicic acids have been found in urine, and are derived from the water drunk. The sodium in the urine occurs chiefly combined with chlorine, but some is united with phosphoric and uric acids. Potassium is chiefly combined with chlorine also. It is said that more potassium is excreted during fevers than sodium, but during convalescence the reverse is the case. Magnesium and calcium occur in normal urine combined with chlorine and phosphoric acid, as chlorides and acid phosphates, if the urine be acid; if neutral, the neutral calcic and magnesic phosphates are precipitated; while if the urine is alkaline, the tribasic calcium phosphate and calcium carbonate are deposited, the magnesium being precipitated as ammonio-magnesium phosphate (Landois). Peroxide of hydrogen was found in traces in fresh urine by Schönbein,§ it disappeared when decomposition set in, accompanied by cloudiness of the urine, while traces of nitrous acid were then detected. (Peroxide of hydrogen is detected by means of tincture of indigo and dilute solution of ferrous sulphate, the colour of the indigo being discharged by the peroxide.)

The *gases* are obtained by means of the gas pump. CO_2 pre-

* Munk: "Verhandl. d. physiol. Gesell. zu Berlin," 1876, No. 9; "Deutsch. med. Wochens.," 1876, No. 46; and "Arch. f. path. Anat.," Bd. lxix., S. 354.

† Gscheidlen: "Pflüger's Archiv," Bd. xiv., S. 401, and Bd. xv., S. 350.

‡ Müller: "Chem. Centralbl.," 1887, S. 807, and "Berl. klin. Wochensch.," xxiv., S. 405—408.

§ Schönbein: "Zitzungsbericht d. Bayer. Acad. d. Wiss," 1864, i., 2, S. 115.

ponderates over the O and N. O and N occur to the extent of not more than one volume per cent.; CO_2 from 4·4 to 9·96 per cent. (Planer), or 14·30 per cent. (Pflüger). CO_2 is increased in the urine in fever, its amount increasing with the rise of temperature (Ewald).

The **Estimation of Ammonia** in urine is generally performed by the method of Schlösing and Neubauer, which consists in placing a measured quantity of urine, to which milk of lime has been added, under an air-tight bell-glass, together with an open vessel containing a measured quantity of titrated acid. In from twenty-four to thirty-six hours all the ammonia has passed out of the urine into the acid, which is then titrated with standard alkali to find the amount of ammonia absorbed. Sutton ("Volumetric Analysis," p. 326) finds, however, that this method is liable to defects, and recommends the following:— "100 c.c. urine are exactly neutralized with decinormal ($\frac{N}{10}$) soda or potash, as for the estimation of free acid; it is then put into a flask capable of holding five or six times the quantity, 10 c.c. of normal alkali added, and the whole brought to boiling, taking care that the bladders of froth which at first form do not boil over. After a few minutes these subside, and the boiling proceeds quietly. When all ammoniacal fumes are dissipated, the lamp is removed, and the flask allowed to cool slightly; the contents then emptied into a tall beaker, and *normal* nitric acid delivered in from the burette with constant stirring, until a fine glass rod or small feather dipped in the mixture and brought in contact with violet litmus paper produces neither a blue nor a red spot. The number of cubic centimeters of normal acid are deducted from the 10 c.c. of alkali, and the rest calculated as ammonia. 1 c.c. of alkali = 0·017 gram of ammonia. *Example:* 100 c.c. of urine were taken, and required 7 c.c. of $\frac{N}{10}$ alkali to saturate its free acid; 10 c.c. of normal alkali were then added, and the mixture boiled until a piece of moistened red litmus paper was not turned blue when held in the steam; 4·5 c.c. of normal acid were

afterwards required to saturate the free alkali ; the quantity of ammonia was, therefore, equal to 5·5 c.c., which, multiplied by 0·017, gave 0·0935 gram in 1,000 of urine." If the urine, however, contains free carbonate of ammonia, Schlösing's method must be adopted. It is described in full in Salkowski and Leube, S. 196.

To Estimate the Lime.—100 c.c. of urine are precipitated with ammonia, the precipitate redissolved in acetic acid, and enough ammonic oxalate added to precipitate all the lime as oxalate ; the precipitate allowed to settle in a warm place, the clear liquid passed through a small filter, the precipitate transferred to the filter and washed with hot water, then dried, and together with the filter ignited in a platinum or porcelain crucible, by which means it is converted into a mixture of calcic oxide and carbonate. It is then transferred to a flask by the aid of a washing bottle, and an excess of $\frac{N}{10}$ nitric acid delivered in with a pipette. The amount of acid, over and above what is required to saturate the lime, is found by $\frac{N}{10}$ caustic alkali, each cubic centimeter of acid being equal to 0·0028 gram of CaO (Sutton).

To Estimate the Magnesia.—The filtrate and washings from the precipitate of calcic oxalate obtained as above are evaporated on the water bath to a small bulk, then made alkaline with ammonia, sodic phosphate added, and set aside for eight or ten hours in a slightly warm place, that the magnesia may separate as ammonio-magnesium phosphate. The supernatant liquid is then passed through a small filter, the precipitate brought upon it, washed with ammoniacal water in the cold, and dissolved in acetic acid, then titrated with uranium solution—see Phosphates—(of which 1 c.c. = 0·005 gram P_2O_5). Each cubic centimeter of uranium solution required is equivalent to 0·002815 gram magnesia (Sutton).

To Estimate the Soda and Potash.—50 c.c. of urine are mixed with the same quantity of baryta solution, allowed to stand a short time, and filtered ; then 80 c.c. (= 40 c.c.

urine) measured into a platinum dish and evaporated to dryness on the water bath; the residue is then ignited to destroy all organic matter, and when cold dissolved in a small quantity of hot water, ammonic carbonate added so long as a precipitate occurs, filtered through a small filter, the precipitate washed, the filtrate acidified with hydrochloric acid and evaporated to dryness, then cautiously heated to expel all ammoniacal salts. The residue is then treated with a little water and a few drops each of ammonia and ammonic carbonate, filtered, the filter thoroughly washed, the filtrate and washings received into a tared platinum dish, then evaporated to dryness, ignited, cooled, and weighed. By this means the total amount of mixed sodic and potassic chlorides is obtained. The proportion of each is found by titrating for the chlorine (see under Chlorides). The weight of chlorine is multiplied by the factor 2·103; from this product deduct weight of mixed salts found above. The remainder multiplied by 3·6288 gives weight of sodic chloride present in the mixture. The weight of sodic chloride deducted from the total chlorides gives the potassic chloride (Sutton).

Sodic chloride × 0·5302 = Soda (Na_2O).

Potassic chloride × 0·6317 = Potash (K_2O).*

* See Sutton: "Volumetric Analysis," p. 115.

PART II.

THE ABNORMAL CONSTITUENTS OF URINE.

CHAPTER IX.

SERUM-ALBUMIN AND OTHER PROTEIDS.

Albumin.—The ordinary albumin met with in urine is the serum-albumin of the blood.

The urine of healthy people is, as a rule, free from albumin, although Posner* maintains that traces are present in healthy urine. He obtained it by adding an excess of alcohol or tannin to the filtered urine, and observed a precipitate which answered to the proteid reactions. Béchamp, in 1865, stated that every urine, on the addition of three times its volume of alcohol, gave an albuminous precipitate, which he named mephrozymose,† and it is possible that this is the substance which Posner found. In any case, this infinitesimal trace is not detectable by the usual clinical methods, and need not be taken into consideration.

There is, however, a condition known as "physiological albuminuria," in which comparatively large quantities of albumin are excreted, and yet no other evidence of kidney disease can be found, nor, indeed, of any other disease. Dr. George Johnson, however, strongly protests against the

* Posner: "Chem. Centralbll.," 1886, S. 730, 731.

† Béchamp: "Compt. Rend.," 1865, ii., p. 251.

name "physiological albuminuria," as he maintains that the most delicate test fails to detect albumin in normal urine, and that albuminuria may occur as a transient condition, unattended by symptoms of disordered health, and apart from evidence of structural changes in the kidney, does not make the condition physiological. In 1842, Simon maintained that albuminuria might be present without disease of the kidneys; and Gigon, in 1858, held that albumin was present in normal urine. Leube* found albumin in 16 per cent. of the urines of soldiers examined after a long march, and Chateaubourg† found it in seventy-six urines out of ninety-four from soldiers after a long march;‡ but Oertels§ experimented on a good number of individuals, some of them invalids, whom he made ascend heights, and found albumin in only 3 per cent. of the cases examined. Ralfe, out of sixty people examined for life assurance, found it in only two.‖ These discrepancies are probably due to the use of different reagents and the confusion of other proteids with serum-albumin. Senator's monograph¶ on "Albuminuria in Health and Disease," contains a mass of information on the excretion of albumin by the apparently healthy.

Besides this "physiological albuminuria," there is "functional albuminuria," which may occur in derangement of the nervous system, derangements of digestion, and altered conditions of the blood, but in these cases there is, of course, absence of the symptoms of Bright's disease, and of tube-casts from the urine.

* Leube: "Virchow's Archiv," Band lxxix.; cf. Band lxxii.

† Chateaubourg: "Recherches sur l'albuminurie physiologique," 1883.

‡ Saundby observes: "This result was obtained with potassio-mercuric iodide, now supplied on blotting paper for the use of busy practitioners. In the existing state of opinion as to the diagnostic value of albuminuria, if such tests become popular I pity the public."—"Glasgow Med. Journ.," June, 1884.

§ Oertels: "Handb. der allgem. Therapie:" von Ziemssen, vol. iv.

‖ Ralfe: "Diseases of the Kidneys," p. 533.

¶ Senator: "Albuminuria in Health and Disease" (New Sydenham Society's translation), 1884.

We may now consider generally the conditions which lead to the presence of albumin in easily-detectable quantity in the urine. Serum-albumin may be present in urine—(1) Under the conditions just referred to, without any detectable disease of the kidneys; this has been named "hæmatogenous albuminuria" by von Bamberger; it may occur in women from suppression of the secretion of milk, and in either sex after a too free use of albuminous food. (2) From an increase of the blood-pressure within the renal blood-vessels, as after copious drinking, in heart disease, emphysema of the lungs, chronic pleuritic effusions, &c. (3) After section or paralysis of the vaso-motor nerves of the kidneys, which leads to congestion of the kidneys. (4) After violent muscular exercise, and in different convulsive conditions, such as epilepsy, and strychnine poisoning; in apoplexy, spinal paralysis, violent mental emotion, and the excessive use of morphia. (5) It may be present in fevers, such as scarlet fever, typhus fever, and in pyæmia and pneumonia. Quincke has found cloudy swelling of the renal epithelium in these cases, which is one of the factors concerned, the others, perhaps, being alteration of the quality of the blood and paralysis of the renal vessels from increase of temperature. (6) In acute and chronic inflammation of the kidneys, and other morbid conditions of these organs. (7) Inflammation and suppuration in the ureters, pelvis of the kidney, bladder, and urethra, in men and women, or vaginal and uterine discharges in women. (8) The use of drugs which irritate the kidney, such as cantharides and carbolic acid. (9) Complete withdrawal of common salt from the food, the albumin disappearing after the salt is given again (Wundt and Rosenthal).* (10) Great reduction of temperature (Lassar). (11) A peculiar condition of

* Albumin sometimes appears in the urine after chloroform narcosis, the inhalation of arseniuretted hydrogen, and carbonic oxide, &c., in lead and phosphorus poisoning, and in cholera, in leukæmia, syphilis, cancer, &c., &c. Semen mixed with the urine may lead to the inference that the patient is suffering from Bright's disease, but in that case spermatozoa are present.

the epithelium covering the Malpighian tufts, such as occurs in scurvy, diabetes, anæmia, &c.

Serum-albumin gives, in common with other proteids, certain reactions, of which only three may be mentioned here :—

(1) With strong, pure nitric acid it becomes yellow, either in solution or in the solid state, and this colour changes to a deep orange on adding ammonia; this is known as the *xanthoproteic* reaction.

(2) On adding Millon's reagent to a solution, a precipitate forms, which after boiling becomes, like the supernatant liquid, a fine red colour. Solid albumin behaves in the same way. If only traces are present, no separation of the albumin takes place, only a red colouration of the fluid, which becomes more distinct after some time.

(3) A solution containing serum-albumin, to which a solution of caustic soda and a few drops of very dilute solution of sulphate of copper are added, shows a violet tint. Other tests* are given, but the most important of these, which serve for the detection of albumin in urine, will be now referred to. It would not help us, in the clinical testing of urine, to commit to memory a series of tests; we must be content with a few reliable ones.

The Tests for Albumin in Urine are the following (in every case, if the urine is at all turbid, it should be filtered before applying any of the tests) :—

(1) Serum-albumin and serum-globulin are the only proteids occurring in urine which are coagulated at a temperature of from 73° to 75° C. or 163·4° to 167° F. If, however, the urine is alkaline, precipitation will not occur on heating, so that it is necessary to acidulate it with a couple or more drops of acetic acid before heating.

* (4) The true albumins, when dissolved in acetic acid, give, on the gradual addition of concentrated H_2SO_4 and cautious warming, a violet solution, with a feeble yellow-green fluorescence, which shows a band between b and F. This is the reaction of Adamkiewicz.

Again, in perfectly normal urine, on heating, as said above, a precipitate of earthy phosphates may fall, which looks to the naked eye very like a precipitate of albumin; but on adding an acid the phosphatic precipitate at once disappears. Saundby* remarks: "In order to obtain the best results with this means the following method and precautions must be adhered to. The urine should be that passed after breakfast; it must be clear, and, if necessary, should be filtered, with or without previous addition of sodium chloride or magnesium sulphate. Putrid urine is unfit for accurate examination. Fill a test-tube two-thirds full of urine, and boil the upper half. *It must be well boiled, not merely heated to boiling-point.* Acidulate with a few drops of *dilute* acetic acid. Hold the tube against a shaded background, with the light falling from above, when the faintest haze may be detected by contrast with the clear fluid below. In a bad light, or by artificial light, the detection of a faint haze with certainty is impossible. . . . . The cloud so obtained, if the urine has been filtered after saturation with sodium chloride or magnesium sulphate, is almost certainly serum-albumin." Further, Kirk and others have asserted that urine containing much mucin, but no albumin, gives a cloud resembling albumin with this test, but Saundby failed to confirm this statement.

Tyson† observes that the addition of two or three drops of acetic acid may diminish the precipitate produced by boiling urine containing albumin; but if a few more drops are added the full amount is again thrown down; hence up to fifteen or twenty drops should be then added. On the other hand, adding too much acetic acid will convert the albumin into the acid modification, which will dissolve on heating the urine, and fail to be detected. Acidulation with acetic acid is preferable to that with nitric acid, as the latter may dissolve

* Saundby: "Glasgow Med. Journ.," June, 1884.

† Tyson: Loc. cit., p. 35.

up faint traces of albumin, converting it into acid albumin. Nitric acid should only be used in the *cold*, and then in considerable amount, as in the next test. Méhu* states that urine charged with oxalate of lime becomes slightly turbid on heating, even after all the lime possible has been filtered off, and that this turbidity will not disappear on adding a few drops of strong acetic acid. But both Tyson and Saundby failed to verify the truth of this assertion.

(2) Serum-albumin may be detected by *Heller's nitric acid test*:—The urine freed, if necessary, from turbidity by filtering, is poured, to the amount of about 10 c.c., into a test-tube, and some strong nitric acid is allowed to trickle down the side of the tube so as not to mix with the urine; or better, the nitric acid is introduced first, and the urine taken up in a pipette, the test-tube being slightly inclined so that the point of the pipette can be made to touch the side of the tube. At the point of contact of the two fluids a white zone or band is seen *varying in thickness according to the amount of albumin present.* In normal urine a brown ring, *due to the oxidation of the chromogens* of the urine, is seen at the point of contact of the fluid, which becomes darker on standing, and in highly coloured urine, such as we find in fever, the ring of albumin may also be coloured. If much indican be present the ring may have a violet or red tint; blood-colouring matter will also cause it to assume a brownish-red, and bile pigment a green or blue colour. If albumin be present abundantly this test answers well enough; but in cases where only small quantities are present it is liable to several fallacies, so much so that it is now but little used. Thus, in urines of high specific gravity a haziness due to acid urates is produced, but in that case the upper edge of the ring is not circumscribed (Landois); it also disappears with heat, but it is not easy to apply heat in such a test. In concentrated urine crystalline nitrate of urea is produced, although rarely; and in the case of patients taking copaiba or turpen-

* Méhu: "L'Urine, Normale et Pathologique," Paris, 1880, p. 326.

tine, resinous matters are present in the urine, which give a yellow-white cloud on applying the test: the cloud disappears on adding alcohol, but there is danger of an explosion when nitric acid and alcohol are mixed together.*

(3) *Picric acid* is used by Dr. George Johnson for the detection of albumin in urine.† Minute traces may, according to him, be detected by pouring into a test-tube, six inches long, a four-inch column of urine; then, while the test-tube is held in a slanting position, an inch column of picric acid solution (saturated) is added by gently pouring the acid on the surface of the urine, with the upper layer of which it mixes. This is rendered turbid, and contrasts with the clear urine below; the fluids must mix in order to get precipitation. Heat increases the turbidity; on standing the coagulated albumin subsides, and, in an hour or so, forms a delicate layer at the junction of the yellow acid and the nearly colourless layer of urine. But other substances are precipitated by picric acid as well as serum-albumin—for example, urates which disappear on heating; creatinin, peptones, quinine, morphia, and other vegetable alkaloids and oleo-resins; but these latter also disappear with heat.‡ If phosphates are present a second cloud masks that due to the albumin. Mucin is also precipitated by this reagent.

(4) *Potassio-mercuric iodide*, introduced by Tanret, and known as Tanret's reagent, is used by some as a precipitant for albumin; but it is *uncertain in its action, and precipitates other bodies besides serum-albumin*, such as peptones and the alkaloids. Brasse§ found that on heating, the peptone and alkaloidal precipitate, produced by Tanret's reagent, dissolve, leaving the albumin insoluble, and that the alkaloidal pre-

* H. Prunier found that this test is not reliable, as it gives a precipitate when no albumin is present, and he found peptones in the precipitate.—"Journ. Pharm.," [5], xiii., S. 501—502.

† Johnson: "Albumen and Sugar Testing," London, 1884, p. 11.

‡ Jaffé finds uric acid and creatinin in the precipitate, produced by picric acid in urine. See "Zeits. physiol. Chemie," x., 391—400.

§ Brasse: "Compt. Rend. Soc. Biol.," [8], iv., 369, 370.

cipitate can be separated from the peptones by means of its solubility in ether. Brasse also found that bile-salts give a precipitate with this reagent insoluble in hot or cold water, but this precipitate can be distinguished from albumin by its solubility in ether. Tanret's reagent is as follows:— Bichloride of mercury, 1·35 grams; iodide of potassium, 3·32 grams; acetic acid, 20 c.c.; distilled water, 64 c.c.* It coagulates the same bodies as picric acid. But its use cannot be recommended, for the reasons mentioned. Méhu states that it precipitates mucin.

(5) *Sodium tungstate*, with or without citric acid, precipitates albumin, but it also precipitates acid urates, peptones, and mucin, and its use cannot be recommended.

Other reagents have been recommended, such as *ferrocyanide of potassium*, which, according to Tyson, is as delicate as nitric acid, but less delicate than heat, also *metaphosphoric acid*. "Ferrocyanic" and "mercuric" test-papers are used by Dr. Oliver for rapid bedside testing,† and for the approximate estimation of albumin, of which Ralfe speaks highly.‡ But from what we know of Tanret's reagent and ferrocyanide as precipitants for albumin, it does not seem advisable to neglect the acetic acid and heat test, which, according to the best authorities, is the most reliable.

Sir W. Roberts has introduced an acidulated brine solution, consisting of a pint of saturated solution of common salt, to which an ounce of HCl is added. Tyson finds this about equal to the nitric acid test in delicacy. Sir W. Roberts now believes that the heat and acetic acid is the best test for the detection of small quantities of albumin in urine.

According to Palm§ the test for albumin by acids may

* Tanret: Cf. Tyson, loc. cit., p. 44.
† Oliver: "Bedside Urine Testing," London, 1885.
‡ Ralfe: "Diseases of the Kidneys," p. 105.
§ Palm: "Zeits. anal. Chemie," xxvi., S. 35—38.

be made much more sensitive by dissolving the acid in alcohol, or better, in alcohol containing 10 per cent. of ether; an excess of the reagent will not then dissolve the precipitate.

Rough Approximate Estimation of Albumin.—An idea of the variation of the amount of albumin excreted in the urine from day to day may be obtained by precipitating the albumin by heat and dilute acetic acid, and allowing the test-tube to stand for some hours—about twelve or more—and during that time shaking it twice, in order to cause a uniform subdivision and precipitation of the particles of albumin. The urine should be filtered from solid urates, epithelium, &c., before testing.

Hoffmann and Ultzmann state that an idea of the amount of albumin present may be gained from the thickness of the ring in Heller's test (with cold nitric acid as described

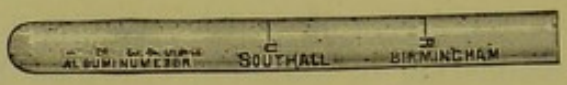


Fig. 36.—Esbach's Tube (Albuminumeter) for the Estimation of Albumin.

above). A white ring of 2 to 3 m.m., or $\frac{1}{12}$ to $\frac{1}{8}$ inch, which is faintly white, has no granular appearance, and is well defined only when placed against a dark background, indicates the presence of less than $\frac{1}{2}$ per cent., usually $\frac{1}{10}$ per cent. If the ring is 4 to 6 m.m. or $\frac{1}{6}$ to $\frac{1}{4}$ inch, granular, white, opaque, and visible without a dark background, the quantity is from $\frac{1}{4}$ to $\frac{1}{2}$ per cent. If it appears granular and flocculent, and sinks in more or less lumpy masses to the bottom of the tube, and if by stirring with a glass rod the fluid looks like cream, the quantity is large, 1 to 2 per cent. (Tyson). I have not found this method reliable.

A much better idea may be gained by the use of *Esbach's tubes* (Fig. 36). These tubes are about 6 inches high and

0·6 inch wide, and are graduated in grams of albumin per liter; there are two marks, the lower one marked U, the upper R. The urine is poured up to the level of the U, and should be diluted previously with one or two volumes of water, if its specific gravity is higher than 1006. The reagent is then poured in up to the level of the higher mark, R. The reagent is prepared by dissolving 10 grams of picric acid and 20 grams of citric acid in 800 c.c. of boiling water, and making up to 1 liter, the mixture being well shaken and laid aside. After adding the reagent the tube should be closed by the cork, and turned upside down several times without shaking, so as to mix the fluids. The tube is then left to stand perpendicularly for twenty-four hours, and the level of the albumin read off; this gives the number of grams of albumin present in a liter—the dilution of the urine, of course, being taken into consideration. The percentage can be obtained by dividing by 10.*

Sir William Roberts† has proposed a *dilution method*, which depends upon the fact that when an albuminous urine is progressively diluted with water, and tested at frequent intervals by Heller's nitric acid test, the precipitate gets less and less, until at last it disappears; at that point the urine contains less than 0·0014 per cent. albumin. With the increasing dilution the opacity also takes a longer time in appearing. The urine is therefore diluted *until it gives no opalescence for thirty seconds after the addition of the acid, but shows it at the forty-fifth second.* The urine is first tested for albumin, so as to form an idea of the amount present. It is then diluted from ten to one hundred times, as may be necessary, and poured into a test-tube about $\frac{5}{8}$ inch wide, to the depth of an inch. About 10 or 12 minims of nitric acid are then taken

* Less than 0·5 gram albumin per liter cannot be accurately estimated by Esbach's tube. Dr. Johnson states that a solution of picric acid containing 5 grs. to the ounce answers for the estimation. If the urine is alkaline it should be acidulated with acetic acid before the picric acid is added.

† Roberts: "Med.-Chir. Trans.," vol. lix., 1876.

up in a pipette, and then, holding the test-tube inclined, the acid is allowed to form a layer beneath the urine a quarter of an inch deep. The time of adding the acid is noted exactly, and the time when the opacity appears, which is best seen by holding the tube against a black background. The experiment is repeated with more or less diluted urine until the reaction appears, in not less than thirty-five and not later than forty-five seconds after adding the acid. The amount of water added is known. If, for example, 5 c.c. of urine have been diluted up to 500 c.c., the urine is supposed to contain 100 degrees of albumin, each degree corresponding to 0·0034 per cent. (0·008 Musculus, 0·0033 Hammarsten); each volume of water added is termed a degree, the zero being the state of dilution necessary for the terminal reaction.

Dr. Oliver, by precipitating all the albumin by a mercuric test-paper from a given quantity of urine and comparing the diminished transparency with that produced by coagulating the albumin from a standard solution containing $\frac{1}{10}$ to 1 per cent. albumin, has introduced an approximate method. Instead of the test solution he uses a piece of ground-glass. But I must refer to his book on "Bedside Urine Testing" for a full account of the method, of which Ralfe speaks highly.

Accurate Quantitative Estimation of Albumin.—To estimate accurately the amount of albumin in urine a good chemical balance is required, and the process takes more time than is usually at the disposal of the practitioner. The urine must be filtered, then 100* c.c. are taken and placed in a beaker of about 200 c.c. capacity, and if the reaction is not acid a drop or two of acetic acid must be added. The urine is then heated on the water bath for half an hour or more (while it is frequently stirred to prevent clotting), until the precipitate settles. A small Swedish ash-free filter-paper is then dried at 100° C. in the hot water oven (Fig. 37),

* It is better to add another 100 c.c. of distilled water to the urine.

and when cool weighed. The precipitate is then collected on the filter paper, taking care to remove every trace of it from the beaker, by a small clean feather, if necessary. It is then washed with boiling water, to which a few drops of ammonia

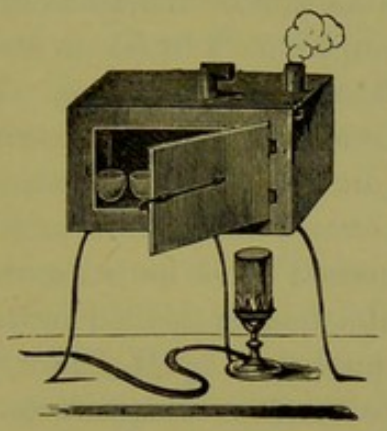


FIG. 37.—HOT WATER DRYING OVEN FOR DRYING PRECIPITATES.

have been added to remove uric acid and urates, and then with hot water alone, until the filtrate gives no reaction with a solution of nitrate of silver, showing that all the chlorides

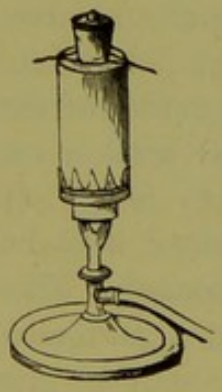


FIG. 38.—ARRANGEMENT OF CRUCIBLE SUPPORTED ON A TRIANGLE OVER A GAS-BURNER (*it should not be placed perpendicularly, but in an inclined position*) FOR INCINERATING RESIDUES.

have been removed. It is then washed with alcohol and ether, then paper and albumin dried in the hot-air bath at 110° C., and when cool weighed in a weighing tube or between clamped watch glasses, this being repeated until it

ceases to lose weight. This weight, less that of the filter paper, is that of the albumin in 100 c.c. of urine. The dried filter, with the albumin on it, can be incinerated (Fig. 38) in a weighed platinum crucible, if necessary, and the weight of the ash deducted from that of the albumin. If the urine is very rich in albumin, from 20 to 50 c.c. should be taken and diluted up to 100 c.c. before boiling. If much mucus is present it is advisable to dilute the urine with twice its volume of water, and add 15 c.c. acetic acid to 100 c.c., allow to stand for twenty-four hours, and filter before boiling. Even this method is not quite accurate.

Very recently a *densimetric* method for estimating albumin in urine has been published by H. Záhoř,* which is said to yield fairly accurate results. Huppert and Záhoř† had previously worked out a method for proteids generally, on which Záhoř's method is based. The filtered urine is mixed with enough acetic acid to ensure the coagulation of all the albumin on subsequent boiling, the right quantity being easily determined by boiling a small quantity of urine with the acid in a test-tube previously; the filtrate obtained from this test-specimen should give no reaction with acetic acid and ferrocyanide of potassium when the right amount of acid is used. A quantity of fresh urine is now placed in a flask firmly closed with a caoutchouc stopper. The flask is hung for ten to fifteen minutes in a large bath, which is filled with water kept boiling. By this means the albumin is precipitated. This is filtered off into a funnel leading through a perforated cork into a flask, the funnel being covered with a glass plate. The specific gravity of the acidulated urine itself, and of the filtrate obtained from it after coagulation of the albumin, are taken with a urinometer graduated to four places of decimals, both fluids being kept at the same temperature. This is done by placing them in two cylinders, both immersed in

* Záhoř: "Zeits. phys. Chemie," xii., S. 484—495.

† Huppert and Záhoř: "Zeits. phys. Chemie," xii., S. 467—483.

a large vessel of water, which should be kept at the same temperature if a series of experiments are to be performed. The best temperature is 17·5° C. The difference between the two specific gravities multiplied by 400 gives the amount of albumin in grams in 100 c.c. of urine.

With regard to the *amount* of albumin found in urine, the maximum, according to Tyson, is probably from 3 to 4 per cent.; since blood-serum itself contains only about 5 per cent. 2 per cent. is a large quantity, and the most common percentage is a half.

Schaumann* has proposed the following modification of the gravimetric method for estimating albumin. A plug of absorbent cotton wool is fitted into an Allihn's filter tube. Tube and plug are dried at 110° C. and weighed. The tube is then fixed on a filter support, and connected with a filter pump. A weighed quantity of urine is acidulated with acetic acid, and heated for half an hour until the albumin is precipitated. The supernatant fluid is then poured into the tube, the precipitate being washed repeatedly with hot water, and the washings poured into the tube before the precipitate is introduced, which is done carefully. It is then washed again by pumping hot water through the filter until the washings give no longer a chloride reaction with silver nitrate. The tube is then closed at the wider end with a cork, perforated for a glass tube; it is then placed in an iron drying box provided with holes at each end, in which the tube is fitted. The end having the perforated cork is connected with a calcium chloride tube and wash-bottle charged with sulphuric acid, whilst the drawn-out end is connected with an aspirator. A moderately rapid stream of dried air is then drawn through the tube for an hour; the temperature of the drying box is raised gradually to 100° C., and after an hour to 110°. After heating at this temperature, dried air still being drawn through, the tube is weighed every half hour until the weight is constant. The difference between this weight and

* "Zeits. f. anal. Chemie," xxvii., Heft 5.

that of the filtered tube before being used represents the weight of dried albumin contained in it. This method is only likely to be used in laboratories, and it does not seem to possess any decided advantage over the ordinary gravimetric method.

Serum-Globulin, or Paraglobulin.—Serum-globulin very frequently accompanies serum-albumin in urine, although it is generally overlooked on account of its similarities to albumin.

According to Senator it always accompanies serum-albumin, the more globulin there is present the more difficult it is to separate it. The urine should be saturated after neutralization with magnesium sulphate in order to separate it from the serum-albumin; if it is then filtered the precipitate will contain the globulin, while the filtrate contains the serum-albumin. Dr. Sidney Martin* has shown, in a recent paper, that the only reliable method for the separation and detection of this and some other proteids met with in urine when present in small quantity is by saturating the urine with ammonium sulphate $[(NH_4)_2SO_4]$. After powdering the crystals in a mortar the salt must be added to the urine in a flask, and shaken well with the hand until no more is taken up by the liquid. This operation takes only a few minutes. By this means albumin (both serum- and egg-albumin), globulin, and albumose are completely precipitated, whether the liquid be acid, neutral, or alkaline; while peptones remain in solution.

Further, he shows that we cannot distinguish by heating and acidulating urine between serum-albumin, egg-albumin, paraglobulin, and hemialbumose. The hemialbumose† met with in urine is peculiar in its behaviour with heat, as it is precipitated on heating, but redissolves on further

* Martin: "Brit. Med. Journ.," April 21, 1888, pp. 842—844.

† Hetero-albumose according to Martin. The other albumoses are proto- and deutero-albumose.

heating, and is precipitated on cooling, provided the urine is not acidulated. If the urine be acidulated with acetic acid it is not precipitated by heat. Egg-albumin is distinguished from serum-albumin by the addition of ether, which coagulates it, but the test should be applied to that obtained by precipitation as described below.

"The precipitate obtained (by ammonium sulphate) is amorphous, and rises to the surface of the liquid; it is readily removed by filtration. When on the filter it may be redissolved by adding a small quantity of distilled water, or, if it is requisite to obtain the proteid in a state of great purity, it may be washed twice with a saturated solution of ammonium sulphate before adding the distilled water. The proteid or proteids are now in concentrated solution containing ammonium sulphate, the presence of which does not interfere with the ordinary tests for proteids. The filtrate, after saturation with ammonium sulphate, may contain peptones, and these are to be tested for in the manner presently to be described."

Martin then goes on to show that the *albumins* may be recognized in the redissolved precipitate by the fact that they are not precipitated by saturation with magnesium sulphate, and that they coagulate by heating their solutions to 73° C. Egg-albumin being coagulated by ether, serum-albumin not. The *globulins* are precipitated by saturation with magnesic sulphate, and the serum-globulin, which is practically the only one met with in urine, is coagulated at 75° C. The *hetero-albumose*, which is the most frequently occurring albumose in urine, is precipitated at a temperature of 43°—50° C., the precipitate being soluble in a few drops of a weak acid, and it is precipitated in the absence of acids, while the albumins and globulins are not. It also gives the biuret reaction (*pink*, not violet, except excess of $CuSO_4$ is added), and the characteristic reaction with nitric acid, a precipitate in the cold which redissolves again on heating, and reappears on cooling; it is also precipitated by

acetic acid and ferrocyanide of potassium, and by saturation with magnesic sulphate. The peptones are the only proteids which remain in solution after saturation with ammonium sulphate, and they are identified by the xantho-proteic and biuret reaction (a *pink* colour being produced in the latter case.)

Paraglobulin may be detected, however, in urine, if present in large amount, by diluting the urine with twice its bulk of distilled water and passing a stream of carbonic acid through it, acidulating if the urine is neutral or alkaline; in from twenty-four to forty-eight hours the globulin falls as a milk-white flocculent substance. It is held in solution in the urine by the sodium chloride and other neutral salts. If present in small quantity this test fails.

When the urine is largely diluted with water the salts are so reduced relatively that the globulin is no longer held in solution by them, and it is, therefore, precipitated. On this fact Sir W. Roberts bases a method for its detection:—A glass vessel is filled with water, and some drops of the urine allowed to fall into it. Sometimes each drop leaves a milky track behind it, and when a number of drops have been added the water becomes opalescent. On adding acetic acid it again becomes clear.

Sometimes paraglobulin is found alone in urine, sometimes it exceeds serum-albumin in quantity. According to Senator it occurs in urine in lardaceous diseases of the kidneys, and it has also been found in excess in the intense hyperæmia following cantharides poisoning, in the early stage of scarlatinal nephritis, and in functional albuminuria associated with marked disturbance of the digestive organs (Ralfe).*

* Dr. Noel Paton ("Edin. Med. Journ., 1888, p. 522) estimates the serum-globulin in urine by first estimating the total proteids by Esbach's method. 50 c.c. of urine are then rendered faintly alkaline with a drop or two of KHO, and agitated with powdered magnesic sulphate repeatedly until the solution is saturated, then allowed to stand in a warm place for twenty-four hours. In this way the globulin is completely precipitated. The

Hemialbumose or Propeptone.—I may recall to mind Kühne's* researches on the splitting up of the albumins by means of acids and by the action of pepsin and trypsin. Albumin is split up into an anti-group and a hemi-group through these influences. Thus, in the case of the pepsin- and trypsin-action, respectively, we have antialbumose and hemialbumose, the former still further splitting up into antipeptone, and the latter into hemipeptone; the hemipeptone, by further action, yielding tyrosin and leucin. The following tables from Krukenberg, after Kühne, show these decompositions better than any description can :—

SCHEMES OF THE SPLITTING UP OF ALBUMIN.

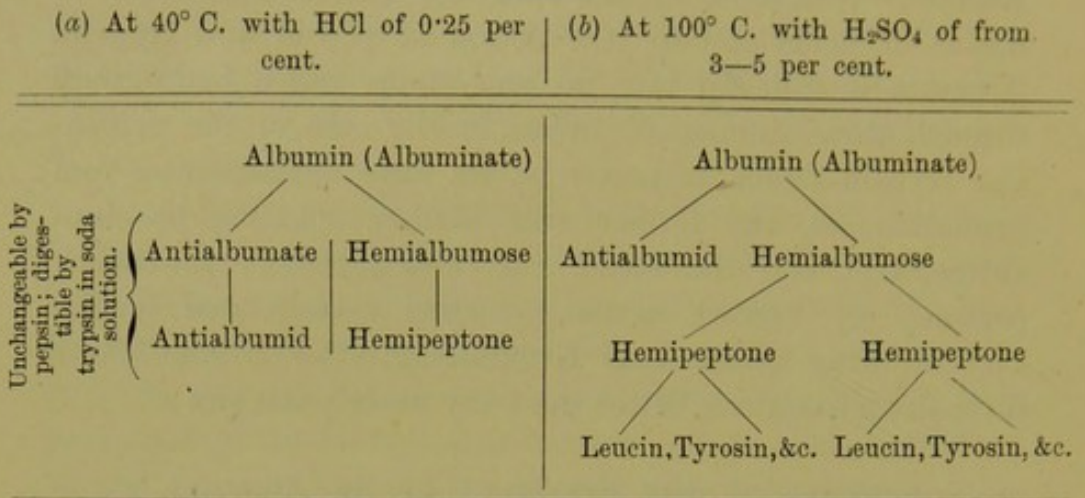
ALBUMIN.

Anti-group.	Hemi-group.
Antialbumose (taken for syntonin). Insoluble in neutral fluids; in certain stages of incomplete pepsin digestion the neutralization precipitate consists almost entirely of this.	*Hemialbumose* (= A-peptone Meissner), easily soluble in warm salt solutions (5 per cent.), with difficulty soluble in cold water; indiffusible.
Antialbumate (= parapeptone Meissner), soluble in dilute mineral acids.	
Antialbumid (= Hemiprotein Schützenberger, or dyspeptone Meissner), insoluble in dilute mineral acids; soluble in soda.	
Antipeptone. Diffusible; cannot be changed further by pepsin or trypsin.	*Hemipeptone.* Diffusible, capable of being split up by trypsin, unchangeable by pepsin.

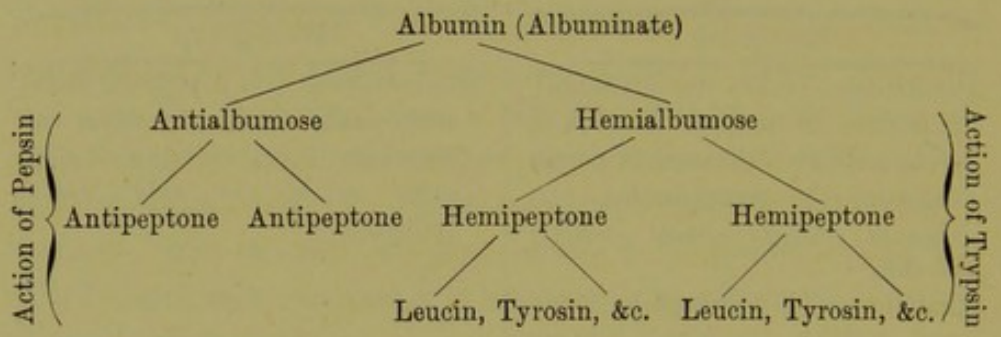
solution is then measured and filtered, and an Esbach's tube is filled up to the mark with the filtrate, and the picric acid solution added; it is allowed to stand five days, and then read. This, deducted from the amount of total proteids, gives the amount of serum-globulin.

* Kühne: "Verhandl. d. naturhistor. med. Vereins zu Heidelberg," Bd. i., Heft 4. Later researches are abstracted in "Journ. Chem. Soc." from time to time.

ACTION OF ACIDS.



ACTION OF ENZYMES (FERMENTS).



Hemialbumose or propeptone is, therefore, a less complete hydration product of albumin than peptone, hence the name propeptone. This body was first met with in the urine of a male patient suffering from osteomalacia, by the late Dr. Bence Jones;* he found that it was precipitated in the cold by nitric acid, but by boiling, with or without the addition of nitric acid, it only gave a precipitate when the urine cooled. Hoppe-Seyler has found it several times in cases of "atrophy" of the kidneys,† and Kühne found it present abundantly for five weeks in the urine of a case of osteomalacia.‡ He

* Bence-Jones: "Philos. Trans.," 1848, i., p. 55.

† Hoppe-Seyler: "Physiologische Chemie," S. 858, in which the other references to this subject are given.

‡ Kühne: "Zeits. f. Biologie," xix., S. 209.

separated it by precipitating the proteids* with alcohol, and washed and dried the precipitate at a low temperature; the residue was only partially soluble in water. The part insoluble in water was heated with a 5 per cent. solution of sodium chloride, and again extracted with water. The watery solution of hemialbumose thus obtained coagulated when heated, provided the solution were free from acid or alkali, for if either of these is present even in traces it will not coagulate. When digested with pepsin it yielded only peptones; with trypsin, peptones, and afterwards leucin and tyrosin, were formed, and it gave all the reactions of hemialbumose artificially prepared from albumin. Oertels† found it in the urine of two people out of thirty-three, who had ascended considerable heights. Virchow‡ found an identical substance in the bone-marrow of cases of osteomalacia, and Lassar in the urine of people rubbed with petroleum.§ Hemialbumose shows these reactions—(1) By strong acidulation of a solution with acetic acid, and the addition of a few drops of solution of ferrocyanide of potassium, a white precipitate falls, which is soluble in an excess of the ferrocyanide. (2) After strong acidulation with acetic acid, the addition of an equal volume of concentrated solution of sodium sulphate (Na_2SO_4) gives a precipitate on boiling. (3) It gives a white precipitate when a solution is heated with metaphosphoric acid in powder. These three reactions distinguish it from the peptones. Further, (4) It gives a precipitate with nitric acid in the cold, soluble on warming, and again precipitated on cooling; and (5) It shows a *pink* colour with sulphate of copper and caustic potash.

Tyson acidifies the urine with a few drops of acetic acid, adds about one-sixth its volume of concentrated salt solution,

* This urine gave a precipitate on heating to 43° to 50° C.

† Oertels: "Ziemssen's Handbuch der Therapie," 1884.

‡ Virchow: "Arch. f. pathol. Anatomie," Bd. iv., S. 309.

§ Lassar: Ibid., Bd. lxxvii., S. 164.

boils, and filters off the precipitate. Albumin and globulin remain on the filter. The filtrate is allowed to cool, and if a turbidity arises by the further addition of salt solution, which disappears by heating and reappears on cooling, then hemialbumose is inferred to be present.

Peptones.—Reference has already been made to the fact that these remain in solution after all the other proteids in urine have been carried down by ammonium sulphate. A definite condition known as peptonuria is now recognized. Peptone in urine was first recognized by Gerhardt* ; but, according to Hoppe-Seyler, the clouding produced by acetic acid and ferrocyanide of potassium (which, however, do *not* precipitate peptones), and the red colouration with caustic alkali and sulphate of copper, indicates at least the presence of other bodies in traces as well as peptones. Schultzen and Riess found peptones in the urine in many cases of phosphorus poisoning ; Maixner† in cases of suppuration and croupous pneumonia—in fact, he maintains, like Hofmeister, that peptone is present in cases where pus-corpuscles are undergoing disintegration in the body, although its presence in fresh pus has been questioned of late by others ; but it probably always occurs in decomposing pus. Peptones have been found, *in loco*, in cases of myomata of the uterus, by Fischel,‡ and by the same observer in hens' eggs during incubation ;§ this, he thinks, lends support to the view arrived at by him that peptones are formed in the tissues of the embryo during development, as he had previously found them constantly in the urine of pregnancy.‖ Peptones have been found in the urine of acute rheumatism, in typhoid fever, variola, scarlatina, miliary tuberculosis, ery-

* Gerhardt : "Deutsch. Arch. f. klin. Med.," Bd. v., S. 215 ; "Wien. med. Presse," 1871, No. 1.

† Maixner : "Prager Vierteljahrs.," 1879, S. 75.

‡ Fischel : "Zeits. f. physiol. Chemie," x., S. 14, 15. But Fischel's results are considered doubtful, as he did not use ammonium sulphate.

§ Fischel : Ibid., S. 11—13.

‖ Fischel : "Archiv Gynäk.," xxiv., S. 425.

sipelas, empyema, carcinoma of the liver and intestines, catarrhal jaundice, parametritis, apoplexy, parotitis, typhus, syphilis, and so on. Peptonuria may occur on very slight irritation of the urinary mucous membrane (Ralfe). An immense amount of work has been done on the distribution of peptones in the body, and their occurrence in urine, during late years, and it is impossible to say more here in this connexion, so I shall only refer to the "Journal of the Chemical Society," where abstracts of all the more important papers are given. We must remember that pepsin occurs in traces in normal urine; and we must not, therefore, attach too much significance to the presence of traces of peptones in urine.

Peptones are not precipitated by acids or alkalies, or by acetic acid and ferrocyanide of potassium, or by acetic acid after saturation with sodium sulphate, or by ammonium or magnesium sulphates. In concentrated solution they give the *biuret reaction*, becoming *rose-red* when the solution is treated drop by drop with solution of caustic soda or potash, and then with a weak solution of cupric sulphate. If a reddish colour appears, the copper solution is to be added until the colour has reached its greatest intensity. Excepting hemialbumose other proteids give a *violet* colour when thus treated. Ralfe* directs a drachm of Fehling's solution to be placed in the bottom of a test-tube, and then a drachm of the urine to be gently floated on its surface: "At the point of contact a zone of phosphates forms, whilst just above this, if peptones are present, a delicate rose-coloured halo will float. Should the peptones be mixed with serum-albumin, the halo will be mauve; if only albumin is present, purple." Peptones obtained in solution by the method recommended by Dr. Sidney Martin, referred to above, are recognized by the biuret reaction; they also give, on adding two or three drops of

* Ralfe: "Brit. Med Journ.," vol. i., 1883, p. 662.

nitric acid, boiling, and then adding, after the solution has cooled, some liquor ammoniæ, a *brown* colour.

Martin observes: "This method of saturation with ammonium sulphate is the only certain one of testing for peptones in urine." The urine may be filtered, after it has been concentrated, into a large excess of alcohol, by which the peptones are thrown down; they are then collected on a filter, and dissolved in water. "If such a solution gives no precipitate on heating, or with nitric acid, or with acetic acid and ferrocyanide of potassium, or on saturation with ammonium sulphate, and yet gives a brownish colour on heating with nitric acid and adding ammonia, and a pink colour with copper sulphate and potash, the urine contains no proteid but peptones, and the case is one of simple peptonuria" (Martin).

I need not, therefore, refer to other methods for the detection of peptones, as they are only with certainty distinguished from other proteids by *completely separating them from the proteids by saturating the urine with ammonium sulphate*, as Dr. Martin directs, and then applying the above tests. There is no doubt now that we may have peptones in urine independent of other proteids, but we must remember that they may arise in the urine itself by the action of ferments, or by the agency of bacteria on the cellular elements of the urine, or on proteids present in it.*

When injected into the blood peptones rapidly appear in the urine; whether under these conditions they do, or do not, produce poisonous symptoms is still a matter of dispute. Normal blood and normal urine seem to be free from them, the peptones taken up from the alimentary canal being built up in the organism into more complex molecules, and only under abnormal conditions appearing in the urine.

Mucin occurs in traces in normal urine, according to

* Or in carnivorous animals by the action of pepsin excreted with the urine (Neumeister: "Zeits. Biol.," xxiv., 272; and "Journ. Chem. Soc.," 1888, 516, 517).

Hofmeister,* and it may be mistaken for albumin. In diseases of the urinary passages it is increased in amount. It is precipitated by alcohol, dilute mineral acids, and certain organic acids, such as acetic and citric, but not by heat. If some acetic acid, or concentrated solution of citric acid, be introduced into a test-tube, and some urine containing mucus be poured over the acid carefully, a cloudiness appears at the point of contact. If albumin is present as well, the mucin cloud is seen above the coagulum of albumin. Oliver uses a test-paper for its detection. The urine is acidified with citric acid and a mercuric test-paper added, when a delicate haze is seen on holding the urine up to the light, which, according to Oliver disappears on applying heat to near the boiling-point, and reappears on cooling, vanishing again on boiling. Salkowski and Leube† add to the urine double its volume of 95 per cent. alcohol, filter off the precipitate, and treat with water. The resulting solution becomes cloudy with acetic acid, and does not dissolve in an excess of this acid, but easily in hydrochloric or sulphuric. It gives the biuret reaction with caustic soda and sulphate of copper. It is precipitated by lead acetate, and the filtrate from this gives no biuret reaction. Besides mucin the alcohol coagulum contains another proteid substance, which, after solution in alkalies, can be recognized by the biuret reaction. This precipitate caused in urine by alcohol was, as I said before, called "nephrozymose" by Béchamp (p. 140), who stated that it possessed diastatic reactions, but Leube‡ found that it is not a simple body, but a mixture of an albumin and a saccharifying ferment.

* Hofmeister: "Zeitschrift f. physiol. Chemie," Bd. iv., S. 261.
† Salkowski and Leube: "Die Lehre vom Harn.," S. 217.
‡ Leube: "Ber. d. physic.-med. Soc. zu Erlangen," 4 Marz, 1878.

CHAPTER X.

BLOOD AND BILE IN URINE.

Blood in Urine is only detected with certainty, when the microscope fails, by the spectroscope* (Fig. 39). It may be present either as oxyhæmoglobin, which shows the characteristic absorption bands between D and E, or as methæmoglobin. Sometimes, however, it seems to be present in a condition nearer to hæmatin, as Lewin and Posner† have pointed out, and as Dr. Armitage and I have observed.‡ In the latter case, on adding ammonium sulphide the absorption bands of reduced hæmatin appear (see Chart of Spectra). In hæmaturia, whether this be of renal, vesical, or urethral origin, the blood-colouring matter is generally present as oxyhæmoglobin, but when of renal origin it may be present as methæmoglobin. The latter, too, soon forms in acid urine after its separation from the body, so that in drawing a conclusion this must be taken into consideration.

In every specimen of urine obtained from cases of paroxysmal hæmoglobinuria which I have examined, I have found methæmoglobin, and Hoppe-Seyler§ has had a similar experience. Sometimes oxyhæmoglobin is present as well. According to the same authority, bile pigments, with few exceptions, occur at the same time in the urine of hæmo-

* For the method of using this instrument and a fuller description of alkaline and acid hæmatin, &c., see "The Spectroscope in Medicine," 1880.

† Lewin and Posner: "Centralbl. f. d. med. Wiss.," No. 20, 1887.

‡ MacMunn: "Brit. Med. Journ.," July 21, 1888.

§ Hoppe-Seyler: "Physiol. Chemie," S. 862.

globinuria. He is confirmed in this opinion by Kühne, Herrmann, and von Tarchanoff, but opposed by Naunyn, Steiner, and others. Methæmoglobin is distinguished by the band in red between C and D, nearer to C, besides others, and the urine generally has a brownish tint, or it may appear almost black. On adding a little ammonia, alkaline methæmoglobin is produced; showing a band just before D. With ammonium sulphide the colour becomes redder, and

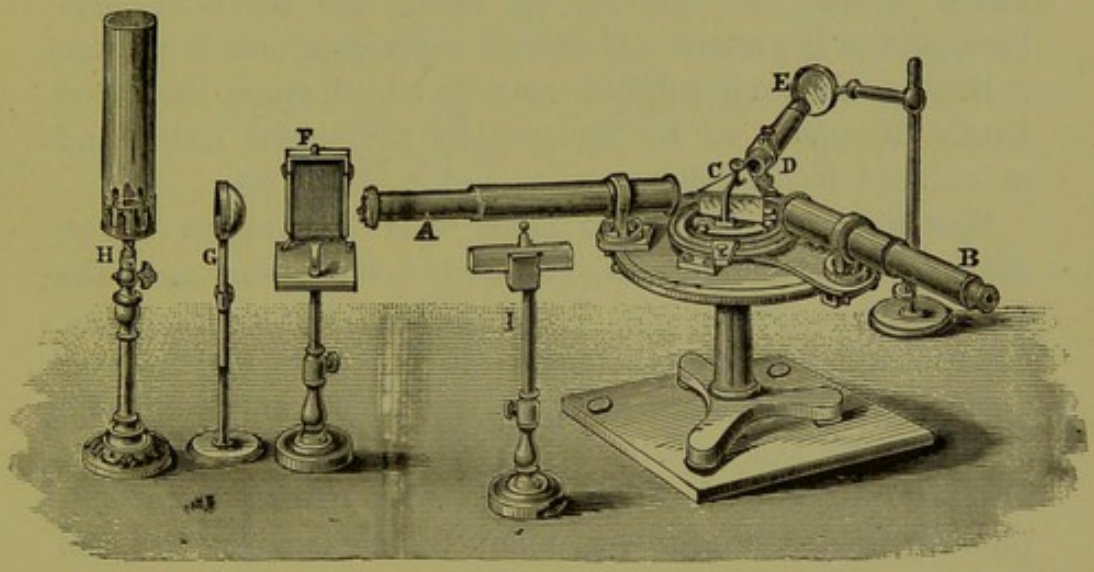


FIG. 39.—ARRANGEMENT OF SPECTROSCOPE.

A, Collimating tube, with adjustable slit. B, Observing telescope. C, Prism. D, Tube for scale. E, Mirror for illuminating scale. F, Preyer's hæmatinometer, with parallel sides (shown with the flat side towards the reader). G, Bull's-eye condenser before slit. H, Argand burner. I, Long spectroscope bottle for examining a deep layer of liquid. — (From M'Kendrick's "Physiology.")

oxyhæmoglobin again forms, which soon changes into reduced hæmoglobin* (see Chart of Spectra).

To detect small traces of blood in urine by means of the spectroscope a deep layer must be examined; this can be done by bringing a long spectroscope bottle (I, Fig. 39) before the slit of the chemical spectroscope, or by looking down through a test-tube full of urine with a direct-vision

* *Acid* urine containing blood is smoky, alkaline urine containing it is a brighter red colour.

spectroscope lighted from below. The bands seen cannot well be mistaken for anything else, especially after a little practice. If the observer has a microspectroscope he can remove the objective of the microscope, place a tube so that its central axis corresponds to the optical axis of his microscope tube and within the latter, and having filled the tube with the urine, slip the microspectroscope over it and turn the microscope mirror so as to throw a good light up through it. There is then no difficulty in seeing the bands of oxyhæmoglobin if present. If enough oxyhæmoglobin is present a little ammonium sulphide may be added, when the blood bands disappear to be replaced by the single hazy band of reduced hæmoglobin.

Sometimes, however, the blood may be present in an *insoluble* state, and then, as I described some years ago,* the following procedure may be required: — Filter the urine, cut the filter paper with the deposit on it into small pieces, digest in a little rectified spirit containing ammonia, and observe with the spectroscope; generally, a faint band at D, due to alkaline hæmatin, is seen, but if ammonium sulphide be added the two very characteristic bands of reduced hæmatin (the so-called hæmochromogen) appear at once (see Chart of Spectra). No other known colouring matter behaves in this way.

Of course the sediment may show blood-corpuscles under the microscope (Fig. 40), and then no further test is necessary; but they are often absent, and we must then have recourse to other tests. One of these is the guaiacum test: To some urine obtained from the lowest stratum in a test-tube a drop of freshly-prepared tincture of guaiacum is added, and a few drops of the so-called ozonic ether, which is merely a solution of peroxide of hydrogen in ether; the mixture is shaken and allowed to stand, when the ether assumes a blue colour. Almén uses oil of turpentine and tincture of guaiacum in equal parts, shakes them till an

* MacMunn: "Brit. Med. Journ.," July 19, 1879.

emulsion forms, and adds the urine; the urine then shows a blue colour. But this test is untrustworthy, as saliva, nasal mucus, probably pus, fibrin (?), and the internal use of iodide of potassium, also cause a blue colour to appear with this test.

When no blood-corpuscles are found microscopically we must not conclude that they have not been present, as they may become dissolved in the urine, especially if it is

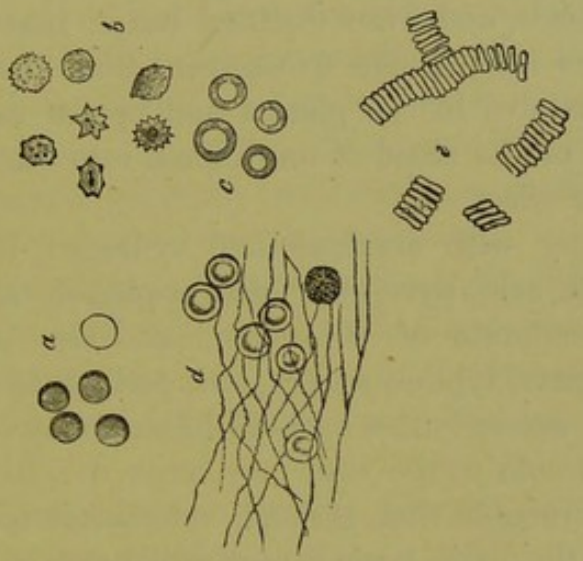


FIG. 40.—HUMAN RED BLOOD-CORPUSCLES.

a, After the action of water, and as they appear in urine freshly passed. *b*, Crenated, as they appear in acid urine. *c*, *d*, and *e* are to be neglected, as blood never appears in these forms in urine.

alkaline. With regard to paroxysmal hæmoglobinuria, Pavy was the first who showed that no blood-corpuscles are present in the urine of such cases. On boiling this urine it generally gives a brown coagulum. If this be extracted with alcohol acidulated with sulphuric acid the colouring-matter goes into the alcohol, and is seen to give, when examined spectroscopically, the bands of acid hæmatin. Sometimes, microscopically, brown masses, composed of granular pigment, and resembling tube-casts, owing to their having been moulded

in the *tubuli uriniferi*, are visible. Sometimes, but rarely, the hæmoglobin occurs in crystals, as Neale first observed.* The albumin found in the urine in these cases is the globin or proteid constituent of the hæmoglobin.† Just as the albumin of egg, by injection into the blood-vessels, appears in the urine, so does hæmoglobin when it is injected, and according to Ponfick's researches,‡ it passes through the epithelium of the tubules, being held merely in solution in the liquor sanguinis. But the injection of glycerin, salts of the bile acids, and even distilled water, into the blood-vessels, causes hæmoglobin to separate from the blood-corpuscles, to dissolve in the plasma and appear in the urine. The injection of the blood of one animal into that of another has the same effect.

In poisoning with arseniuretted hydrogen, hydrochloric acid, sulphuric acid, pyrogallic acid, naphthol, carbolic acid, phosphorus, chlorate of potassium, &c., also in cases of jaundice, pyæmia, typhus, scurvy, and perhaps in fat embolism and after severe burns, hæmoglobinuria may occur. The application of cold to the skin may cause it. In some cases it is not improbable that through vaso-motor disturbances, especially in the liver, some poison which has a destructive action on the blood-corpuscles may pass over into the circulation. Hoppe-Seyler's statement, referred to above, that bile pigments occur in the urine of such cases, and the well-known clinical fact that patients suffering from hæmoglobinuria are often slightly jaundiced, would go to support this idea.

Bile Pigments and Bile Acids in Urine: Choluria.—Urine containing *bile pigments* is coloured from yellow-brown to green, and by shaking the urine the froth appears yellow or greenish, whereas that of normal urine

* Neale: "Lancet," 1879, vol. ii., p. 725.

† In two cases examined by Dr. Halliburton there was serum-albumin in addition.

‡ Bridges Adams found it in the Malpighian capsules.—"Diss. Bern.,' 1880.

is colourless. A strip of white filtering paper soaked in the jaundiced urine becomes coloured yellow; and if urine be filtered through such paper, the stained paper can be used for the reaction with nitric acid (Rosenbach), or its colouring matter can be extracted by chloroform, and the chloroform used for further reactions. The bile pigments may be present in urine in two kinds of jaundice: (1) that due to obstruction of the bile-ducts, and (2) that in which no such obstruction occurs or can be detected *post mortem*. In cases of extravasation of blood into the tissues in large quantity, the hæmoglobin may be converted into bilirubin by the action of the connective-tissue corpuscles on it; and bile pigment may then appear in the urine (Quincke). Dog's urine in summer generally contains bile pigment, and yet no evidence of disease may be present.*

Bilirubin, $C_{16}H_{18}N_2O_3$, and biliverdin, $C_{16}H_{20}N_2O_5$ (Städeler), or $C_{16}H_{18}N_2O_4$ (Maly)—into which the former changes when exposed to the air in alkaline solution—or further oxidation products of these, may be present in the urine.

Bile pigments in urine may be detected by the following tests:—(1) If some nitric acid be poured into a test-tube and the urine gently poured over its surface, a coloured ring appears, which is first green, then blue, violet, red, and, lastly, yellow. This is known as the Gmelin-Heintz reaction. Each of these colours corresponds to the presence of an oxidation product of bilirubin, the final yellow corresponding to choletelin. If, as is often recommended, the yellow nitric acid is used, these colours follow each other too quickly to be observed (Emerson Reynolds). The initial green must be seen in order to enable one to be sure that bile pigment is present. A red colour may be given by the oxidation of some chromogen, probably one belonging to skatol; and the blue and violet by the oxidation of indican We have, however, in the spectroscope a certain guide in

* Salkowski and Leube: "Die Lehre vom Harn," S. 246.

this case, for, as I have shown,* this colour-change is accompanied by a change of spectrum. A broad shading composed of two distinct bands appears at D, then a black band close to F. As the colour-changes progress the band after D fades away, then that before D; and when the yellow stage is reached one band, that of F, is alone visible. In the case of indican the action of nitric acid may, in urines containing an excess of indican, produce a blue or violet colour. And, as I have shown,† this is characterized by the presence of a band before D and one after D. The bluer the colour the more distinct is that before D, while the redder it is the more distinct is that after D (see Chart of Spectra). But in the case of indican these bands persist, whereas in the case of bile pigment they fade away. These spectrum-changes are best studied in a chloroformic solution of the bile pigment, got either by extracting a filter paper through which it has been filtered, or by shaking the urine acidulated with acetic acid, with chloroform, and separating off the coloured chloroform; but in this case it is difficult to make the chloroform separate itself from the urine. When the chloroform is then treated with nitric acid the above changes of spectrum can be easily studied.

(2) Another test is that by means of iodine, first described by Maréchal‡, and re-introduced by Dr. Walter G. Smith, of Dublin.§ A drachm of the urine is placed in a test-tube, and a couple of drops of the tincture of iodine of the B.P. allowed to trickle down the side of the tube, held nearly horizontally, so that the two fluids may touch, but not mix. "If bile pigment be present, a fine green colour will almost immediately be developed below the red layer of iodine

* MacMunn: "Spectroscope in Medicine," 1880, pp. 160, 161. Dr. Quinlan has described the spectrum of bilious urine in "Proc. Roy. Irish Acad.," 2nd Ser., vol. iii. (Science).

† MacMunn: "Proc. Roy. Soc.," No. 226, 1883.

‡ Maréchal: "Journ. de Pharm. et de Chimie," Mars, 1869.

§ Smith, W. G.: "Dub. Journ. Med. Sc.," 1876, p. 452.

tincture." If the urine be very dark it is to be diluted with water before applying the test. It may also be necessary to shake the urine with chloroform, and on separation and evaporation of the chloroform to test the residue. The green colour is due to oxidation of the bilirubin, and no other ingredient of urine, normal or pathological, behaves in this manner (Smith).*

Sometimes, especially in febrile cases, the bile pigment present in urine refuses to give the Gmelin-Heintz reaction, the bilirubin being converted into more oxidized products.

(3) Fleischl† mixes the urine with nitric acid, or with a concentrated solution of nitrate of sodium, and adds concentrated sulphuric acid, so as to form a layer at the bottom of the test-tube; the colour-changes are then well seen.

(4) When, to a chloroformic solution of the bile pigment, obtained as described above, bromine water is added, a ring of similar colours is obtained (Maly).

In all these tests a very darkly coloured urine should be diluted with water before applying the test.‡ The presence of albumin does not interfere with these tests. In cases where they fail, some method must be adopted by means of which the bile pigments can be precipitated out of the urine. Thus, we may adopt Hoppe-Seyler's,§ which has been modified somewhat by others:—Add milk of lime to the urine, which precipitates the bilirubin, but leaves urobilin and indican in solution, pass a stream of carbon dioxide through it until no further precipitation takes place, and search for bilirubin in the precipitate after it has stood

* Saundby dilutes the urine and the iodine solution (liq. iodi 1 to 10): "Lectures on Bright's Disease," p. 123.

† Fleischl: "Centralbll. f. d. med. Wiss," 1875, No. 34.

‡ The nitric acid test may be done by spreading out a thin film of the urine on a white plate; a drop of the acid placed in the centre of this get surrounded by the rings of colour.

§ Hoppe-Seyler: "Physiol. Chemie," S. 864.

some time; by adding a little water to it, and shaking with chloroform and acetic acid, bilirubin colours the chloroform solution yellow, biliverdin the watery solution green; both solutions give Gmelin's reaction with nitric acid.

The Bile Acids occur only in small quantity in urine even in cases of jaundice due to obstruction, where they are present in largest amount. We do not yet know exactly in what form they occur in human urine, probably as anthropocholic acid, $C_{18}H_{28}O_4$, or combinations of this acid with glycocoll or taurin (Salkowski and Leube). But this is of little consequence, as all the bile acids behave similarly in presence of certain precipitants, and all give the reaction of Pettenkofer. Vogel and Dragendorf* found 0·8 gram of these acids in 100 liters of normal urine. Since it is probable that a small quantity of bile acids is formed in the intestine, their presence in normal urine is not surprising.

It is not so easy to detect the bile acids by Pettenkofer's reaction as is commonly supposed, even when they are present in cases of jaundice. Indeed, Tyson goes further, and states that Pettenkofer's test will not detect bile acids either in urine or other animal fluid when applied *directly*. Besides, when we have got the colour reaction, we do not know that it is due to these acids, as an immense number of substances give the same reaction.† In the spectroscope, however, we have a means of helping the diagnosis, as the spectrum is peculiar, but variable, changing as the reaction progresses. Pettenkofer's test has to be carefully applied in this manner: Six or eight ounces of urine (180—240 c.c.) are evaporated to dryness on the water bath. The residue extracted with absolute alcohol, filtered, and an excess of ether added (12 to 24 times the bulk of alcohol used); by this means the bile acids are precipitated out. The precipitate is then removed by filtering, and redissolved in distilled water,

* Dragendorf and Vogel: "Zeits. f. analyt. Chemie," Bd. xi., S. 467.

† Cf. Udranszky: abstr. "Journ. Chem. Soc.," 1888, pp. 863, 878.

decolourized by filtering through animal charcoal, and the filtrate treated by Pettenkofer's test. A single drop of a solution of syrup of cane sugar, diluted with water, so as to form a 20 per cent. solution, is then added to a drachm or two (3·7 to 7·4 c.c.) in a test-tube. Sulphuric acid is then added drop by drop, while the test-tube is kept immersed in a beaker of cold water, as the temperature must not exceed from 50° to 70° C. (122° to 158° F.). As the quantity of acid approaches the amount of fluid treated, a fine cherry-red or purple-violet colour appears. If the fluid becomes yellow, then the sugar is becoming charred by the acid, and the purple colour does not appear; this is prevented by keeping the temperature down (Tyson).

Krukenberg* directs 1 to 2 c.c. of the urine to be evaporated on the water bath, the residue to be treated with three to five drops of 0·2 per cent. cane-sugar solution, and with the same number of drops of common red sulphuric acid; by warming then to about 70° C. the marginal zone of the liquid becomes purple-violet; after longer digestion at 50° C. the purple colour becomes more intense.

The bile acids may be precipitated out of the urine by means of acetate of lead and a little ammonia; the precipitate is washed with water, then boiled with alcohol and filtered hot. The lead salts of the bile acids dissolve in the hot alcohol; the solution is then treated with a few drops of soda-solution and evaporated to dryness on the water bath. From the residue boiling absolute alcohol extracts the soda-salts of the bile acids. The alcohol is put into a closed flask, and treated with an excess of ether. The resinous precipitate is then dissolved in water and tested as before (Salkowski and Leube).

Sometimes, however, bile acids in urine may be detected by the method of Strassburg.† Some cane sugar is dissolved in the urine, a piece of filter paper is then dipped in the

* Krukenberg: "Grundriss med. chem. Analyse," S. 91.

† Strassburg: "Pflüger's Archiv," Bd. iv. S. 461.

urine and allowed to dry. If it be now touched with concentrated sulphuric acid, in about a quarter of a minute a violet-red spot appears.

If to the fluid showing Pettenkofer's reaction a sufficient amount of acetic acid be added, a clear fluid is obtained, appearing with transmitted light a violet-red colour, and with reflected a dirty green colour. The latter is due to fluorescence; it is seen best by letting the fluid stand from half to one hour before adding the acetic acid.

Schenck* states that the spectrum of the purple fluid obtained by Pettenkofer's test consists of a band between D and E near E, and a second at F; but I found a spectrum, from human bile decolourized with animal charcoal and dissolved in alcohol, which showed two bands, one before D, and another beginning about half way between D and E, and covering E and b.† Lately, I have again examined this spectrum, and find that owing to the strong absorption of the violet end of the spectrum, and the use of gaslight, another band was concealed which covers F. On observing by good daylight this third band can be seen. But in using decolourized bile for this purpose we have other things present, such as cholesterin, and I therefore prepared some pure bile-salts by dissolving the residue left by the evaporation of a decolourized alcohol solution in alcohol, and then adding ether. The alcohol solution of the precipitate was then treated by Pettenkofer's test, and then three bands were seen—one at D, one covering E and b, and the darkest at F. On evaporation of the alcohol and solution of the bile salts in distilled water, the same series of bands was seen—a feeble band at D, with its darker portion towards violet, another beginning midway between D and E and covering E and b, and a dark one at F (see Chart of Spectra). I was, therefore, correct in describing a band at D, which seems to have been missed by others. It must

* Schenck: "Maly's Jahresb. f. Thierchemie," 1872, S. 232.

† MacMunn: "Spectroscope in Medicine," p. 165.

be remembered that, in applying Pettenkofer's test to urine containing albumin, this must be removed.

Dr. Oliver has proposed a qualitative and quantitative test for biliary acids in urine by means of a solution of peptone, and also a peptone test-paper, for which his book, previously referred to, may be consulted.

Hay finds that sublimed sulphur sinks in urine containing bile salts, as the latter *lower the surface-tension* of fluids.

CHAPTER XI.

SUGARS IN URINE.

Grape Sugar in Urine.—Grape sugar, or dextrose, $C_6H_{12}O_6$, occurs in traces in normal urine (about 0·5 gram in twenty-four hours).* When in larger quantity, whereby it is easily recognizable, it may be due to a transient glycosuria, or, if persistent, to diabetes mellitus. Such diabetic urine contains mostly more sugar than all the remaining solid constituents together, possesses a sweet taste, undergoes the alcoholic fermentation with beer yeast, rotates the plane of polarized light to the right, and yields on evaporation crystallized grape sugar (Fig. 41) in crusts. If these are pressed, washed with alcohol, and recrystallized repeatedly from boiling alcohol to remove the accompanying salts, the sugar may be obtained purer. It occurs when crystallized from water in knotty aggregates of fine needles, having the composition $C_6H_{12}O_6 + H_2O$. Out of hot ethyl- or methyl-alcohol it crystallizes free from water in warty masses of hard needles, which melt at 146° C. It is easily soluble in cold and hot water, less in alcohol, and is insoluble in ether. In alkaline solutions it is capable of abstracting oxygen: acting as a strong reducing means, upon which property the various tests for grape sugar are based. It has been found in the urine due to a transitory glycosuria in cholera, intermittent fever, cerebro-spinal meningitis, heart and lung diseases, cirrhosis of the liver, and gout, and in poisoning by, or after the use of, morphia,

* See Wedenski: abstr. "Journ. Chem. Soc.," 1889, p. 293.

carbonic oxide, chloroform, chloral, curara,* and after the injection of amyl nitrite and ether into the blood. In diabetes mellitus its presence in urine is accompanied by polyuria and polydipsia, and by a high specific gravity of the urine, 1030 or more; so that in a case where a large quantity of urine of a pale colour, and having a specific gravity of 1030, is passed day after day, this disease is probably present.

It does not lie within my province to describe the pathology of diabetes, so I shall merely refer to two theories

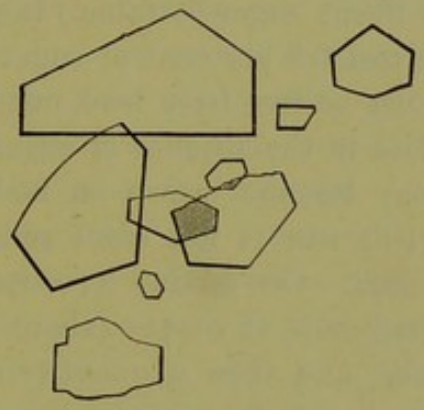


Fig. 41.—Tabular Crystals of Grape Sugar obtained from Honey.

which have been advanced for explaining the presence of sugar in the urine in excess in all those cases where it has been caused to appear by *experiment*.

(1) The glycogen in the liver may be transformed into glucose by the blood passing through the liver giving up its ferment† to the liver cells. The normal function of the vaso-motor system of the liver, and the centre for this system in the floor of the fourth ventricle, may be regarded, if this hypothesis be correct, as more or less an "inhibitory system" for the formation of sugar.

(2) If we suppose that, under normal conditions, there is a small amount of sugar passing into the hepatic vein from the liver, we might explain the diabetes as due to the

* Which paralyze the hepatic vaso-motor centre.

† See Ransom's paper in "Journ. Physiol.," viii., pp. 112, 113.

disappearance of these decompositions—diminished burning up of sugar in the blood—which are constantly removing the sugar from the blood (Landois and Stirling).

The following are the more important *tests* for grape sugar in urine:—

(1) *Moore's (or Heller's) Test* is now little used. The urine is boiled in a test-tube with an equal amount of liquor potassæ or liquor sodæ, when, if sugar be present, a yellowish-brown colour appears, which is darker the larger the quantity of sugar present. On adding dilute sulphuric acid the smell of burnt sugar (caramel) is perceptible. For the success of this test 0·3 per cent. of sugar must be present. The alkali used must be free from lead, otherwise the colouration may take place in the absence of sugar. The colouring matters of bile may become brown on boiling with caustic alkalies. Hence this test is not *alone* reliable.

(2) *Trommer's Test.*—On adding a couple of drops of a weak solution of sulphate of copper (about 1 to 30) to some urine in a test-tube, and then a quantity of liquor potassæ or liquor sodæ, equal in bulk to the urine, a blue precipitate of hydrated cupric oxide, in combination with the sugar, forms, which redissolves, if the urine contains sugar, on agitation; but if no sugar be present the fluid is turbid and greenish. If the fluid is now heated, even before it reaches the boiling-point, yellowish-red streaks of precipitated hydrated suboxide of copper are seen, or they may be pure red from anhydrous suboxide. The occurrence of one or the other depends on different conditions—purer solutions give the red precipitate, the more impure the solution the yellower is the precipitate. It is advisable to set aside a specimen of urine treated in the same way, but without heating, for over twenty-four hours; if then the copper becomes reduced, as before, we know that the reduction is not due to other substances, but to sugar (Neubauer). Albumin, if present, should be removed. As little copper solution as possible should be used; the precipitation should begin to take place

before the heat reaches the boiling-point, as by prolonged boiling other substances may act as reducing agents the earthy phosphates which are precipitated must not be mistaken for the suboxide; and there must be a distinct yellow or red precipitate. In order to eliminate the effects of the phosphates, the caustic alkali may be added before the copper solution, and the precipitate filtered off. Other substances reduce the cupric to cuprous oxide, such as uric acid, hippuric acid, urates, mucus, indican, hypoxanthin, glycuronic acid combinations, and milk sugar in the urine of nursing mothers. Besides, the presence of other substances in urine, such as creatinin, peptones, the pigments, &c., sometimes prevents the reduction of the cupric to the cuprous salt, a yellowish-green precipitate only forming. Hence other tests may be required. Trommer's test is capable of detecting from $\frac{1}{2}$ to 1 per cent. of sugar in urine.*

(3) *Böttcher's Bismuth Test.*—An equal volume of liquor potassæ or liquor sodæ is added to the urine, and then a pinch of subnitrate of bismuth, the solution being shaken and boiled. If sugar be present, first grey oxide, Bi_2O_3, then black metallic bismuth is deposited on the sides of the tube. As albumin and other sulphur-containing substances, *e.g.*, pus, blood, mucus, &c., may produce the black sulphide of bismuth (Bi_2S_3), it is advisable to adopt Brücke's modification.† Freshly precipitated basic bismuth nitrate amounting to 1·5 grams is mixed with 20 c.c. water and heated to boiling, then 7 grams of iodide of potassium and 20 drops of hydrochloric acid are added. Equal quantities of urine and of water are put into two separate test-tubes; to the one containing water hydrochloric acid is added until a drop of the above reagent (Frohn's) no longer produces cloudiness. In this way we learn how much hydrochloric acid is to be added to the urine. The

* Trommer's test often fails, a yellowish solution being produced. See Brunton: "St. Barth. Hosp. Reports," xvi., 235.

† Brücke: "Wien. acad. Sitzungsber.," 1875, Bd. lxii., 2 Abth.

latter is then acidified with that amount of hydrochloric acid, the reagent added, and the mixture filtered. The filtrate, which should not now become cloudy on adding hydrochloric acid or the reagent, is boiled for a few minutes with an excess of a concentrated solution of caustic soda, or potash. If a grey or black colour results, sugar must be present (Tyson). Or a portion of urine may be treated with plumbic oxide (PbO) instead of the bismuth salt, after being treated with the caustic alkali, and if no blackening results then no sulphide is present.

(4) *Braun's Test.*—When grape sugar is boiled with picric acid and caustic soda or potash, it reduces the yellow picric acid to the blood-red picramic acid. Dr. George Johnson* has again called attention to this test, and uses it not only for qualitative, but also for quantitative purposes. In applying it for detecting grape sugar in urine—to a drachm of the urine, 10 minims of a saturated solution of picric acid are added, half a drachm of liquor potassæ, and enough water to make up two drachms. If albumin be present a turbidity is produced, but this does not interfere with the test. On boiling the mixture a dark mahogany-red colour is produced if sugar be present. Or a third of a grain of picric acid, or as much as can be carried on the point of a penknife, may be introduced into a test-tube, graduated into three drachms, then half a drachm of water added, and the water heated to dissolve the picric acid. Half a drachm of urine is then added, and if albumin is present this is at once detected; on then adding a little caustic potash and boiling the liquid, if sugar be present a dark colouration appears. But the colour alone is not sufficient, the fluid must become *opaque*. For *quantitative* testing, Dr. Johnson uses a standard solution, made by boiling together a fluid drachm of a solution of grape sugar (containing one grain to the ounce), half a drachm of liquor potassæ, and 40 minims of a saturated solution of

* Johnson, G.: "Lancet," November 18, 1882.

picric acid, and made up to four drachms with water. The liquid is kept boiling for a minute, when it changes to a claret colour. It is then cooled, and, if necessary, water added to make it up to four drachms. This corresponds to one-quarter grain of sugar to an ounce. But as the colour is not permanent, it is imitated by a solution of ferric acetate, made by mixing one drachm of solution of ferric chloride of sp. gr. 1·44, four drachms of solution of acetate of ammonia,* four drachms of glacial acetic acid (sp. gr. 1·065), and one drachm of liquor ammonia, which is to be added last, the solution being diluted with distilled water up to four ounces. This solution corresponds to a quarter of a grain of sugar to an ounce. It should be kept in the dark. In estimating the sugar in a sample of urine, a drachm of urine is heated with 40 minims of a saturated solution of picric acid and half a drachm of liquor potassæ, and diluted up to four drachms. The fluid is boiled, and kept boiling for a minute. It is then cooled by immersing the test-tube in cold water, and, if necessary, the fluid is diluted with water up to four drachms. Its colour is then compared with that of the standard solution by means of the picro-saccharimeter, introduced by Dr. Johnson. This consists of two tubes having the same internal diameter. One contains the standard solution of ferric acetate, the other is graduated, and is for the urine treated as above. If, on comparing the depth of colour in the two tubes, it is found to be the same, then the urine contains one quarter of a grain of sugar to the ounce. If, on the other hand, the urine (and picric acid solution) has to be diluted in order to make it of the same colour as the standard solution, by reading off on the graduated tube the amount of water added, the amount of sugar in grains per ounce is easily calculated. Certain precautions are necessary, which I have not space to mention here.

* B.P. solution may be used: "Liq. ferri perchloridi," and "Liq. mmoniæ acetatis."

(5) *Indigo-carmine Test.*—This test, first proposed by Mulder, has lately been revived by Dr. Oliver. It depends upon the fact that, when a solution of indigo carmine is made alkaline by sodium carbonate and heated, the colour remains blue; but when heated with grape sugar a series of colours appears—violet, purple, red, yellow, and finally a straw colour. The solution does not keep, however, so that Oliver has brought out a test-paper impregnated with indigo-carmine and sodium carbonate. By means of this, sugar can be estimated quantitatively as well as qualitatively, for which Dr. Oliver's "Bedside Urine Testing" may be consulted.

(6) For *qualitative* as well as *quantitative* testing, either *Fehling's solution*, or a modification of it introduced by Dr. Pavy, may be used. Pavy's fluid differs from Fehling's in containing caustic potash, instead of caustic soda, the ingredients being present in a different proportion, and, calculated in English measures, 100 minims correspond to half a grain of grape sugar, which Pavy assumes to consist of $C_6H_{14}O_7$, whereas Fehling puts it at $C_6H_{12}O_6$. Pavy adds ammonia to the copper solution to prevent the precipitation of the cuprous oxide produced by the reducing action of the grape sugar. In this way the disappearance of the blue tint of the fluid during the performance of the quantitative analysis becomes more apparent.* Pavy's latest solution is described on p. 185.

Fehling's solution is thus prepared:—

(*a*) Pure sulphate of copper in crystals (preferably prepared by recrystallization a few days previously) is roughly powdered, pressed between folds of dry filter paper, and 34·639 grams weighed off. This quantity is then dissolved in moderately warm distilled water, and the solution

* In decomposing urine, which contains ammonia or ammonic carbonate, Pavy's method must be adopted. 100 c.c. of Pavy's fluid = 0·05 gram of glucose. 10 c.c. of the urine are diluted as described, and delivered from a burette into Pavy's liquid, amounting to 50 to 100 c.c. (previously heated to boiling), until the colour is discharged.

diluted to 500 c.c. at the usual temperature, the solution being then kept in a well-stoppered flask.

(*b*) 173 grams of Rochelle salt—sodium potassium tartrate—in pure crystals, are dissolved in 100 c.c. of a solution of caustic soda of sp. gr. 1·34, and diluted to 500 c.c. with distilled water. This is preserved in a stoppered bottle, the stopper being smeared with paraffin so as to exclude the air.

For using the solutions, exactly equal volumes of both are mixed, and measured off with a pipette. On agitating, a deep-blue liquid is obtained, of which 10 c.c. = 0·05 gram grape sugar.

Before employing this solution for either qualitative or quantitative testing, some should be diluted with four times its volume of water and boiled. If no precipitate forms, it is fit for use. The two solutions should only be mixed before they are required, as the mixture soon decomposes.

Fehling's solution is liable to the same sources of error as Trommer's, hence all the precautions mentioned before must be adopted.*

Quantitative Estimation of Grape Sugar by means of Fehling's Solution.—The urine must be filtered, and if albumin be present it must be removed by boiling, and acidulation with acetic acid if necessary. The urine must be diluted, so that its contained sugar does not exceed 0·5 per cent. Diabetic urine requires to be diluted generally with ten times its volume of water, and it is then introduced into the burette.

10 c.c. of Fehling's solution are then measured off with the pipette into a deep porcelain dish or a small flask, and 40 c.c. of water added. This is heated on a wire gauze over a spirit lamp or Bunsen burner, the burette being placed over it. When the solution begins to boil the diluted urine is allowed to run into it gradually. Very soon a red precipitate of the suboxide, or a yellow of the hydrated suboxide, begins to form. On further addition of the urine,

* Cf. Sutton's "Volumetric Analysis," pp. 259—268.

the suboxide separates out more and more, the blue colour gradually disappearing. The solution is boiled again, and the urine added until the blue colour disappears. This is best seen by tilting the white dish, or, if the flask is used, by taking it to the window and rotating it. If the right point* appears to have been reached, a few cubic centimeters of the fluid are filtered through a small, thick Swedish filtering paper. The filtrate, which must be quite clear, is then acidulated with acetic acid, and a few drops of a solution of ferrocyanide of potassium added. If copper be present a brownish colour appears. If this is the case, more urine must be run in, say half a cubic centimeter, and the above test tried again.

A second estimation should be made, doing this as rapidly as possible, as, according to Worm-Müller and Hagen, small quantities of sugar are destroyed by the hot alkaline liquid; moreover, the precipitated suboxide is apt to reoxidize and dissolve. If only a small quantity of urine has been used up for the estimation, a fresh portion should be taken and diluted to a greater extent than before; while, if a large quantity, it should be diluted less than before.

Calculation.—Suppose the urine has been diluted with seven volumes of water, and that 10 c.c. of Fehling's solution have been required for 11·9 c.c. of diluted urine (10 c.c. of Fehling = 0·05 gram sugar), then the *percentage* is—

$$x = \frac{0{\cdot}05 \times 100}{11{\cdot}9} = \frac{5}{11{\cdot}9} = 0{\cdot}4202.$$

But the urine was diluted to eight times its volume, therefore the percentage of sugar in the urine was—

$$0{\cdot}4202 \times 8 = 3{\cdot}3616.$$

Or expressed in words: The number of volumes used to dilute the urine multiplied by 5, and divided by the number of cubic centimeters of urine used, gives the amount

* Cf. Hagemann: "Pflüger's Archiv," xliii., 377—384; and "Journ. Chem. Soc.," 1889, p. 535.

of sugar in percentages. According to Worm-Müller and Hagen, the results are $\frac{3}{10}$ths per cent. too high.

The presence of alcohol, benzoate of soda and chloral, &c., in the urine vitiates the accuracy of this method.

In diabetic urine containing little sugar, the oxide of copper does not settle down, but goes through the filter paper. To avoid this, Pavy adds ammonia. His latest test is composed of 34·65 grams cupric sulphate, 170 grams Rochelle salt, 170 grams caustic potash, dissolved to 1 liter with distilled water; 120 c.c. are then mixed with 400 c.c. of ammonia (sp. gr. 0·88) and diluted to 1 liter. 10 c.c. of this = 1 c.c. Fehling.

For various improvements in this method, see Salkowski and Leube, "Die Lehre vom Harn."

Fehling's solution, in solid form, may be applied to urine by means of Pavy's test-pellets, and Dr. Oliver has introduced cupric test-paper.

Sugar may also be estimated by Knapp's* method, which is based upon the reduction of an alkaline solution of mercuric cyanide by grape sugar, but it possesses no advantage over Fehling's.

(7) Sugar may be detected qualitatively and estimated quantitatively by *fermentation*. Under the influence of beer yeast, the sugar splits up into ethyl alcohol and carbonic anhydride, about 95 per cent. of the sugar undergoing this change. The fermentation takes place best at 35° C. The estimation is made either by the loss of weight of the apparatus caused by the escape of the CO_2 produced, or by the gain in weight of an absorption tube containing caustic potash to absorb this gas; but Gréhant and Quinquaud† have recently shown that estimations of glucose by fermentation are vitiated by the fact that CO_2 is given off by the respiration of the yeast itself, but this amount is easily determined, and the results then become accurate.

* Knapp: "Annal. d. Chem.," Bd. cliv., S. 252.

† Gréhant and Quinquaud: "Compt. Rend.," cvi., pp. 1249 to 1250.

1 molecule of grape sugar gives 2 molecules of CO_2, or 100 CO_2 = 204·54 sugar. The apparatus required is figured in Salkowski and Leube's "Die Lehre vom Harn" and other text-books.

To detect sugar by means of its fermentation, about a drachm of bakers' or brewers' yeast is added to about 4 ounces of urine in a six-ounce bottle, which is lightly corked, and left to stand at a temperature of from 15°—25° C. In twelve hours or so, the fluid gives off CO_2, and becomes lighter in colour, and its specific gravity is diminished. Sir W. Roberts states that urine containing less than 0·5 per cent. does not show signs of fermentation when thus treated. It is, therefore, by no means a delicate test.*

Upon the loss in specific gravity which the fluid undergoes on fermentation, Sir W. Roberts has based a quantitative method for estimating grape sugar. *Every degree lost in the specific gravity corresponding to one grain of sugar in a fluid ounce.* Four ounces of the urine are placed in a twelve-ounce bottle, a piece of German yeast ("Vienna yeast") as large as a small walnut is added. The bottle is then closed with a cork cut with nicks to allow the CO_2 to escape, and placed on a mantelpiece, or any other warm place. Beside it is placed another bottle containing urine only. In from eighteen to twenty-four hours fermentation is completed. The specific gravity is then taken, and at the same time and under the same temperature that of the unfermented urine. The percentage is calculated by multiplying the degrees of specific gravity lost by 0·23. Manassein gives this number as 0·219. But Budde† has recently shown that this factor must necessarily be a variable one, increasing as the percentage of sugar diminishes; and, practically, the method gives very fallacious results.

* Roberts: "Pract. Treatise on Urinary and Renal Diseases," &c., 4th edition.

† Budde: "Pflüger's Archiv," xl., S. 137—172.

(8) Grape sugar in urine may be estimated by the polarizing saccharimeter, such as Laurent's shadow instrument, Jellet's, Duboscq's, Soleil-Ventzke's, or other, but the expense of these instruments is so great that at present they are not likely to be used for clinical work; moreover, Worm-Müller has shown the polarization method to be useless for diabetic urine.

To distinguish lævulose, or fruit sugar, from glucose, which it very frequently accompanies in cases of diabetes, this instrument is required, as lævulose reduces the salts of copper like glucose, but in a less degree. Its specific rotatory power is − 108·3 at 0° C.; − 99·44 at 14°; − 97·1 at 17·5°; and − 52·5 at 87·2°, according to Tuchschmied.

It is not, however, within the scope of these lectures to discuss polarimetry, for which I must refer to the different text-books, such as Salkowski and Leube, so often referred to before.

(9) Von Jaksch* has introduced a test which Halliburton† has found useful in other cases. On adding to urine containing sugar a solution of phenyl-hydrazine hydrochloride, containing a small quantity of sodium acetate, a yellow precipitate of needles of phenyl-glucazone forms in a few minutes. These should be examined with the microscope. They melt at 204° to 205° C.

(10) Crismer‡ finds that if 2 to 3 c.c. of a 1 to 1000 solution of safranine be heated with a fluid containing sugar (dextrose) and 2 to 3 c.c. of soda, the safranine becomes reduced, as the solution becomes colourless and opalescent.

Detection of Small Traces of Sugar in Urine.—Sometimes urine containing grape sugar refuses to respond to the tests for that substance. In that case, it may be necessary to evaporate down half the daily quantity of urine, and to get the sugar from it in pure solution. One way of

* Von Jaksch: "Zeits. klin. Med.," Bd. xi., S. 20.
† Halliburton: "Journ. Physiol.," vol. x., No. 4.
‡ Crismer: "Pharm. Zeit." Bd. xxxiii., S. 651.

doing this is to evaporate the urine to the consistence of a syrup on the water bath, and extract the residue with absolute alcohol. Evaporate this, extract once with alcohol; add to this a solution of potassium hydrate in 80 per cent. alcohol. The precipitate which forms, after pouring off the alcohol, is dissolved in water, neutralized exactly with acetic acid, and precipitated with acetate of lead, filtered, the filtrate treated with sulphuretted hydrogen. The filtrate, after this treatment, which frees it from sulphide of lead, is evaporated on the water bath, and used for the reactions of grape sugar (Salkowski and Leube). Or Brücke's method may be adopted —namely, the urine is precipitated with neutral lead acetate, filtered, the filtrate treated with basic lead acetate and ammonia. The last precipitate contains the grape sugar; it is decomposed by sulphuretted hydrogen, and, after filtering, the filtrate is used for the reactions.

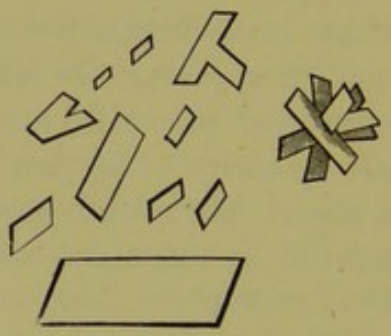


FIG. 42.—CRYSTALS OF MILK SUGAR.

Milk Sugar, $C_{12}H_{22}O_{11} + H_2O$, is sometimes found in the urine of nursing mothers. Hofmeister recognized it in such urine* by precipitating with neutral acetate of lead, the filtrate and wash-water being then treated with ammonia, the filtrate again precipitated with lead acetate and ammonia. The washed precipitate was suspended in water, decomposed by sulphuretted hydrogen, the filtrate shaken with silver oxide, the filtrate from this freed from silver by sulphuretted

* Hofmeister: "Zeits. f. physiol. Chemie," Bd. i., S. 101.

hydrogen; and barium carbonate being added, it was evaporated. Alcohol removed from the residue milk sugar, which was obtained crystallized by evaporation over sulphuric acid *in vacuo* (Fig. 42). A strong reducing power of the urine for the copper salts and strong right-handed polarization suggest its presence in the absence of symptoms of diabetes.

Inosit, or Muscle Sugar, $C_6H_{12}O_6+2H_2O$, is sometimes found in the urine of diabetes mellitus, diabetes insipidus, and albuminuria; in that of phthisis, syphilis, and typhus fever. It has also been detected in healthy urine after the use of large quantities of water or of diuretics,

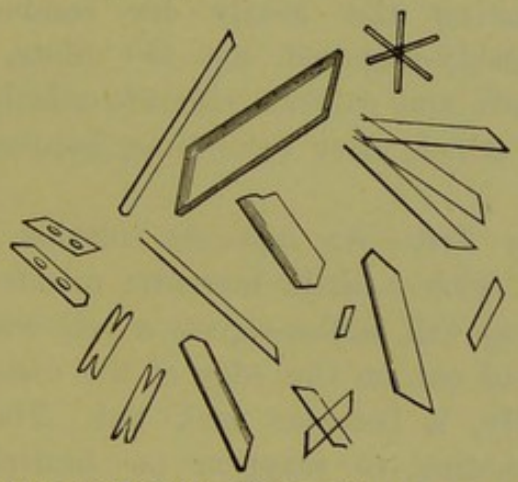


FIG. 43.—CRYSTALS OF INOSIT—MUSCLE SUGAR—FROM THE MUSCLES OF THE HUMAN HEART.

also in animals after puncture of the diabetic centre in the medulla. Hence its presence in urine is of some clinical importance.

In the pure condition it crystallizes in large fine rhinorhombic prisms, or monoclinic tables, which easily effloresce (Fig. 43). They are sometimes grouped in rosettes. From a solution in boiling spirit it crystallizes in shining lamellæ, somewhat like cholesterin. The crystals sometimes assume a red colour, when the fluid containing them is evaporated. In the impure condition it crystallizes in cauliflower-like masses. It is soluble in 16 parts water at 10° C., insoluble

in absolute alcohol and ether. It fuses at 210° C. It has a sweet taste, does not rotate the plane of polarization, and does not ferment. In alkaline solution it retains hydrated cupric oxide in solution, *and does not reduce it.* Fermented with putrid albumin it furnishes sarco-lactic acid. The watery solution is not precipitated by neutral lead acetate, but is by basic, especially on heating. Two reactions distinguish inosit when present in solution, namely:—

(1) *Scherer's Test.*—On evaporating a solution containing inosit on the water bath nearly to dryness in a porcelain dish, having added a few drops of common nitric acid,* and then treating the nearly dry residue with some drops of a freshly-prepared, not too dilute, watery solution of ammonia, and calcium chloride solution, a rose-red mass remains, which, after some time, becomes changed in colour.

(2) *Gallois's Test.*—An aqueous solution treated in a porcelain dish with a little mercuric nitrate $(NO_3)_2Hg$ in solution—a drop will suffice—gives a yellowish precipitate. If this is spread out on the edge of the dish quickly, and heated carefully, it becomes dark red. The colour disappears on cooling, to reappear on heating. Albumin, sugar, and tyrosin interfere with the reaction. Danilewsky obtained inosit as a product of pancreatic digestion (probably).†

Detection in Urine.—A large quantity of urine (several liters) is feebly acidified, precipitated completely with neutral acetate of lead, filtered, and the warmed filtrate treated with basic acetate of lead, as long as a precipitate forms. After standing for forty-eight hours this precipitate is filtered off, washed, suspended in water, and treated with a stream of sulphuretted hydrogen. From the filtrate, after some hours, uric acid separates, from which the fluid is

* The HNO_3 is omitted by Salkowski and Leube.

† Danilewsky: "Ber. d. deutsch. chem. Ges.," Bd. xiii., S. 2132.

poured off. The solution is then evaporated to a syrup on the water bath, and precipitated with absolute alcohol. The precipitate is dissolved in hot water, and three or four times as much alcohol of 90 per cent. as water added. The alcohol is then treated with ether until the clouding is permanent, when the inosit crystallizes out. The watery solution can be used for Scherer's reaction directly (Salkowski and Leube).

CHAPTER XII.

ACETONE AND DIACETIC ACID IN URINE.

Acetone, C_3H_6O, or CH_3COCH_3, sometimes occurs in urine. Chemically, acetone is dimethyl ketone. It is obtained artificially by the oxidation of secondary propyl alcohol, by replacing the chlorine in acetyl chloride by methyl, by the distillation of calcium acetate, or by passing the vapour of acetic acid through a red-hot tube. It is a colourless liquid, boiling at 56° or 58° C., and has a peculiar smell, somewhat reminding one of chloroform. It is miscible with water, alcohol, and ether, and gives some reactions which help to detect it in solution:—

(1) If agitated with an excess of a concentrated watery solution of acid sulphite of sodium, a combination of acetone and sodium bisulphite separates out in shining scales.

(2) Treated with a little solution of caustic potash and a few drops of a concentrated watery solution of iodine in iodide of potassium, crystals of iodoform separate, which, under the microscope, are six-sided plates, or of a star shape (Lieben's iodoform test). The latter test may be used to detect its presence in urine in excess by adopting Ralfe's modification: About a drachm of liquor potassæ, containing 20 grains of iodide of potassium, is placed in a test-tube and boiled. A drachm of the urine is then carefully floated on the surface of this. At the point of contact a ring of phosphates is formed, and, after a few minutes, if acetone or its allies are present, the ring becomes yellow and studded with yellow

points of iodoform; but lactic acid and ethyl alcohol act similarly.

(3) *Le Nobel's Test.**—If an alkaline solution of sodium nitro-prusside, so dilute as to have only a red tint, be added to a fluid containing acetone, a ruby-red colour is produced, which, in a few moments, changes to a straw-yellow. On boiling, after adding acid, the colour changes to greenish-blue. One-fourth of a milligram of acetone may be detected by this reaction, but with very small amounts of acetone the colour may only be yellow, yet on adding an acid it becomes violet.

(4) *Legal's Test*† (almost identical with Le Nobel's).—Some drops of a concentrated solution of sodium nitro-prusside are added, and the mixture made alkaline by a little caustic potash. A red colouration is produced, which disappears after some time; when this has taken place some acetic acid is added, and, if acetone be present in the urine, a deep violet colouration appears.

(5) Chautard‡ adds a drop of an aqueous solution of magenta decolourized by sulphurous acid. If acetone be present, a violet colour is produced, the intensity of colour being proportional to the amount present. In dilute solution, four or five minutes elapse before the colour appears. In urine containing only a little acetone 200 c.c. should be distilled at a gentle heat, and the first 15 c.c. of the distillate tested. In this way 0·01 per cent., according to Chautard, may be detected.

(6) *Baeyer and Drewsen's Indigo Reaction.*—A few crystals of nitro-benzaldehyde are heated until dissolved; the solution allowed to cool, when the aldehyde separates out as a white cloud. The mixture, or distillate if necessary, is made alkaline with dilute caustic soda. If acetone be present, yellow, then green, followed by an indigo-blue colour, occurs

* Le Nobel: "Chem. Centr.," 1884, S. 626.
† Legal: "Journ. Pharm.," [5], xviii., pp. 206, 207.
‡ Chautard: "Bull. Soc. Chim.," xlv., pp. 83—86.

within ten minutes. By agitating with chloroform, and testing this, smaller quantities of acetone may be made to yield the reaction. One part acetone present in 2,500 can be detected in the distillate from urine by this means. Pyroracemic acid, aldehyde, and acetophenone are the only other bodies which give this reaction, but these are not found in urine.

Acetone has been confused with, and mistaken for, diacetic acid in urine. I shall, therefore, now refer to the latter before pointing out the pathological conditions under which either, or both, may be found in urine.

Diacetic Acid, or Ethyl-diacetic Acid, $C_6H_{10}O_3$, is a thick syrupy fluid miscible with water, decomposable below 100° C. into carbonic acid and acetone * :—

$$\underset{\text{(Diacetic acid.)}\dagger}{CH_3.CO.CH_2.CO_2H} = \underset{}{CO_2} + \underset{\text{(Acetone.)}}{CH_3.CO.CH_3}$$

It strikes a Bordeaux-red with a solution of ferric chloride, and by this test its presence in urine is generally inferred. Gerhardt‡ first found that the addition of this reagent to the urine of diabetics produced a brown-red colouration, which appeared to him to point to the presence of ethyl-diacetic acid.§ Subsequently, Rupstein‖ detected in the urine of diabetics, which gave this reaction, alcohol and acetone, so that it seemed probable that ethyl-diacetic acid was present in the urine and that, under the influence of alkalies, and by taking to itself one molecule of water, it split up into acetone and alcohol : according to this equation :—

$$\underset{\text{(Ethyl-diacetic acid.)}}{C_6H_{10}O_3} + H_2O = \underset{\text{(Acetone.)}}{C_3H_6O} + \underset{\text{(Alcohol.)}}{C_2H_6O} + CO_2$$

* Morley : "Organic Chemistry," p. 195.

† This formula is given by Morley, and also occurs in the 1888 edition of Watts' "Dictionary of Chemistry." I give it here with all reserve.

‡ Gerhardt : "Wien. med. Presse," 1871, No. 1.

§ Gerhardt attributed acetone also to the presence of aceto-acetic ether.

‖ Rupstein : "Centralb. f. d. med. Wiss.," 1874, No. 55.

But Fleischer* showed, in 1879, that the body which gives this ferric chloride reaction in urine is not taken up by agitation with ether after the urine has been treated with sulphuric acid, whereas ethyl-diacetic acid should, if present, go over into the ether, and can be found partly undecomposed in the distillate, by distilling a watery solution. This red reaction with ferric chloride, the acetone smell of the breath, and the presence of acetone in the urine in diabetics, have led to a theory that the peculiar train of symptoms, now known as Küssmaul's coma, which sometimes occurs in diabetes, is due to the presence of acetone in the blood; and hence the condition has, without sufficient reason, been called acetonæmia. Now, Salomon and Brieger† have found that acetone in large doses produces no effect on animals or men, even in diabetic cases. The urine in these cases gave no smell of acetone, nor its reactions; so that the acetone appeared to be destroyed in the body. Be that as it may, there is no doubt that acetone is present in the breath and in the urine of a certain number of cases of diabetes, and when Küssmaul‡ tried to show that diabetic coma is often accompanied by the body giving the ferric chloride reaction in urine, and of acetone, he was not altogether wrong, although we now know that diabetic coma may apparently occur independently of their presence. Saundby§ and others maintain that the train of symptoms included under Küssmaul's coma may be met with in several diseases, especially in those in which the state of the blood has undergone profound pathological alterations. And Saundby is supported in this view by the observations of Senator‖ and Riess.¶

Acetone is not limited to diabetic urine, where it was

* Fleischer: "Deutsche med. Wochenschr.," 1879, No. 18.
† Salomon and Brieger: "Zeitschr. f. klin. Med.," Bd. vi., Heft 1.
‡ Küssmaul: "Deutsch. Arch. f. klin. Med.," 1874, Bd. xiv.
§ Saundby: "Birmingham Med. Review," Feb., 1885.
‖ Senator: "Zeits. f. klin. Med.," Bd. vii., Heft 3.
¶ Riess: Ibid., Bd. vii., Supp. Heft, S. 34.

first found, as well as in the breath and blood of such cases, by Petters,* in 1857, as it has since been found in many other diseases. Indeed, there are very few practitioners who have not noticed the smell of acetone in the breath of children especially, suffering from different febrile conditions. Kaulisch, in 1860, noticed the smell of acetone in the urine of patients suffering from variola, typhus, and pneumonia; and this statement is confirmed by von Jaksch. This latter author† shows that he had observed the ferric chloride reaction of Gerhardt in the urine of a case where no sugar was present, and further research taught him that when the ferric chloride reaction is present the distillate gives Lieben's iodoform reaction. But he also obtained the same result with the distillates of urine which gave no ferric chloride reaction, especially with the urine of fever patients, as Deichmüller‡ showed to be the case with scarlet fever. Acetone was found by von Jaksch in the urine of healthy people up to 0·01 gram in twenty-four hours; hence he assumes a condition of physiological acetonuria, acetone being a normal product of tissue change, this being much exaggerated in febrile states. Acetone, he says, may be increased in diabetes without any other clinical evidence of its increase. In some rare cases of this disease the ferric chloride reaction may occur, and acetone may be obtained from the urine. In these cases he thinks diacetic acid is present, but these reactions may also be present in measles, scarlatina, and pneumonia. But the association of diacetic acid in the urine and of acetone is exceptional. In another communication von Jaksch§ gives the preference to Lieben's iodoform reaction, but we must remember that alcohol and lactic acid also respond to this test. Windle‖ obtained the

* Petters: "Prager Vierteljahrs.," 1857, Bd. lv., S. 81.

† Von Jaksch: "Zeits. f. physiol. Chemie," vi., S. 541—556.

‡ Deichmüller and Tollens: "Annal. d. Chem.," Bd. 209, S. 22 and 30.

§ Von Jaksch: "Chem. Centr.," 1884, 674, 675. See also his "Acetonurie und Diacetonurie," Berlin, 1885.

‖ Windle: "Liverpool Med.-Chir. Journ.," July, 1884.

ferric chloride reaction in several cases not diabetic, while he found it absent in many cases of diabetes. Saundby* sums up the cases wherein it has been obtained as follows:—

(1) In many acute diseases, *e.g.*, measles, scarlatina, and pneumonia, without symptoms of coma being present.

(2) In cancer, Bright's disease, perityphlitis, strangulated hernia, after minor surgical operations, and sulphuric acid poisoning, without dyspnœa or coma.

(3) In the urine of diabetics for weeks and months without coma or dyspnœa supervening.

(4) In a case of cancer of the stomach dying from coma (v. Jaksch). Acetone, and diacetic acid were present, but no sugar, in the urine of this case.

(5) In a case of Litten's after scarlatina, in which albuminuria was present.

(6) In diabetic urine during or just before the peculiar terminal dyspnœa sets in.

According to Legal,† the urine of patients who have taken thalline, antipyrine, salicylic acid, and phenol may give the ferric chloride reaction. So also do, according to Le Nobel, β-oxybutyric acid, thiocyanates, acetic and formic acid compounds, but in the case of diacetic acid the colour disappears on boiling. Besides, if the urine be previously boiled, it will not show the ferric chloride reaction if diacetic acid be present, whereas it does when most of the above substances are present.

The occurrence of acetone in diabetic urine has been placed beyond doubt by the observations of the authorities quoted, and by others such as those of Rupstein‡ and Cantani;§ while, as stated above, Gerhardt showed that urine yielding acetone gave the ferric chloride reaction (although this statement is too general). Rupstein also, on

* Saundby: "Birm. Med. Review," Feb., 1885.

† Legal: "Journ. Pharm." [5], xviii., 206, 207.

‡ Rupstein: "Centralb. f. d. med. Wiss.," 1874, S. 865.

§ Cantani: "Diabetes," 1878.

acidulating the urine with acetic acid and agitating with ether, was able to obtain the ferric chloride reaction in the residue from the ether extract. On the other hand, Salkowski found that this reaction occurred in diabetic urine in eight out of fourteen cases, and yet these cases gave off no acetone smell, and the ferric chloride reaction disappeared on boiling and the addition of acids.* He could never recognize the reaction in the ether extract, whereas control experiments, performed by adding diacetic acid to normal urine, enabled him to detect this reaction in the urine and in the ether extract from it. Hence he assumes with Fleischer that any ferric chloride reaction in urine in the majority of cases is not caused by ethyl-diacetic acid, but is due to the presence of another unknown substance. Hence the origin of acetone must be attributed to another source —something which arises from the fermentation of the grape sugar, and which furnishes alcohol and acetone. Alcohol itself, besides being often present in the urine of children and old people, has been also found in diabetic urine.

Von Jaksch† has shown that acetone alone may be found in cases whose course is usually favourable, whereas diaceturia is a most dangerous condition. Acetonuria may result from a continued high temperature, its excretion running parallel to the range of temperature. It often is present in diabetes, but does not necessarily accompany either it or glycosuria, while its occurrence in the former may sometimes be followed by diaceturia. He maintains that diabetic coma is the result not of acetone, but of diacetic acid in the blood. We know, however, that diabetic coma may be unaccompanied by the presence of the substance which gives the ferric chloride reaction in urine. Moreover, von Jaksch maintains that some convulsive attacks in children are due to *diaceticæmia*. This

* Salkowski and Leube : "Die Lehre vom Harn," S. 397.

† Von Jaksch : "Chem. Centralb.," 1884, S. 674—675. See also his "Acetonurie und Diacetonurie," Berlin, 1885.

condition in adults is accompanied by vomiting, dyspnœa, and jactitation, soon terminating in coma and death; whereas in children it is not so serious, the child feeling weak, having a thickly-coated tongue, often conjunctival catarrh, sometimes vomiting, generally constipation, and very little or no fever. But, as stated before, it is not certainly known that diacetic acid is present in these cases.

Minkowski* discovered an acid in the urine of a case of diabetes which he afterwards found was nearly identical with the β-hydroxybutyric acid of Wislicenus, but differing from it in being optically active; and he calls attention to the toxic effects of such acids when introduced into the body. It is suggestive, as Saundby remarks, that this acid is capable of breaking up to form diacetic acid, and is therefore nearly related to acetone. Wolpe† has also found it in the urine of diabetes, but he has shown that there is no fixed relation between the quantity of acetone and of hydroxybutyric acid eliminated; the latter, however, and diacetic acid vary in a parallel degree.

It would appear, from Saundby's accurate observations,‡ that constipation has been a marked feature in many of his own cases of Küssmaul's coma, and he attributes to it an important *rôle* in these cases. If this be so, may not we have to do with the absorption of poisons such as ptomaines, or perhaps of certain acids such as that referred to, from the intestine, which, in consequence of vaso-motor disturbances in the liver, escape its destructive action, and, passing over into the hepatic vein, get into the general circulation and poison the nerve centres?

Whether the body in urine which gives the ferric chloride reaction in cases of diabetes is, or is not, diacetic

* Minkowski: "Archiv f. exp. Pathologie," Bd. xviii., and "Chem. Centralbl.," 1884, S. 406, 407 and S. 672.

† Wolpe: "Chem. Centr.," 1887, S. 277, 278; see also Kulz: "Zeits. Biol.," xxiii., S. 329—339; "Journ. Chem. Soc.," 1887, 290; Stadelman: "Zeits. Biol.," xxxii., S. 456—459; "Journ. Chem. Soc.," 1887, 464.

‡ Saundby: Loc. cit., p. 21.

acid, we must remember that this reaction is of very grave import, often foretelling the onset of diabetic coma and death.

The following method, from Salkowski and Leube, may be used for procuring and recognizing acetone and alcohol in urine in cases of doubt. Large amounts of urine, as much as 50 liters, are successively distilled fresh, the distillates united, and, after acidulation with sulphuric acid, distilled fractionally (fractionized)—the lighter volatile parts collected. After three or four fractionizings in this way, the distillate is treated with fused calcium chloride in excess, and distilled on the water bath; the distillate repeatedly treated with chloride of calcium, and again distilled. For the recognition of the acetone in the distillate, use is made of the boiling-point (58° C. or 56° C. Roscoe), specific gravity (·814 at 0°), the smell, and the iodoform reaction. If alcohol be present, as it sometimes is with acetone in urine, it is contained in the chloride of calcium residues, and gives it off by distillation over the free flame. To test for alcohol the iodoform reaction is applied, and the formation of aldehyde and acetic acid by oxidation. Diacetic *ether* may also occur in urine, according to Gerhardt.

CHAPTER XIII.

FATTY BODIES IN URINE: LIPURIA AND CHYLURIA.

Fatty matters may be present in urine under two conditions —(1) When fat passes over into the urinary passages without disease of the kidneys; and (2) In those cases where it arises *in loco* from fatty degeneration or other disease of the urinary organs and passages. Claude Bernard* found that a dog which had been fed for eight days with mutton fat passed fat in its urine; and that fat can pass into the urine when in excess in the blood is proved by the observations of Wiener† and Scriba.‡ In "fat embolism," which sometimes occurs after fractures, the fat may appear in the urine. In diabetes mellitus the urine has been found to contain fat by Kobert,§ and both it and the sugar disappeared on a scanty diet. Fatty urine has also been seen by Ermann‖ in phosphorus poisoning accompanied by atrophy of the liver. It has been also observed in phthisis, pyæmia, and long-standing suppuration. In these cases the blood contains an excess of fatty matters. The second class of cases of lipuria occurs in Bright's disease, where fats may appear sparingly in the urine. The fatty tube-casts of that disease are familiar to the physician. In pyonephrosis fat has been found in the urine by Ebstein,¶ which collected

* Claude Bernard: "Leç. sur les propriet. des liqu., &c.," 1859, tom. ii., p. 86.

† Wiener: "Arch. f. exper. Pathol.," 1879, Bd. xi.

‡ Scriba: "Deutsch. Zeits. f. Chir.," Bd. xii., S. 118.

§ Kobert: "Rassmann, Diss. inaug.," Halle, 1880, S. 22.

‖ Ermann: "Vierteljahrsschr. f. ger. Med.," 1880, Bd. xxxiii., S. 61.

¶ Ebstein: "Deutsch. Archiv f. klin. Med.," 1879, Bd. xxiii., S. 113.

in drops on the surface of the urine; and hæmatoidin crystals were also present in the sediment.

The recognition of fat in the urine is made by means of the microscope, and by extracting the fat with ether, and examining by the usual tests the residue left after it evaporates.

Chyluria, however, is the disease, above all others, in which large quantities of fats appear in the urine, and these are usually accompanied by lecithin and cholesterin. This disease is now known to be due to the *Filaria sanguinis hominis* (Lewis), a filiform worm from 8 to 10 centimeters long, which, according to Manson,* inhabits the lymphatics, especially those of the scrotum and lower limbs. The embryos, which are about 0·35 m.m. long, pass from the lymphatics into the blood, and chyluria and hæmaturia occur, both resulting from the presence of the embryos in large numbers in the kidneys. The embryos are sometimes present in the urine, and will be found figured in Zeigler and Macalister's work on Pathology (Vol. I., p. 331, Fig. 98).

Manson has found that these worms are spread by mosquitos, which suck them up with the blood of diseased animals. They then pass through a phase of their life-history in the body of the mosquito, from whence they escape into drinking-water, and then into the intestines of those who drink the water. The disease is limited to the tropics, and is often found in soldiers who have been on foreign service. According to Hoppe-Seyler we find, in chylous urine, serum-albumin, serum-globulin, and fibrinogen, fats in a fine state of division, besides traces of soaps and of the constituents mentioned above — lecithin and cholesterin. Peptones may be also present. The urine

* Manson: "Lancet," 1878; "Trans. Path. Soc.," 1881. See also Lewis: "On a Hæmatozoon inhabiting Human Blood, &c.," Calcutta, 1872; "Lancet," 1877; "Quart. Journ. Micros. Sc.," 1879; also Art. "Chyluria" in Quain's "Dict. of Medicine," 1882.

contains, in fact, all the constituents of chyle. The fat may be removed by agitation with ether, which causes the urine to be less cloudy, and it can be made to appear clearer by adding some alkali. A trace of an albumin—also present in chyle—may prevent it becoming clear by the ether treatment alone. Chylous urine generally deposits flocks of fibrin. Its chemical composition has been investigated by Hoppe-Seyler,* Eggel,† and Brieger.‡ Hoppe-Seyler examined the blood of Eggel's case, and at a time when very milky urine was passed. The blood did not contain much fat, and the serum had only a faint cloudiness, such as normal serum shows during digestion. Brieger found that the fat in the urine was diminished or increased by diminishing or increasing the amount of fat in the food. The night urine remained albuminous in spite of the diminution of fat. In cases where the chyle has escaped into the urine by means of a fistulous communication, chyluria has also been observed (Odenius, Lang, von Hensen).

For the *recognition* and *estimation* of the fat, lecithin and cholesterin, the methods of Hoppe-Seyler, Eggel, and Brieger may be adopted:—

A large amount of urine (5 liters) is divided into different parts and repeatedly agitated with ether, then made alkaline with caustic soda, and again shaken with ether; the united ether extracts are distilled, the residue again dissolved in ether, the solution poured into a small weighed flask, and distilled, then the flask and residue are dried at 100° C., and weighed as an ether extract (Salkowski and Leube).

For estimating the individual components, the residue should be boiled with hot baryta water for some time. The cholesterin is unchanged by this process; the fat, however,

* Hoppe-Seyler: See "Physiol. Chemie," S. 870, 871.
† Eggel: "Deutsch. Arch. f. klin. Med.," Bd. vi., S. 421.
‡ Brieger: "Zeits. f. physiol. Chem.," Bd. iv., S. 407.

becomes converted into baryta soaps of the fatty acids and glycerin; the lecithin into the baryta soaps of the fatty acids, glycerin-phosphate of barium and neurin, $C_5H_{15}NO_2$. The liquid is then filtered; the precipitate contains the insoluble barium soaps and some cholesterin, the filtrate contains glycerin, glycerin-phosphate of barium, and neurin. The dried baryta soaps are then extracted with ether, and from the evaporated ether extract cholesterin crystallizes, after repeatedly taking it up into water-free ether and absolute alcohol and spontaneous evaporation of these.

The *cholesterin*, $C_{24}H_{44}O + H_2O$, crystallizes from alcohol

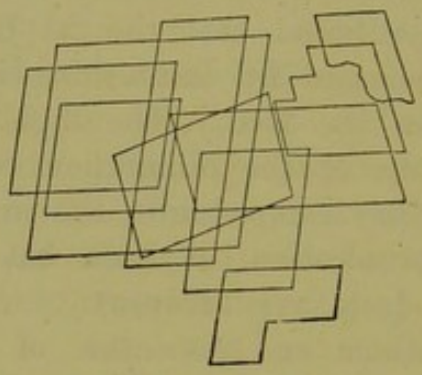


FIG. 44.—CRYSTALS OF CHOLESTERIN.

(Fig. 44) in large, very thin rhombic plates, which are easily recogized microscopically and by certain chemical tests:—

(1) By concentrated sulphuric acid cholesterin is changed into a red mass, which becomes green by heating on the water bath.

(2) On adding to the crystals some not too concentrated sulphuric acid, and then iodine, they become coloured violet, blue, green, red, yellowish, and, lastly, brown.

(3) If the cholesterin crystals are rubbed up with sulphuric acid, and chloroform added, a blood-red to violet solution is obtained, which, on exposure to the air, becomes violet, blue, and green, and, lastly, colourless. Fuming sulphuric acid brings about this change momentarily.

(4) If cholesterin be dissolved in chloroform and an equal volume of concentrated sulphuric acid be added, the solution becomes blood-red, then purplish, later blue and green, finally yellow (a trace of water decolourizes the solution at once). The lower layer of sulphuric acid shows a green fluorescence, and, on adding acetic acid, becomes purple, without losing the fluorescence.

(5) If a trace of cholesterin be evaporated to dryness on a porcelain crucible cover, with red nitric acid, a yellow spot is left, which becomes red with ammonia, but is not perceptibly altered by caustic soda.

In the filtrate from the baryta soap neurin may be detected. For the method of detecting this and for that of estimating lecithin, see Salkowski and Leube: "Die Lehre vom Harn," S. 258, 259. Cf. also Hoppe-Seyler: "Handbuch d. physiol. und pathol. chem. Analyse" (4th ed.), S. 374.

Beale* found cholesterin in the urine in four cases of fatty degeneration of the kidney, Salisbury† in the urine of diabetes and jaundice, and Pöhl‡ in the urine of an epileptic treated with bromide of potassium.

To recognize cholesterin, the urine should be agitated with ether; this should be saponified with caustic potash, and the watery solution again shaken with ether, which removes cholesterin and soaps. On evaporation of the ether the residue should be tested by the above tests.

Sir W. Roberts found oil in the urine of three patients, two of whom were taking cod-liver oil; and Dr. Henderson in cases of cardiac disease.§

* Beale: "Archives of Medicine," 1857.

† Salisbury: "American Journ. Med. Sciences," 1863, vol. xlv.

‡ Pöhl: "Petersbourger Med. Wochenschr.," 1877, No. 1, Jahrb. i., S. 171.

§ Henderson: "Brit. Med. Journ.," May 22, 1858.

CHAPTER XIV.

CYSTIN, TYROSIN, LEUCIN, AND OXYMANDEL ACID.

Cystin, $C_3H_7NSO_2$.—Sometimes, though rarely, cystin may be recognized as a urinary deposit. Its crystals are characteristic—they are colourless, beautiful *hexagonal plates* (Fig. 45), easily soluble in ammonia, caustic potash, or caustic soda, in mineral acids; insoluble in water, alcohol, ether, and dilute acetic acid. It sometimes occurs as a constituent of urinary or renal calculi. At times it may occur in the urine independent of disease, as Toel, Pletzer,

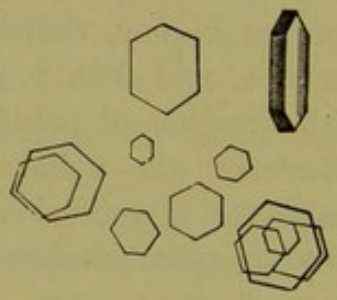


FIG. 45.—CRYSTALS OF CYSTIN.

Bartels, Marowsky, Ultzmann, Löbisch, Niemann, Heath, Southam, and others have found.

Tollens and Niemann suppose that, in the normal condition, cystin is changed in the organism into taurin. As some cystin remains in solution in the urine, the statements of the amount present are not reliable. Its origin in the body is unknown. Some maintain, and others deny, that it is a constituent of normal urine. It may be obtained crystallized from an ammoniacal solution, and may be distinguished from uric acid in that the latter occurs as an

amorphous deposit on evaporation of the ammonia. In a mixed deposit composed of cystin, phosphate of lime and triple phosphate, the cystin may be recognized by the solubility of the two latter in acetic acid, the six-sided plates of cystin remaining unchanged. This acid precipitates it out of alkaline solutions* (see under Urinary Deposits, *infra*).

Tyrosin, $C_9H_{11}NO_3$, and **Leucin,** $C_6H_{13}NO_2$.—Both these occur in the urine in cases of acute yellow atrophy of the liver and in phosphorus poisoning. They are found, physiologically, in the axilla, and between the toes accompanying certain odoriferous matters. They have been found in the urine also in cases of typhus and variola, and are frequently accompanied by biliary pigments and albumin. They seem to replace urea, to a certain extent, in disease.

Tyrosin is formed, artificially, by the decomposition of horn and albumin by means of acids and alkalies, and occurs then, mixed with leucin, as well as in the products of digestion of proteids, by means of trypsin. It is recognized in urine by its peculiar crystals, which occur in silky colourless sheaves of needles (Fig. 46). Both leucin and tyrosin can be precipitated out of urine by means of basic acetate of lead (Frerichs). The urine, as fresh as possible, is precipitated by this reagent, freed from lead by passing in sulphuretted hydrogen, filtered, and the filtrate concentrated by evaporation to a syrupy consistence. Tyrosin and leucin crystallize out in the cold. After standing some days, the leucin may be separated by boiling absolute alcohol,

* Cystin may be obtained from urine, and estimated quantitatively by the method of Löbisch ("Annal. d. Chemie," Bd. clxxxii., S. 231). 500 c.c. urine are treated with 20 c.c. acetic acid of 20 per cent., and kept in a cool place. A sediment is formed after twenty-four hours, which is mostly cystin, mixed with uric acid, oxalates, &c. This is collected on a weighed filter, washed with dilute acetic acid, dried, and weighed. The weighed filter placed in a funnel, the cystin dissolved by a few drops of dilute HCl, then dried, and weighed again. The difference in weight gives the amount of cystin. From the acid filtrate cystin may be obtained by neutralizing with ammonic carbonate.

and recognized by the reactions described below. Tyrosin, when quite pure, is insoluble in ether as well as alcohol; with difficulty soluble in cold water (1 part in 1900), more easily in boiling water, and easily soluble in dilute acids and alkalies. It may be recognized, when isolated, by the following tests (besides the crystals):—

(1) If touched on a watch-glass with common red sulphuric acid, and heated on the water bath for from ten to

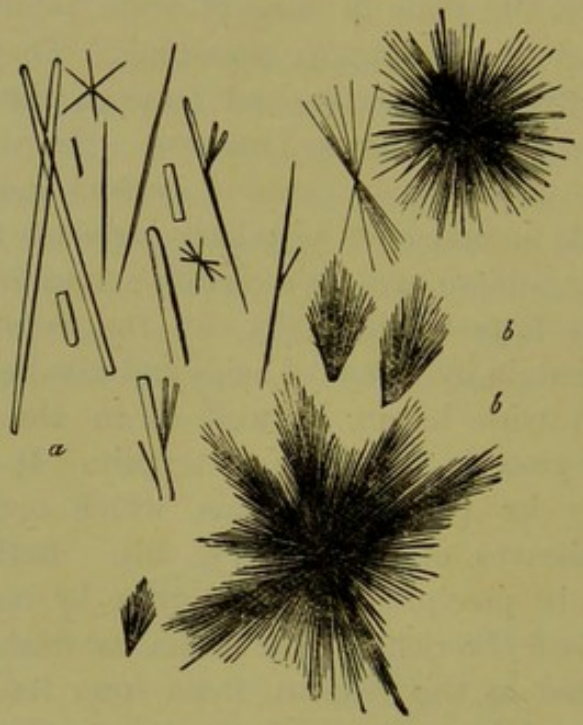


FIG. 46.—ACICULAR CRYSTALS OF TYROSIN.
a, Single crystals. *b*, *b*, Smaller and larger groups of the same.

fifteen minutes, it becomes red from the formation of tyrosin-sulphuric acid. If the fluid be then diluted with water, and neutralized by boiling with baric carbonate ($BaCO_3$), and filtered, the filtrate, when cold, gives, on adding a drop of dilute ferric chloride (one drop liq. ferri perchlor. to 10 c.c. water), a violet colour. This colour is evanescent, and destroyed by an excess of ferric chloride. (This is known as Piria's test, also Städeler's.)

(2) If some tyrosin be dissolved in as small a quantity

as possible of boiling water, and treated with mercuric nitrate free from acid, a white precipitate forms after some time, which, on heating, becomes yellow. If, to the hot solution, potassic nitrate with a little nitric acid be added, the fluid becomes a fine red, and on cooling red-brown flocks separate (Hoffmann's modified test).

(3) A tyrosin solution boiled with Millon's reagents shows a red colouration, and after some time a red precipitate forms. Excess of reagent is to be avoided.

(4) Scherer evaporates some tyrosin with nitric acid on

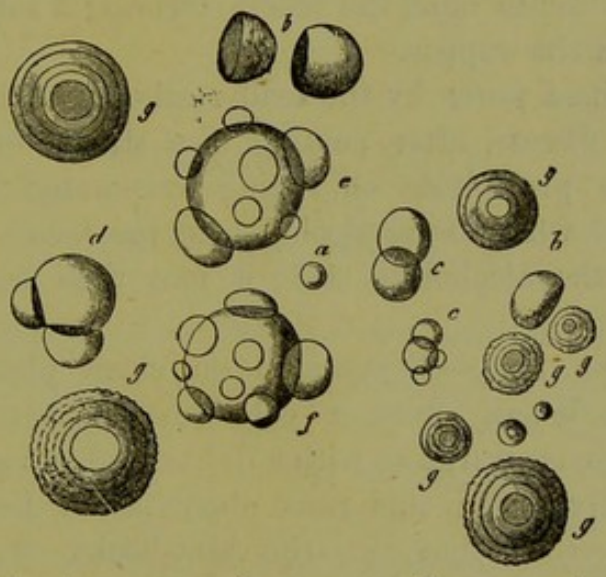


FIG. 47.—SPHEROIDAL CRYSTALLINE MASSES OF LEUCIN.
g, Laminated globules, in which form it is generally seen in urine.

a porcelain dish to dryness. A yellow spot remains, which becomes red-yellow with caustic soda.

Leucin may occur as a urinary sediment in the form of yellowish-brown balls (Fig. 47), or it may be necessary to evaporate a little of the urine and extract it with alcohol. The alcohol solution, on evaporation, leaves the leucin balls, which are best seen after twenty-four hours, and are often marked concentrically, or may have fine spines projecting from them. Sometimes Frerichs' lead method may be required for its separation, as described above. It is procured artificially by the same method as tyrosin, but mucus-

yielding tissues furnish no tyrosin. It may be obtained by digesting fibrin with a trypsin solution. In the pure state it forms white, glistening leaflets, which dissolve in 48·8 parts of cold, and easily in hot water, less in alcohol,* not in ether. In the presence of impurities it is more easily soluble, and only when these are present does it form the leucin balls. By cautious heating in a tube open at both ends, it sublimes without fusing into woolly masses, and a smell of amylamin is given off. Boiled with cupric hydrate, an aqueous solution, holding leucin in suspension, becomes an intense blue, the leucin forming a soluble combination with the copper.

It is obtained purer by the lead method described under tyrosin, the filtrate, after passing in a stream of H_2S and filtering the precipitate off, being evaporated down, the residue boiled with strong alcohol, and the leucin separated by allowing the alcohol to cool; it may then be tested as fallows:—

(1) *Scherer's Test.*—Evaporated on clean platinum foil slowly, pure leucin gives a colourless, almost invisible, residue, which, on warming with a drop of caustic soda, forms a kind of oily drop; this runs about, as in Leidenfrost's phenomenon (described in the text-books of Physics), without touching the surface of the platinum.

(2) Heated in a glass tube open at both ends, it behaves as described above.

Oxymandel Acid, $C_8H_8O_4$, is nearly related to tyrosin, and was observed in many cases of acute yellow atrophy of the liver by Schultzen and Riess.† It was found in the ether extract, got by agitating the urine with ether, after acidulating with sulphuric acid. It forms colourless glistening needles, which fuse at 162°, soluble with difficulty in cold, easily in hot water, in alcohol, and ether. By distillation with lime it furnishes phenol.

* Except when hot.

† Schultzen and Riess: "Chem. Centralbl.," 1869, S. 680.

PART III.

URINARY DEPOSITS AND CALCULI.

CHAPTER XV.

URINARY DEPOSITS.

URINARY deposits are divided into two classes, according to the text-books, namely, *unorganized* and *organized;* but these terms are apt to lead the beginner astray, as he associates them with the terms inorganic and organic. I propose to call them, here, *Chemical* and *Anatomical.*

Chemical, or Unorganized Deposits.—The chemical urinary deposits are either crystalline or amorphous. These deposits are substances which, owing to some change in the chemical composition of the urine, are no longer held in solution, but become precipitated out. To collect them a conical glass should be used, covered by a ground-glass plate, and when the deposit has settled down the supernatant urine should be poured off and the deposit taken up in a pipette or "medicine dropper," and then placed on a microscope slide and covered in the usual manner with a cover-glass. When we wish to apply a reagent, such as an acid or an alkali, a little bit of bibulous paper, such as blotting- or filtering-paper, should be applied so as to touch the fluid at one edge of the cover-glass, and a drop of the

reagent applied on a glass rod to the opposite side of the cover-glass; it then runs in easily.

Some of these sediments can only occur in *acid* urine,

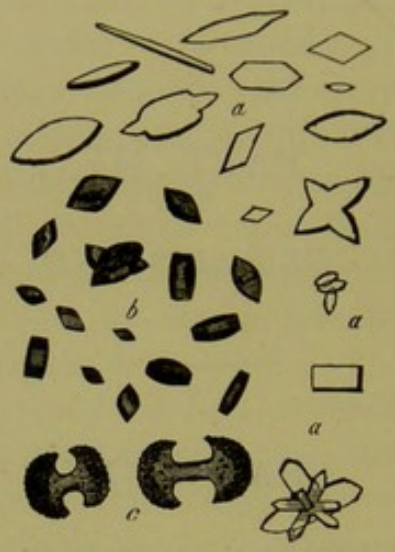


FIG. 48.—VARIOUS FORMS OF URIC ACID CRYSTALS.

either in the amorphous or crystalline condition. Others are only found in alkaline urine, and nearly all these latter occur in the crystalline condition.

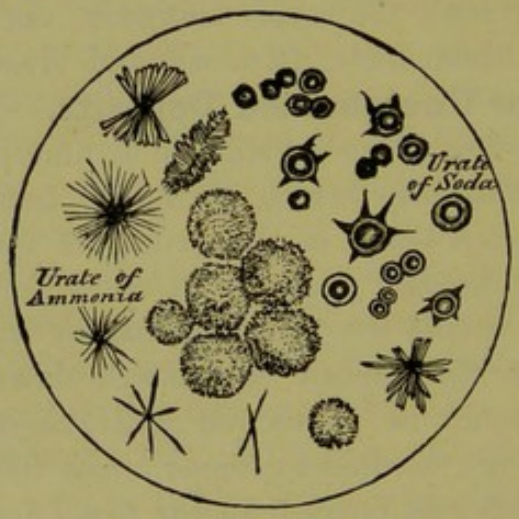


FIG. 49.—ACID URATES.

I.—In *acid* urine we may have a deposit which appears under the microscope—

A. *Amorphous* and *granular*. This may be caused by—

(1) *Urates* of sodium, potassium, and ammonia. This sediment (Fig. 49) seldom occurs colourless, being often tinted red by uroerythrin, or other colouring matter. It is found abundant in fever urine, and separates out of any human urine, not too dilute, by cooling to 0°. The sediment is recognized by heating it either on the slide, or, better, in a test-tube, when it dissolves and reappears on cooling. If the urine is albuminous it should be carefully heated, when the urates disappear, before the albumin comes down. To confirm this proof, a little hydrochloric acid should be run in under the cover-slip, when after some time crystals of uric acid are seen. (See Chapter IV.)

(2) If the sediment does not disappear on heating, but disappears with acetic acid without effervescing, it is probably *tribasic calcium phosphate*. But this is not likely to fall except when the urine is alkaline.

(3) Small bright refracting granules may be present, which dissolve on adding ether; if so, they are due to *fatty matters*.

B. Or the deposit in *acid* urine may be *crystalline*. There may then be present—

(1) *Uric acid*. This appears in the form of a sandy, generally coloured, reddish or yellow powder, like cayenne pepper, on the bottom or sides of the containing vessel, very often mixed with acid urates (Fig. 49). The presence of such a deposit generally indicates an abundance of uric acid. According to Voit and Hofmann* the separation of uric acid arises through a decomposition of the acid urate of sodium into free uric acid and free alkali, the latter diminishing the acid reaction. This sediment may be recognized by its crystalline form (see Fig. 48 and Fig. 17, p. 59) and by the murexide reaction (p. 66).

* Voit and Hofmann: "Zeits. f. analyt. Chem.," Bd. vii., S. 397.

(2) *Oxalate of calcium*, $C_2CaO_4 + 2H_2O$, occurs in octahedra or dumb-bells (Fig. 50); insoluble in acetic acid, but soluble in hydrochloric acid.

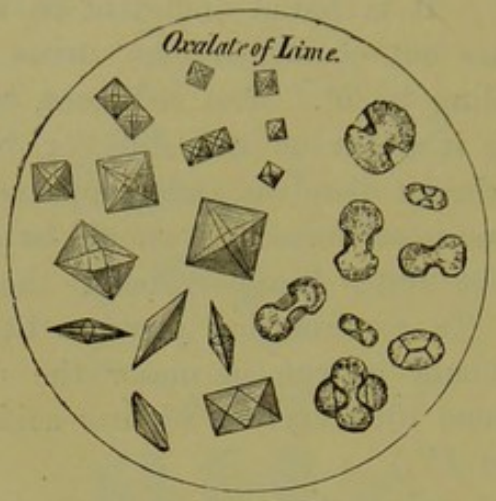


FIG. 50.—OXALATE OF LIME IN OCTAHEDRA AND DUMB-BELLS.

(3) *Cystin* is recognized by its crystals—six-sided plates (Fig. 51 and Fig. 45). It is insoluble in water, alcohol, ether, and dilute acetic acid; easily soluble in alkalies, also in ammonia, as well as in acids.

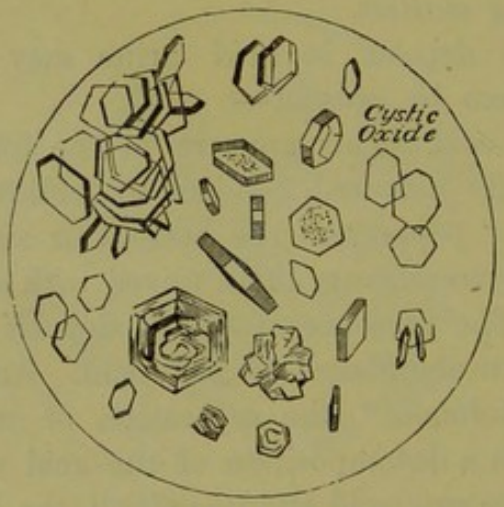


FIG. 51.—CRYSTALS OF CYSTIN.

If this precipitate be boiled with strong caustic potash solution, it undergoes decomposition. If a trace of nitro-prusside of sodium be added to the alkaline

solution the fluid becomes violet. If the caustic potash solution be treated before boiling with a drop of acetate of lead, the fluid becomes black on boiling by the formation of sulphide of lead. A drop of the cystin solution in caustic soda, warmed in a silver dish, gives a black spot of sulphide of silver. (See Cystin, Chap. XIV.)

(4) *Tyrosin* and *leucin* (Figs. 52 and 53) are recognized by their peculiar crystalline form. The reactions



Fig. 52.—Tyrosin Crystals.

of tyrosin, already described, may be tried. Frerichs and Städeler dissolved the sediment in ammonia, and obtained the tyrosin in a pure condition by evaporation.* (See Tyrosin and Leucin, Chap. XIV.)

* (5) Xanthin, when present in excess, is recognized by its lemon-shaped crystals, insoluble on heating, soluble in liquor potassæ, insoluble in acetic acid. Evaporated with nitric acid, and the residue touched with liquor potassæ, it gives a deep purple colour.

II.—In *alkaline* urine we may have—

A. An *amorphous* granular deposit:

Of *tribasic calcium phosphate*, $Ca_3(PO_4)_2$. This dissolves in acids without effervescing. It is found in neutral

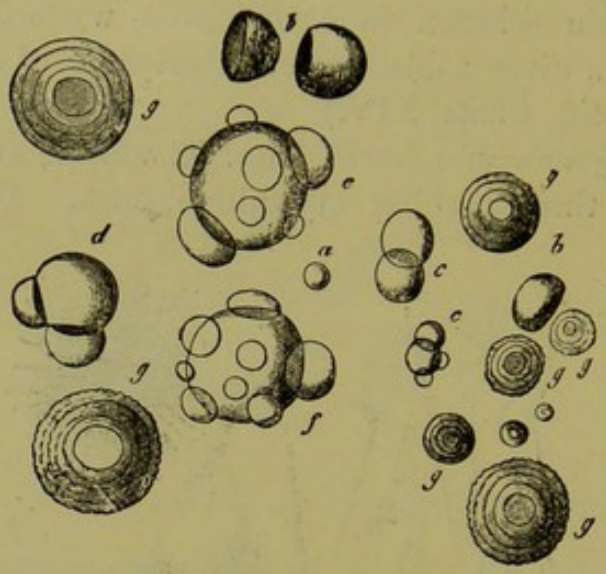


FIG. 53.—LEUCIN IN CRYSTALLINE MASSES.

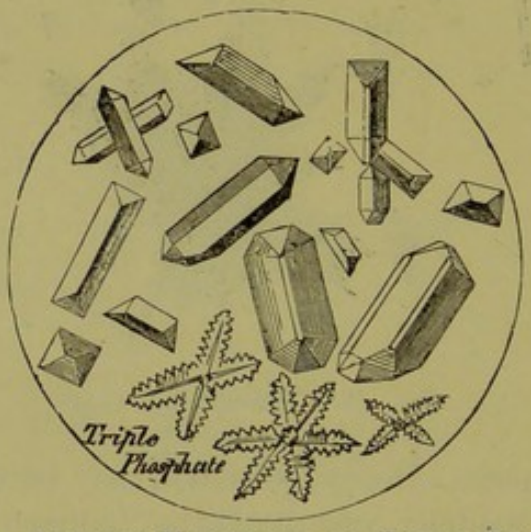


FIG. 54.—AMMONIO-MAGNESIUM OR TRIPLE PHOSPHATE.

or alkaline urine, mostly in the form of a greenish and reddish shining, iridescent pellicle on the surface of the urine, also in the form of granules in decomposing urine. Acetic acid dissolves this easily.

Or we may have—

B. A *crystalline* precipitate. This may be:

(1) *Ammonio-magnesium*, or *so-called triple phosphate*, $MgNH_4PO_4 + 6H_2O$. This is recognized by the peculiar "coffin-lid" forms of its crystals (Fig. 54). It is easily soluble in acetic or other acid. It may also occur in feathery and other crystals intermediate between these and the coffin-lid forms. (Figs. 33 and 34, pp. 120 and 121.)

(2) Sometimes *crystalline phosphate of calcium*, $CaHPO_4$ (Fig. 55), is met with, either alone or accompanying triple phosphate, in *rosettes*, or the crystals may be wedge-shaped or conical, or in spherules or *dumb-*

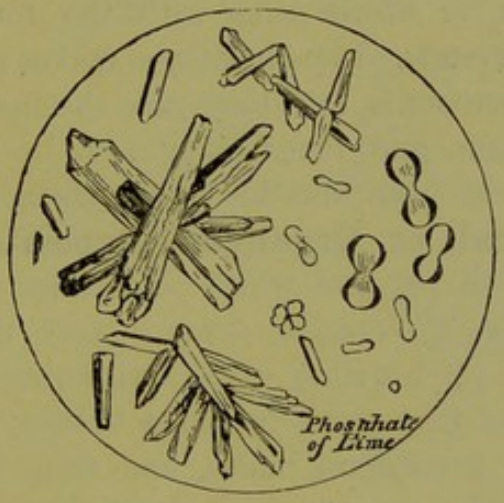


FIG. 55.—PHOSPHATE OF LIME.

bells. This sediment is insoluble in alkalies, or by heat, but is dissolved by acids, which distinguishes it from urates. Long plates of *tribasic magnesic phosphate*, $Mg_3(PO_4)_2 + 22H_2O$, may also be present. This, as Stein showed, is distinguished from triple phosphate by its behaviour with a solution of common ammonium carbonate (1 to 5). Triple phosphate remains unchanged with this solution, while the magnesic phosphate crystals become faint, and after some minutes eaten away at the edges. It is dissolved by acids.

(3) *Calcium carbonate*, $CaCO_3$, rarely occurs in human urine; when it does it appears as whitish balls, or biscuit-shaped bodies, or false dumb-bells. It dissolves in hydrochloric acid, and gives off carbonic acid with *effervescence*, and also with acetic acid.

(4) *Acid ammonium urate*, $C_5H_2(NH_4)_2N_4O_3$, occurs only in fermenting urine with alkaline reaction, in the shape of the "thorn-apple" spherules, or in irregular, club-shaped forms, almost always associated with triple phosphate. It may be that some of these are formed in part from urate of magnesia. These crystals are soluble in hot water, and dissolve in hydrochloric or acetic acid with the formation of uric acid crystals. Liquor potassæ makes these urates part with ammonia, and they give the murexide reaction.

(5) Tyrosin and leucin may also be present in the sediment from alkaline urine, though very rarely.

Anatomical, or Organized Deposits. — (1) Blood-corpuscles, both red and white, sometimes accompanied by fibrin, may be present in urine. Acid urine containing blood is smoky; alkaline urine containing blood is much redder. Blood may come from the kidney, ureters, bladder, prostate, and urethra in males, and from the uterus and vagina, as well as from the kidney, ureters, bladder, and urethra in females. In acid urine blood-corpuscles remain undissolved for a long time, but they rapidly dissolve in ammoniacal urine. In diluted urine they soon become biconvex instead of biconcave, then spherical, until finally the corpuscle loses its colour and disappears. In concentrated urine the corpuscles shrink and become crenated (Fig. 56, *b*) at the edges. The method of detecting dissolved blood in urine has been described before (Chapter X.).

(2) *Pus* and *mucus* may be present. The mucus-corpuscle is only a young epithelial cell, and measures from 0·008 to 0·10 of a millimeter ($\frac{1}{3000}$ to $\frac{1}{2500}$ of an inch). Both small mucus- and pus-corpuscles are indistinguishable from white

blood-corpuscles. A nucleus is generally visible, however, in a mucus-corpuscle; whereas the addition of acetic acid is necessary to make it appear in the case of a pus-corpuscle.

Urine containing pus, as in gonorrhœa, cystitis, pyelitis, &c., deposits a white opaque sediment, which generally sinks to the bottom of the vessel, if the reaction of the urine be acid, and no mucus present. On shaking this urine it becomes opalescent. If the urine be heated, albumin is deposited; whereas, if the opacity be due to urates, it will disappear on heating. Pus may be dis-

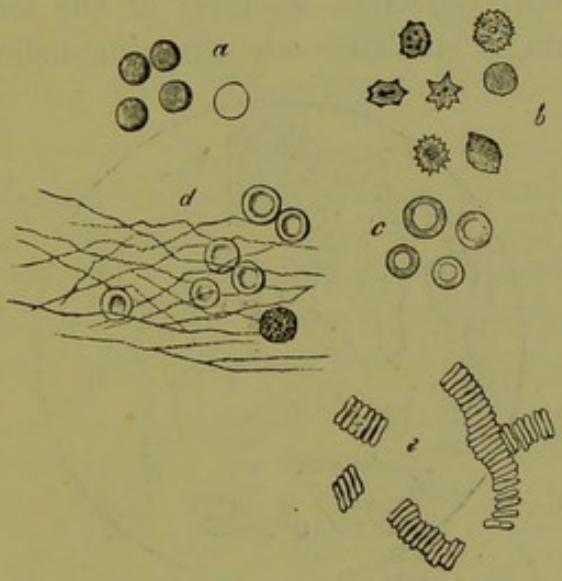


FIG. 56.—BLOOD-CORPUSCLES.
The lower part of the figure to be neglected.

tinguished from mucus by Donné's test. The supernatant urine is poured off, and some *liquor potassæ* added. The pus becomes changed into a sticky, gelatinous, ropy mass, resembling mucus, which adheres so firmly to the bottom of the test-tube that it may be inverted without allowing this mass to escape, and when it does escape it comes out, like white of egg, in one piece. Mucus, on the other hand, becomes more fluid when thus treated and mixed with flocculi.* Pus may come from any part of the genito-urinary

* For the detection of mucus see Chapter IX.

tract, and the presence of epithelium, which differs in microscopic character according to its source, may help the diagnosis.

In disease of the prostate, especially after gonorrhœal inflammation of that gland, thread-like white bodies, which microscopically are made up of united pus-corpuscles, may appear in the urine.

(3) *Epithelium* from any, or all, parts of the genito-urinary passages may be present, but it is not always possible to trace it to its source. *Small round epithelial cells* come from the *tubuli uriniferi* of the kidney. Each cell has a single nucleus, which distinguishes it from a



Fig. 57.—Epithelium from Ureters, Kidney, and Urethra.

pus-corpuscle. *Columnar* cells are derived from the pelves of the kidneys, from the ureters and urethra; *spindle* and *conical* cells from the ureters and urethra (Fig. 57); *flat* or *pavement* epithelium cells from the bladder or vagina (Fig. 58). Epithelium cells are destroyed after some time in alkaline urine.

(4) *Spermatozoa* may occur in the sediment of the urine of healthy individuals. They require a $\frac{1}{5}$th objective for their recognition (Fig. 58). To detect them for medico-legal purposes, a drop of mucus from the vagina should be placed on a glass slide, a drop of water or salt solution added, then

a cover-glass applied, and the specimen examined with a $\frac{1}{5}$th or higher objective. If a seminal stain occurs on linen, this should be cut up into small bits and soaked in water

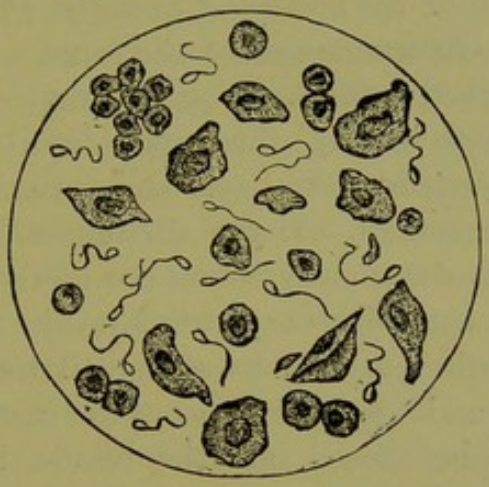


FIG. 58.—VAGINAL (PAVEMENT) EPITHELIUM AND SPERMATOZOA.

or artificial serum in a watch-glass for an hour, the bits of linen squeezed out, and the sediment examined.

(5) *Tube-casts* (Fig. 59) are moulds of the uriniferous

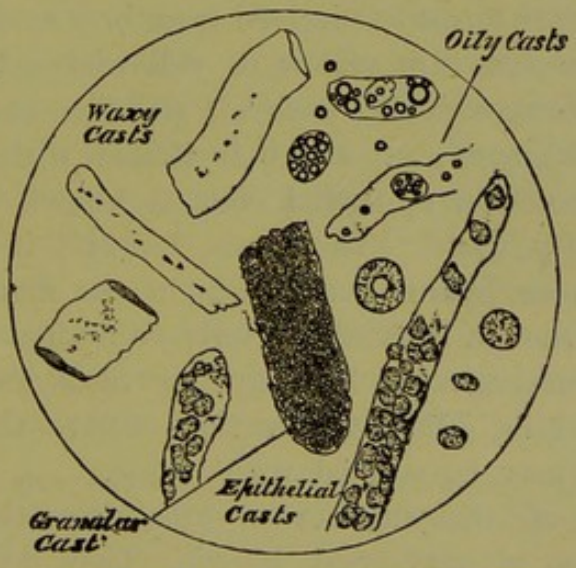


FIG. 59.—TUBE-CASTS FROM KIDNEY.

tubules, produced by the escape of a coagulable constituent of the blood into these tubules; this solidifies, and entangles whatever may have been contained within them. The

pressure of the water behind these plugs forces them on into the pelvis of the kidney, and they are excreted in the urine. Some maintain that tube-casts are produced as a result of the disintegration of the epithelial lining of the tubules, and others that they are a secretion of the same. If a tubule is filled with detached epithelium at the time the coagulable material is poured into it, then epithelium is carried out adhering to the surface of the casts, and appears in the urine as an *epithelial* tube-cast. If the albuminous mould formed by the exudation comes away before the epithelium becomes detached, then a *small hyaline* cast is formed; but if, through disease, the tubule has lost its epithelial lining, then the mould is larger, and appears as a *hyaline cast of large diameter*. If blood be effused into the tubule, the corpuscles adhere, and a *blood-cast is formed*, though casts containing only a few blood-corpuscles are also called *blood-casts*. If a cast contain granular *débris*, formed by the degeneration of the epithelial lining of the tubule in which it is moulded, it is known as a *granular cast*. When the material from which granular casts are formed is derived from broken-down blood-corpuscles, the casts are yellow or yellowish-red. Hyaline casts are sometimes dotted with oil globules, and are then known as *fatty casts;* or any cast loaded with oil drops, either free or within epithelial cells, is known as an "oil cast," or "fatty cast." The term "waxy" is sometimes applied to those hyaline casts which are more solid in appearance, although it should strictly be limited to *amyloid* casts. In detecting some faint hyaline casts, the light has to be reflected in different directions before they can be seen, or they may be stained with iodine.

Mucus-casts of the uriniferous tubes sometimes occur, which are often of very great length. They do not necessarily indicate disease, and generally arise from irritation of the kidney, extending from the ureters (Tyson). *Casts of the seminal tubes* are also sometimes found in urine; these, however, contain spermatozoa.

The *amyloid casts*, which occur in amyloid disease of the kidneys, are stained blue by sulphuric acid and iodine.

Leucocyte casts may occur in purulent diseases involving the renal tubules.

Tube-casts are rapidly dissolved in alkaline urine, while they remain much longer unchanged in acid urine.

To detect tube-casts,* the urine should be shaken up, and then placed in a tall vessel with a bottom sloping to a point (conical urine glass), and allowed to remain covered with a glass plate for twelve hours. The urine should then be carefully poured away from the sediment. Several drops of the sediment are then taken up by means of a pipette, and placed on a glass slide, covered with a cover-slip, and examined with the microscope. Fine hyaline, epithelial, and blood-casts are indicative of acute Bright's disease. Broad hyaline, waxy, granular, and fatty casts indicate that the disease is becoming chronic. In the later stages of Bright's disease only fatty and the larger hyaline casts are found.

(6) *Fungi and Entozoa.*—Normal urine is free from fungi of all kinds if passed into sterile flasks with antiseptic precautions, and does not then become alkaline as it does when exposed to the air (Woodhead and Hare). In pathological conditions *schizomycetous* fungi may pass from the blood into the urine: thus *micrococci* have been found in diphtheritic urine; and, as I stated above, a micrococcus is the cause of the change of urea into ammonium carbonate in cases of cystitis, after the use of dirty catheters, or in the alkaline fermentation which urine undergoes outside of the body.† *Sarcinæ*

* Acid sodium urate may assume the form of tube-casts, but in such cases the application of heat causes the urate to dissolve. These forms have no clinical significance.

† For the method of detecting bacteria in urine, any recent work, such as that of Crookshank on bacteriology, may be consulted. A few drops of urine may be evaporated on a glass slide, and the residue stained by Gram's or other method. Lately, bacteria have been found in the urine of many diseases, such as tuberculosis of the urinary passages (Rosenstein), typhoid fever, charbon, &c. (Berlioz), diphtheria, ulcerative endocarditis, and others.

have sometimes been found in urine and in the kidney, but more abundantly in the bladder. They occur in the characteristic wool-packs—cubical masses, constricted off into four, or eight, or more, in an optical section. These *sarcinæ* are smaller than those found in the stomach—the *Sarcinæ ventriculi*—having a diameter of from 0·001 to 0·01 m.m., and are found in acid as well as alkaline urine. Of the *saccharomycetes*, cells of the *Saccharomyces urinæ* have been found in the urine of diabetics. This is probably identical with the ordinary yeast fungus, *Saccharomyces cerevisiæ*. Some members of the *Phytomycetes*, or moulds, also may occur in stale urine, such as *Penicillium glaucum*, but their presence is of no clinical importance. Of *entozoa*, the hooklets of the *Echinococcus hominis* may be present in urine; and the ova of *Bilharzia hæmatobia*, which causes the "endemic hæmaturia" prevalent in some tropical countries. The embryos of *Filaria sanguinis hominis* may occur in chyluria (Chapter XIII.). Thread-worms may escape into the vagina and be passed in the urine of women; and other parasites accidentally introduced into the urine have been described.

Portions of cancerous and other tumours may be present in urine from growths in various parts of the urinary apparatus. The microscopic examination of the sediment may help the diagnosis in such cases.

CHAPTER XVI.

URINARY CALCULI.

SOLID masses, formed by the deposition of certain inorganic or organic constituents of urine, may occur of any size, from granules, like those of sand, to masses as large as the fist. According to their size these concretions are called sand, gravel, or stones. They may occur in the pelvis of the kidney, ureters, bladder, and sinus of the prostate gland. Probably all urinary calculi, except those deposited upon extraneous substances, or in the prostate, are formed at first in the kidney; they are all built upon a nucleus, which may be (1) either the sedimentary deposits which occur in *acid* urine, and they are then said to be "primarily" formed; or (2) they may be formed from the sediments of *alkaline* urine, or the nucleus may be some *foreign body;* they are then said to be "secondarily" formed. Those primarily formed are at first built up in the kidney, those secondarily in the bladder. The "*primary formation*" begins with the deposition of uric acid in sheaves, as a nucleus, upon which oxalate of lime is deposited in concentric layers. The "*secondary formation*" may occur in *neutral* urine by the deposition of carbonate, and crystalline phosphate of, calcium; or in *alkaline* urine from the deposition of acid urate of ammonium, ammonio-magnesium phosphate, and amorphous phosphate of calcium.

Although several varieties of calculus have been described,

yet, as a rule, only *three* kinds are likely to be met with, whether they be renal or vesical, namely:—

(1) Those formed from uric acid and its combinations.

(2) Those formed by the combination of phosphoric acid with volatile alkali and alkaline earths.

(3) Those formed from oxalate of lime.

"Among these uric acid and the urates form about three-fifths in number, a few of these having a slight admixture of phosphates; while nearly two-fifths are phosphates, either alone or in combination with some uric acid, in which latter case the term 'mixed' is applied; lastly, about 3 per cent. of the entire number are composed of oxalate of lime. It is necessary only to remember, further, that very rarely a

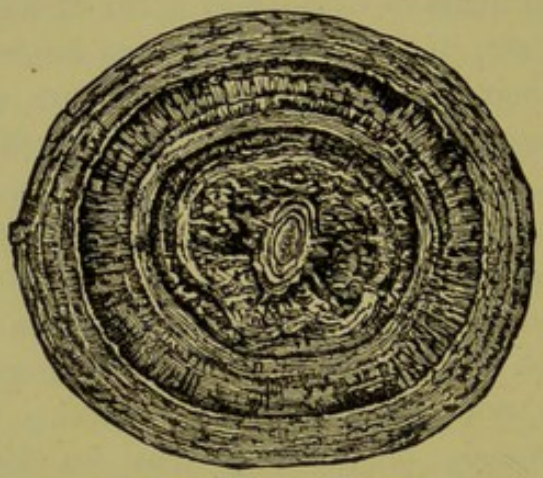


FIG. 60.—SECTION OF URIC ACID CALCULUS WITH NUCLEUS OF OXALATE OF LIME (*Bryant*).

calculus may be formed of pure phosphate of lime or of cystin." Sir Henry Thompson, from whom this extract is taken, adds, "It has fallen to my lot to operate on one case of each (of the latter two) in my life." *

These three kinds of calculi, then, (1) that composed of uric acid and its compounds, (2) oxalate of lime, and (3) the mixed phosphates, are the most common, while calculi of xanthin and cystin are rarely met with.

* Sir Henry Thompson: "Clinical Lectures on Diseases of the Urinary Organs," 6th ed., 1882, p. 67.

(1) *Uric acid calculi* (Fig. 60) are coloured generally red or some shade of red, and are usually smooth on their surface, or they may be tuberculated. On ignition they leave only a trace of residue.

(2) *Oxalate of lime calculi* are often of a dark-brown or gray colour, and are generally of a mulberry shape (Fig. 61), from which they are called "*mulberry calculi*";



FIG. 61.—OXALATE OF LIME, OR MULBERRY CALCULUS, AND SECTION (*Bryant*).

sometimes they are smooth, and are then known as hemp-seed calculi. After ignition they leave a considerable amount of residue, which effervesces with acids. The powdered calculus dissolves, however, in mineral acids without effervescing.



FIG. 62.—CALCULUS COMPOSED OF TRIPLE PHOSPHATE IN SECTION, THE "NUCLEUS" BEING FORMED OF A PIECE OF CATHETER (*Bryant*).

(3) *Phosphatic calculi* may be composed of phosphate of lime, of ammonio-magnesium phosphate (Fig. 62), or of phosphate of lime with magnesia, which last is the commonest of all the phosphatic calculi, and is known as the *fusible* calculus. It is sometimes so soft as to resemble moist chalk.

(4) The other varieties are the *carbonate of lime* calculus,

which, according to Thudichum, is generally that found in the prostate. This dissolves with effervescence in hydrochloric acid.

(5) The *xanthic oxide* calculus, which is very rare.

(6) The *fibrinous calculus* of Marcet and Prout, probably composed of inspissated albuminous matter exuded from an irritated kidney. It has a glassy appearance on fracture.

(7) The *cystin calculi* are mostly small, of a yellow colour, changing to greenish on keeping, and with a smooth surface (Fig. 63).

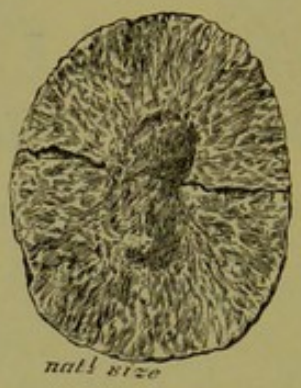


Fig. 63.—Section of Cystin Calculus (*Bryant*).

(8) The *uro-stealith calculus* is probably composed of some fatty material. It has been described in Heller's "Archiv," 1844—5, and by Moore in the "Dublin Quart. Journ." for 1854.

(9) *Blood calculi* only occur in cases of renal hæmaturia, and have been described by Marcet and Sir W. Roberts.

Large calculi are generally not composed of the same materials throughout, the constituents being arranged in concentric layers. A nucleus is always present upon which the other constituents have been deposited. This is most frequently composed of oxalate of lime, or it may be uric acid, but is hardly ever, if ever, phosphatic. Sometimes small blood-clots or other organic matter may form a nucleus, and this gives off the smell of burnt horn on ignition. Bits of pencil or glass, or other foreign body, especially in females, may form nuclei.

The following method of analyzing calculi is taken from Salkowski and Leube. It must be remembered, however, that since different layers may be due to different substances, a little should be scraped off from each layer and examined separately.

Analysis of Urinary Calculi—The calculus is powdered, or a portion of each layer, or any layer scraped off is powdered, and burnt on a piece of platinum foil over a Bunsen flame or spirit lamp.

A. If it burns away *completely*, or only leaves a *little ash*, the powder consists generally either of *uric acid*, or *urate of ammonium*, or *cystin*, or *xanthin*, or of the other substances mentioned below. If it does not burn completely, there may be present *uric acid* and *uric acid salts*, *phosphate of lime* and *phosphate of magnesium*, or *ammonio-magnesium phosphate*, or *oxalate of lime*. The further method of analysis is based on this difference.

B. The powder becomes *black*, but does not burn away. A slight blackening indicates the presence of a small amount of organic urinary components.

The further analysis is then carried out as follows:—

A. *The powder burns completely.*

If this be so, some more powder is taken and digested with *dilute hydrochloric acid*, and gently warmed.

(*a*) If it now *dissolves completely, or nearly completely*, the stone consists of either *cystin* or *xanthin*. To prove the presence of *cystin*, dissolve a portion of the powder in ammonia, and filter. This extract leaves cystin in crystals on evaporation, which may be further tested. To prove the presence of *xanthin*, dissolve some powder in a porcelain vessel in nitric acid, and try the xanthin reactions (see above).

(*b*) The powder is *not quite soluble in the dilute acid* and gentle heating. The solution is then filtered, and the residue on the filter washed.

(1) The *residue* = *uric* acid, gives the murexide and other reactions (see Uric Acid, p. 66).

(2) The *filtrate* may contain chloride of ammonium, xanthin, and cystin.*

To prove the presence of ammonia, some of the powder should be warmed with sodic carbonate; when, if ammonia be present, it will be detected by its smell and its action on litmus paper, and it gives, when a glass rod wet with hydrochloric acid is held over it, fumes of ammonium chloride. If ammonia be detected, then ammonium urate was present.

Albuminous substances develope the smell of burnt horn when burnt on platinum foil; so also do fibrin and coagulated blood. They are soluble in caustic potash solution, from which they are precipitated by acids.

Urostealith, when dry, is brittle and hard, brown or black. When warm it softens, and further heated it melts. It is soluble in ether, and the residue from this becomes violet on heating. It dissolves in warm caustic potash, forming a soap. As it melts, it gives off an aromatic smell.

An *indigo* calculus was found once by Ord. If such should occur again, it would, on heating, give off purple-red vapours, which sublime on a cool surface, forming a crystalline sublimate, and would dissolve in concentrated sulphuric acid with a blue colour, showing a characteristic band before D in the spectroscope (p. 97).

B. If the powder *blackens*, but does *not burn away*, and *leaves a residue*, then a portion of the powdered calculus is digested with dilute hydrochloric acid and warmed.

If it effervesces, *carbonic acid* is present (*carbonate of lime*).

(*a*) If it *dissolves completely*, uric acid is absent.

(*b*) If it *dissolves incompletely*, the residue may consist of uric acid or albuminous substances.

In *every* case, the solution in HCl is filtered, the residue on the filter tested for *uric* acid by the murexide reaction;

* To detect cystin here: neutralize the acid solution with carbonate of ammonium, whereby the cystin separates. To detect xanthin, add ammonia to the acid solution, filter after some time from the urate of ammonium, precipitate the filtrate with nitrate of silver solution, treat precipitate with H_2S, filter again, evaporate filtrate, and test the residue for xanthin.

the filtrate is then made feebly alkaline with ammonia, cooled, and acidulated with acetic acid. There is then obtained either a *clear* solution, or a gradually-forming *white*, pulverulent *clouding*. The yellowish-white flocks which are formed in the generally *clear* solution, consist of *ferric phosphate*. To confirm this, dissolve the filtered-off flocks in pure hydrochloric acid, and, on adding ferrocyanide of potassium, a blue colour becomes developed.

The *white insoluble precipitate is oxalate of calcium*. To prove this, the precipitate is filtered off, washed, dried, and the precipitate incinerated on platinum foil: the residue consists of carbonate of calcium, with which some caustic lime is mixed. It reacts, therefore, alkaline, and dissolves with effervescence in hydrochloric acid.

The solution from which the precipitate has been filtered off may contain *phosphoric acid*, *calcium*, *magnesium*, *potassium*, and *sodium*. To detect these—

Add to one portion some *uranium solution*, or solution of *ferric chloride*, to a slight amount: a yellowish-white precipitate indicates *phosphoric acid*.

Add to the greater part of the remaining solution *oxalate of ammonia*: a white precipitate indicates *lime*. Filter off this precipitate after warming the fluid gently. To one part of this filtrate add some drops of phosphate of sodium, made alkaline with ammonia: a crystalline precipitate, which often only forms gradually, indicates *magnesia*.

If necessary, though this is unimportant, we can detect *sodium* and *potassium* by the usual reagents. By evaporating down some of the fluid and dissolving in a little hydrochloric acid, then dipping a platinum loop in it, and bringing this into a Bunsen flame, we can detect *sodium* by means of the yellow line in the spectroscope. *Potassium* may be detected by giving a yellow precipitate in neutral solution with *platinic chloride*, and a violet line in the spectroscope. *Ammonia* may be detected by warming a part of the original hydrochloric acid solution with sodium carbonate.

The **quantitative analysis** of the constituents of urinary calculi may be carried out as follows:—

(1) The *water* is estimated by drying a weighed portion of the powdered calculus at 100° until its weight is constant.

(2) For estimating *uric acid*, the powder is digested with dilute hydrochloric acid for twenty-four hours, the uric acid collected on a weighed filter; then proceed as described under uric acid (p. 67).

(3) The *phosphoric acid* is estimated in half the hydrochloric acid solution, after making it alkaline, and then acidulating with acetic acid, and titrating with uranium solution as described under phosphates (pp. 128—130).

(4) *Lime* and *magnesia* are estimated in the other half of the hydrochloric acid solution, by removing the lime as oxalate in the acetic acid solution [see under B. (*b*)], and precipitating the filtrate with phosphate of sodium and ammonia (see p. 138).

(5) *Oxalic acid*, if present, separates in combination as oxalate of lime. It is converted into caustic lime, and then weighed. If the amount of calcium is to be estimated in a calculus, the weight of the caustic lime so obtained must of course be added.

(6) *Ammonia* may be estimated by Schlösing's or Sutton's method (p. 137).

The **qualitative** composition of calculi may be *roughly* arrived at as follows:—

If the powder ignited on platinum foil leaves a residue, as described under B above, to a part of the original powder the murexide reaction may be applied:—

(1) A purple colour is developed, showing at once that *uric acid* or *urates* are present; or

(2) No purple is developed, then note whether the calculus melts under the blow-pipe flame.

(*a*) If it does, then the calculus is composed of *ammonio-magnesian phosphate*, also probably of some calcium phosphate, and is the "*fusible* calculus."

(*b*) If not, treat some of the powdered calculus with acetic acid after incinerating; if it then effervesces, the calculus is composed of *oxalate of lime*. If the powder, before incineration, dissolves and effervesces with acetic acid, then the calculus is composed of *calcium carbonate*.

If it does not leave a residue when burned on platinum foil, then the calculus may consist solely of uric acid, or ammonium urate, or of any of the constituents described, under A, above—the presence of uric acid or urates being proved by the murexide test.

This rough method of analysis may serve to enable an opinion to be formed when a more accurate analysis cannot be carried out.

CHAPTER XVII.

BEHAVIOUR OF VARIOUS SUBSTANCES ON INTRODUCTION INTO THE ORGANISM AND DETECTION OF DRUGS AND POISONS IN URINE.

A GREAT number of **inorganic** substances, when introduced into the organism, pass over in greater or less amount into the urine, and leave the organism almost solely in this way. Iodide of potassium, bromide of potassium, and chloride of sodium appear unaltered in the urine. Nitric acid, chloric acid, and boric acid are soon recognized in the urine; so are also cæsium, rubidium, lithium, and thallium soon detectable in it, when these have been introduced by mouth or under the skin. Arsenic acid, arsenious acid, and antimony-combinations pass over only in slight amount into the urine, and even after a long mercurial course of treatment only traces of mercury can be recognized, except large quantities of urine are examined. The excretion of lead by means of the urine is much increased by the administration of iodide of potassium.* Silver, after the long use of silver salts, such as the nitrate, forms a combination soluble in potassium cyanide, which can be detected in the glomeruli of the kidneys as well as in the *rete Malpighii* of the skin. Heavy metals occur in the urine only in slight amount (Hoppe-Seyler).

The alkaline carbonates diminish the acid reaction of urine or make it alkaline; acids appear as neutral salts. Iodine appears as an iodate, sulphur as a sulphate.

* Annuschat: "Arch. f. exper. Pathol.," Bd. x. S. 261.

Of the **organic** substances *alcohol*, unless taken in large amount, is completely oxidized in the body, only traces being detectable in the urine. *Chloroform* is changed into uro-chloralic acid, while, as stated above, the chlorides are increased in amount. *Chloral* also appears as uro-chloralic acid, the urine having the reducing properties of sugar and of lævo-rotatory polarization. The *organic acids* are, as a rule, *oxidized;* their alkaline salts make the urine alkaline. Gallic, pyrogallic, hippuric, and picric acids are only slightly changed. Tannic acid appears in the urine as gallic acid, and benzoic is converted in the kidney into hippuric acid.

The amido acids go to form urea in part. Uric acid appears as allantoin and urea, or it may increase the amount of carbonic acid, oxalic acid, and urea.

Quinine, *strychnine*, and *morphia* are excreted for the most part unchanged in the urine, although morphia may be totally destroyed in the organism.

Aromatic Substances may undergo reduction or oxidation, or they may form new combinations. *Carbolic acid* appears as phenol sulphate, pyrocatechin, and hydrochinon; *benzol* as phenol and phenol-sulphuric acid; *indol* appears as indoxyl-sulphate of potassium; *thein*, *theobromin*, *alloxantin*, and *allantoin* appear as urea. *Xylol* is oxidized to toluylic acid. *Indigo-blue* is reduced in the intestines of dogs to indigo-white. A great number of aromatic substances combine with glycocine in passing through the organism. But anyone who desires further information on this subject may consult Salkowski and Leube's "Die Lehre vom Harn" (S. 268 to S. 274).

Detection of Drugs, &c., in Urine.—(1) *Iodide of Potassium.*—Add to the urine some drops of fuming nitric acid or chlorine water and a few cubic centimeters of chloroform or carbon bisulphide, and shake the mixture. Iodine is set free, and colours the chloroform or bisulphide violet.

(2) *Bromide of Potassium.*—Only large quantities can be detected by the method used for iodide of potassium,

and then the chloroform or bisulphide becomes yellow. If smaller quantities are present, it is better to form a combination of hydrobromic acid and silver. For which see Salkowski and Leube, S. 275, 276.

(3) *Lithia.*—Evaporate 100 c.c. of urine to dryness, and incinerate in a platinum crucible: add a little water and a couple of drops of hydrochloric acid; evaporate this solution. Extract the residue with strong alcohol; evaporate this. Dip a loop of platinum wire into it, or, better, its solution in pure hydrochloric acid, and bring before the slit of the spectroscope.

(4) *Arsenic.*—A very large quantity of urine is to be evaporated to one-eighth its volume, about the same amount of pure hydrochloric acid, free from arsenic, added, and the mixture warmed on the water bath; chlorate of potassium is gradually introduced up to a few grams, until the fluid is bright yellow, the free chlorine removed by evaporation, the fluid well diluted with water, and for several hours a stream of sulphuretted hydrogen allowed to pass through it. The precipitate of sulphide of arsenic is collected by filtering, washed, and dried; the filter is then put into a porcelain evaporating dish and a few drops of fuming nitric acid poured over it, then warmed on the water bath; concentrated sulphuric acid is added, and the fluid warmed until every trace of smell is gone. The fluid being then diluted with water can be tested by Marsh's or other test.

(5) *Lead, Silver,* and *Mercury.*—To detect *lead,* evaporate the urine down, destroy the organic matter by hydrochloric acid and potassic chlorate, dissolve in water, make the solution alkaline, and pass in sulphuretted hydrogen. The lead sulphide is then tested in the usual manner. Or it may be detected by the electrolytic method. To detect *silver,* evaporate the urine, destroy the organic matter by fusing the residue with nitrate of potassium and sodium hydrate; extract with water, and dissolve what is left over in nitric acid, filter the last solution, evaporate, dissolve

in water, and precipitate with hydrochloric acid. Chloride of silver is obtained, which is then tested in the usual manner. To detect *mercury*, evaporate down the urine; free from organic matter by means of chlorate of potassium and hydrochloric acid, and obtain the mercury by electrolysis. Convert the mercury into iodide by introducing the electrodes into a glass tube, which is drawn out into a capillary tube at one end, and sealed at the other. Heat the wide part of the tube, and the mercury sublimes and passes into the capillary tube. Open the bottom of the wide part of the tube and introduce a little solid iodine, and then seal it up again. The vapour of the iodine rises into the capillary tube, converting the sublimed mercury into iodide (Charles).

(6) *Chloroform.*—A stream of air is to be drawn through the slightly warmed urine by means of an aspirator, and then through a red-hot porcelain tube filled with fragments of porcelain, and through a Will-Varrentrap's apparatus or a set of Liebig's bulbs, filled with nitrate of silver solution acidulated with nitric acid. The chloroform is decomposed, and the free hydrochloric acid produces a clouding in the silver solution (Salkowski).

(7) *Iodoform.*—Distil the urine with steam until 50 c.c. have passed over, mix the distillate with a little solution of caustic potash, and agitate with ether in a separating funnel. Evaporate the ether to dryness, treat the residue with absolute alcohol, and test the alcohol solution by pouring it into a short test-tube, in the bottom of which is a little "alkali phenate"; cautiously heat the mixture over a small flame, and in a few seconds a red deposit appears, which is soluble in a few drops of dilute alcohol, with a crimson colour (Charles).

(8) *Salicylic Acid.*—If much be present in urine it may be detected by adding a solution of ferric chloride, which causes a blue-violet colour to appear. If in less quantity 30 c.c. of the urine are to be acidulated with sulphuric acid,

and when cold agitated with a like volume of ether in a separating funnel, the ether separated and treated with ferric chloride solution. The blue-violet colour is then apparent if even a very small amount of salicylic acid be present (Salkowski).

(9) *Carbolic Acid.*—Acidulate the urine with sulphuric acid and distil. The distillate gives a blue colour with ferric chloride; or if bromine water be added, a white crystalline precipitate of tribromo-phenol.

(10) *Chrysophanic Acid*—which may appear after its use as an ointment, and after the use of rhubarb and senna—colours the urine, after it has stood some time, a brownish-yellow colour, which, on adding sodic hydrate, becomes purple-red: this colour lasts for a long time.

(11) *Santonin* often colours the urine greenish-yellow, and this is reddened for only a short time by adding caustic soda or ammonia. This can be distinguished from the colour produced by chrysophanic acid by agitating the urine with amyl alcohol after the caustic soda has been added. The santonin colour goes into this, and gradually changes back to yellow; whereas the other—when produced by adding caustic soda to the chrysophanic coloured fluid—will not go into the amyl alcohol, or, if it does, only slightly, and the colour persists for a long time (see p. 23).

(12) *Rosanilin.*—After the use of hydrochlorate of rosanilin the urine is coloured red. To detect it, make the urine alkaline with ammonia, and shake with ether. Separate the ether into a dish, introduce some threads of white wool, and allow the ether to evaporate spontaneously. The wool becomes dyed red. Some wines are coloured by this salt, which may contain arsenic, and may produce poisonous symptoms.

(13) *Morphia and other Alkaloids.*—These are best obtained from urine by the method of Dragendorff and Wislicenus. The urine is evaporated on the water bath to a syrup, and the residue extracted several times with

absolute alcohol; the united alcohol extracts evaporated, the residue extracted with water to which a few drops of acetic acid have been added, and the solution then repeatedly shaken with amyl alcohol warmed to 70° C. in a separating funnel until the fluid is clear and colourless. The watery solution, by this treatment, parts with urea and so on, which go over into the amyl alcohol, but it retains the morphia. This watery solution is now made strongly alkaline with ammonia, and shaken twice or thrice with hot amyl alcohol, which, on evaporation, leaves morphia behind. To purify the residue, it is dissolved in dilute acid, and the treatment with amyl alcohol several times repeated.

To detect the morphia:—

(1) Dissolve in strong sulphuric acid, add a drop of water and a small fragment of red chromate of potassium. A mahogany colour is produced.

(2) Heat the sulphuric acid solution in an air bath at a temperature over 100° C. up to 150° C. for ten minutes allow to cool, and then add nitric acid. A fine dark-violet colouration is produced, which becomes gradually blood-red.

The same method will apply for the detection of the other alkaloids, such as *atropia* and *strychnine*. These should be tested by their action on frogs. *Atropia* produces dilatation of the pupils; *strychnine* produces convulsions and so on.

We must remember, however, that morphia, even when absorbed from the stomach, may not appear in the urine, or if it does, only in minute traces, as Landsberg has shown and Börntrager has confirmed.

Owing to the discovery of *animal* alkaloids in human urine, too much importance should not be attached to the physiological tests for the vegetable alkaloids.

APPENDIX I.

LIST OF THE MORE IMPORTANT REAGENTS REQUIRED IN URINARY ANALYSIS AND THEIR STRENGTH. LIST OF APPARATUS.

Reagents.

Acetic acid : Glacial, and dilute 1 to 7.
Alcohol : 90 per cent., and absolute.
Ammonia : "Liquor Ammoniæ," B.P.
Ammonium carbonate : 1 part of the common carbonate, 1 part liquor ammoniæ, 4 parts water.* Allow to stand some days before using.
Ammonium chloride : 1 to 8 of water.
Ammonium oxalate : 1 to 24 of water.
Ammonium sulphide : Common yellow (for detecting blood).
Barium nitrate : 1 to 12 of water.
Barium carbonate : Precipitated by ammonium carbonate from chloride of barium, and well washed.
Barium chloride : 1 to 10 of water.
Baryta water : 1 part caustic baryta, crystallized, dissolved in 15 parts water by warming, filtered when cold.
Bismuth subnitrate : In powder.
Bromine water : 1 part bromine, 30 parts water.
Calcium chloride : 1 part of crystals to 5 of water.
Cupric sulphate : 1 part to 12 of water, and 1 to 30.
Ether : Pure, free from acid.

* By water is meant distilled water ; and by parts, parts by weight.

Ferric chloride: 1 part "Liquor Ferri Perchloridi," B.P., to 10 of water.

Fehling's solution: See under Sugars, Chapter XI.

Ferrocyanide of potassium: 1 part to 12 of water.

Hydrochloric acid: Pure, strong.

Iodine solution: "Tinctura Iodi," B.P.

Lead acetate: *Neutral*, 1 part to 4 water.

Lead acetate: *Tribasic*, 1 part to 4 water.

Magnesia mixture: 1 part crystallized magnesium sulphate, 2 parts ammonium chloride, 4 parts liquor ammoniæ, and 8 parts water.

Magnesium sulphate: Cold saturated solution.

Mercuric chloride: 1 part to 16 parts of water.

Millon's reagent: Dissolve 1 part mercury in 2 parts nitric acid by means of a gentle heat, and add 2 parts water.

Nitric acid: Common fuming nitric acid and pure concentrated nitric acid.

Nitro-muriatic acid: 2 parts hydrochloric, 1 part nitric acid.

Phospho-molybdic acid: Common sodium phospho-molybdate 1 part, water 10 parts, strongly acidulated with hydrochloric acid,

Picric acid: See Chapters IX. and XI.

Platinic chloride: 1 part to 10 parts water. Must give no precipitate in alcohol.

Potassium chromate: Yellow chromate 1 part, water 20 parts.

Potassium hydrate: 1 part to 2 parts water.

Potassium nitrate: Pure crystals free from chlorides.

Potassium thiocyanate: 1 part to 20 parts water. (This is the Rhodan-lösung of the Germans.)

Rosolic acid: 1 part of the common acid in 100 parts alcohol.

Silver nitrate: Fused nitrate of silver 1 part, water 30 parts.

Sodium carbonate: Saturated solution.

Sodium hydrate: 1 part fused sticks to 2 parts water.

Sodium phosphate: (Na_2HPO_4) 1 part to 10 parts water.

Sulphuric acid: Pure concentrated, and dilute 1 to 5.

Tartaric acid: In powder, to be dissolved as required.

Water: Pure distilled.

Apparatus.

For ordinary qualitative analysis, the following are required :—

A dozen thin test-tubes of assorted sizes, and stand for the same.

Conical urine glasses, to collect sediments.

Red and blue litmus paper, and filtering paper, assorted sizes.

Funnels of assorted sizes, and stand for them.

Urinometer, and glass to hold it without touching.

Ground-glass covers to cover the urine glasses.

Spirit lamp.

Porcelain dishes for evaporation.

Beakers of glass, of different sizes.

Watch glasses.

A measuring glass to hold 500 c.c.

Glass stirring rods, thin.

Pipettes to take up sediments, &c.

A urine glass of over 2,000 c.c. capacity, to collect the urine of twenty-four hours.

A wash-bottle with distilled water.

Retort stand.

Water bath.

Iron tripods, and wire gauze.

Blow-pipe (for fusible calculus).

Test-tube cleaning brushes.

A microscope with 1 inch and $\frac{1}{5}$th inch objectives; also glass slides and cover-glasses, an erecting camera lucida, such as Zeiss's or Swift's; a stage and eye-piece micrometer, a drawing block, and inclined slope for use with the camera lucida; pencils, paints, and brushes.

It is also advisable to be provided with :—

A hot-water bath, with arrangement for filtering hot.

A hot-water oven.

A hot-air oven.

A chemical thermometer.

A gas-regulating apparatus for the hot-water and hot-air ovens.

Several porcelain crucibles.

A platinum crucible, platinum-tipped forceps to lift it, and platinum foil and wire.

Triangles to support the crucibles, made of wire, surrounded by bits of the stems of clay tobacco-pipes.

A distilling apparatus.

A exsiccator for drying precipitates over sulphuric acid.

A dialyzer.

Bunsen's burners with "rose" flame, and tube for blow-pipe.

Glass pestle and mortar.

Sulphuretted hydrogen apparatus.

Carbonic acid apparatus.

Glass flasks of different sizes.

A small direct-vision spectroscope.

If the student can afford it, a *one*-prism chemical spectroscope, of which the best is Hilger's "Student's Kensington Spectroscope," with a microscope mirror mounted on the scale-tube to illuminate the scale. An argand burner for the same. Spectroscope bottles and a stand to hold them. Logarithm-paper, supplied by Letts & Co., for reducing the measurements of the arbitrary scale to wave-lengths. Or a microspectroscope may be used, such as Browning's, provided with a photographed scale, and a slit whose jaws open equally.

For quantitative analysis are required, in addition:—

Mohr's burettes, and stand.

A set of pipettes to hold 5, 10, 15, 20, 30, and 50 c.c.

A dropping pipette holding 1 c.c., graduated in $\frac{1}{10}$ths and fractions of $\frac{1}{10}$th c.c.

A half-liter and a liter flask.

Volumetric solutions.

A chemical balance and weights.

A Gerrard's urea apparatus and forceps.

A water filter pump, with arrangement for drying *in vacuo*.

Weighing tubes and clamped watch-glasses.

Specific gravity bottle.

Separating funnels.

Platinum spatula.

APPENDIX II.

EXPLANATION OF SOME TERMS USED IN VOLUMETRIC ANALYSIS, AND TABLES FOR CONVERSION OF METRIC INTO ENGLISH WEIGHTS AND MEASURES, &c.

c.c. = cubic centimeter (1 gram distilled water at 16° C) = 0·2817232 fluid drachm.

1 liter = 1000 c.c. at 16° C. = 35·2154 fl. ounces.

Normal solutions are those which contain 1 gram atom of reagent (taken as monobasic), or an equivalent in some active constituent (*e.g.*, oxygen) in the liter. Thus, in the case of a *normal* solution of hydrochloric acid, HCl: (H = 1, Cl = 35·37), 36·37 grams of hydrochloric acid must be present in a liter. Or in the case of a normal solution of caustic soda, NaHO (Na = 23 and H = 1, O = 16,), 40 grams must be present in a liter.

Decinormal solutions are $\frac{1}{10}$th of that strength, which is thus expressed: $\frac{N}{10}$.

Centinormal, $\frac{1}{100}$th, or $\frac{N}{100}$.

Empirical standard solutions are those which contain no exact atomic proportion of reagent, but are constructed generally so that 1 c.c. = 0·01 gram (1 centigram) of the substance to be estimated.

A *titrated* solution means a solution whose strength or chemical power has been determined by experiment. The term titration is only used in a *quantitative* sense (Sutton.)

Conversion of Grams into Grains,* and Vice Versâ.

Grams to Grains.	Grains to Grams and Milligrams.
1 = 15·43235	1 = 0·6480 or 64·80
2 = 30·86470	2 = 0·12958 ,, 129·58
3 = 46·29705	3 = 0·19437 ,, 194·37
4 = 61·72940	4 = 0·25916 ,, 259·16
5 = 77·16175	5 = 0·32395 ,, 323·95
6 = 92·59410	6 = 0·38874 ,, 388·74
7 = 108·02645	7 = 0·45353 ,, 453·53
8 = 123·45880	8 = 0·51832 ,, 518·32
9 = 138·89115	9 = 0·58311 ,, 583·11

Comparison of Measures (English into Metric)

1 minim = 0·05916 c.c.
1 fluid drachm = 3·5495 c.c.
1 fluid ounce = 28·396 c.c.
1 pint = 567·92 c.c. = 0·56792 liter.
1 quart = 1·13584 liter.
1 cubic inch = 16·386 c.c.

Comparison of Weights (Metric into English).

1 milligram = 0·01543 grain (= nearly the $\frac{1}{65}$th).
1 centigram = 0·15432 grain.
1 decigram = 1·54323 grains.
1 kilogram = 35·27395 ounces (avoirdupois).
or 2·2046213 lbs. (avoirdupois).

To convert grams per liter into grains per gallon, multiply by 70.

To convert grains per gallon into grams per liter, multiply by 0·014286.

To convert grams per fluid drachm into grains per fluid ounce, multiply by 123·46.

1 millimeter = 0·03937 inch.

25·4 mm. = 1 inch.*

* From Johnson's "Analyst's Laboratory Companion"—a very useful little book.

To convert degrees of *Fahrenheit's* thermometer into those of the *Centigrade* scale, subtract 32, and multiply by $\frac{5}{9}$, or C. = (F.—32) $\frac{5}{9}$; and conversely, to convert Centigrade readings to the Fahrenheit scale, multiply by $\frac{9}{5}$ and add 32, or

$$F. = \frac{9}{5} C. + 32.$$

TENSIONS OF AQUEOUS VAPOUR FROM 10° TO 25°, FOR CORRECTIONS USED IN THE HYPOBROMITE PROCESS FOR UREA, IN M.M. OF Hg.

10° = 9·126	14° = 11·882	18° = 15·351	22° = 19·675
11° = 9·751	15° = 12·677	19° = 16·345	23° = 20·909
12° = 10·421	16° = 13·519	20° = 17·396	24° = 22·211
13° = 11·130	17° = 14·409	21° = 18·505	25° = 23·582

FIG. 64.—LUNGE'S NITROMETER.

The accompanying figure should have been inserted on p. 51. It represents a nitrometer devised by Lunge, which can be used for urea estimation. To the tube projecting sideways from the three-way stop-cock (in left-hand tube) an india-rubber tube connected with the bottle holding the hypobromite and urine is attached, as in Gerrard's apparatus described on p. 51.

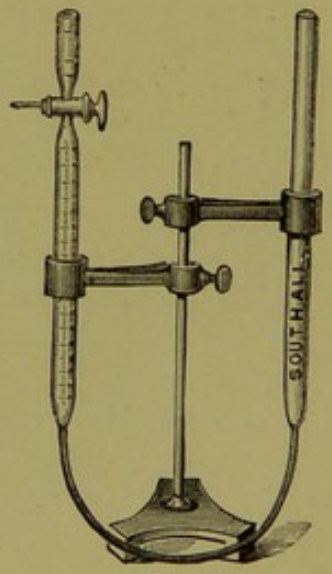


FIG. 64.

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