ON THE DEVELOPMENT

OF

TORULÆ IN THE URINE,

AND ON

THE RELATION OF THESE FUNGI TO ALBUMINOUS AND SACCHARINE URINE.

BY

ARTHUR HASSALL, M.D., LOND. M.R.C.P.

Received November 16th.—Read November 23d, 1852.

PART I.

BEFORE proceeding to offer any remarks of my own on the subject of the present communication, I propose to place before the Society the opinions and observations in relation to torulæ in urine, entertained and recorded by others up to this time.

"Diabetic urine," writes Dr. Griffith,[1] "if left in a warm place, becomes covered with a frothy white layer, as if its surface had been sprinkled with flour. This is quite characteristic, and when once seen cannot be mistaken. This white froth is composed of a number of minute vegetable organisations, which have been denominated *torulæ;* they occur in all fermenting liquids, and their growth is by some considered, in relation to fermentation, in the light of cause and effect. They are figured in pl. ii, fig. 35; their development is very interesting. When first formed they are very minute spherical globules, composed of two coats, and filled with a liquid containing in suspension a number

[1] Practical Manual, pp. 49, 50.

of extremly minute granules; the globules enlarge, rise to the surface, and form the white scum. Some of the internal granules also enlarge, and become distinct nuclei. These continue expanding, the primary globule becomes elongated, and one of the enlarged nuclei bursts through the envelopes of the maternal cell and appears as a bud; this enlarges, others increase in the same manner. As the globules enlarge they become elongated, finally forming long, slender, jointed vegetables, as in pl. ii, fig. 35. These contain several nuclei, which are ready to bud out in the same manner as their parents have originally done. They seem to increase in two distinct ways: one is the budding process above mentioned, the other is the division of the parent cell. It is first divided by the increase of two, three, or more nuclei into as many separate parts. It then becomes contracted opposite the spaces between the continuous extremities of the internal young cells, finally forming distinct and independent plants, capable of further propagation in a similar manner."

Under the head of Torulæ in Diabetic Urine, Dr. G. O. Rees states,[1] "This fungoid vegetable growth, which is delineated on the plate, fig. 15, is characteristic of the existence of fermentation, and its presence may be regarded as a very correct microscopic test of the presence of sugar."

Dr. Golding Bird[2] gives the following account of "Torulæ" in saccharine urine:—"It is well known that in all saccharine fluids undergoing the alcoholic fermentation, minute confervoid, or fungoid vegetations, called torulæ, appear, and pass through certain definite stages of development. There is, indeed, considerable reason to believe that these vegetations bear to fermentation the relation of cause and effect. The arguments lately advanced by Professor Liebig, in opposition to this opinion, do not, to my mind, afford a satisfactory answer to the observations previously made on this subject.

"When urine contains but very small portions of sugar,

[1] Analysis of the Blood and Urine, 2d edit., p. 217.

[2] Urinary Deposits, 2d edit., p. 289.

too little even to affect its specific gravity materially, or to cause it to assume a diabetic character, certain phenomena are developed connected with the production of the vegetation of the genus torulæ or saccharomyces, which will at once point out the presence of sugar. These indications are of very great value, as a saccharine condition of the urine is not uncommon in dyspepsia and some other affections, and is, of course, of the highest importance in directing our treatment.

"When saccharine urine is left in a warm place, a scum soon forms on its surface, as if a little flour had been dusted upon it. This consists of minute oval bodies which soon enlarge from the development of minute granules visible in their interior. These continue expanding, and dilate the oval vesicle containing them into a tubular form; soon afterwards the internal granules become larger and transparent, and project from the exterior of the parent vesicle-like buds. The whole then resembles a jointed fungoid or confervoid growth, which ultimately breaks up, and a copious deposit of oval vesicles or spores fall to the bottom. All these stages of development, fig. 46, require but a few hours for their completion. If the deposited spores be placed in weak syrup, they rapidly germinate, and exciting fermentation, produce a new crop of torulæ. During the growth of the torulæ, bubbles of carbonic acid gas are evolved, and the urine at length acquires a vinous odour, sometimes accompanied by an odour of butyric acid. There are two kinds of urine which may be mistaken for saccharine, by the occurrence of a kind of fermentation not unlike that of fluids really containing sugar. I refer to the form of viscous fermentation which occurs in urine, and ending in the appearance of much ropy mucus. This has occurred to me repeatedly in specimens of urine containing cystine, the odour evolved being, however, disagreeable and sulphurous, quite distinct from the vinous odour of the alcoholic fermentation. Somewhat similar phenomena are occasionally presented by the urine of persons exhausted in health from scrofulous or syphilitic cachexia."

"Torulæ," observes Dr. Bence Jones,[1] "are by no means diagnostic of saccharine urine; but though they form very soon and very plentifully in diabetic urine, yet they may be constantly found in urine which contains no trace of sugar; and though they may lead you to look for sugar, they must never lead you to assert that sugar is certainly present in the urine in which they occur."

In his 10th Lecture on Albuminous Urine,[2] Dr. Jones also makes the following remarks:—

"There is a peculiar microscopic appearance in acid albuminous fluids to which M. Andral has directed attention. In the 'Annales de Chemie,' vol. lxxxiii, p. 385, there is a paper on the development of the penicilium glaucum, under the influence of acidification, in the albuminous fluids of health and disease, by MM. Andral and Gavarret. Serum of the blood diluted with twice its volume of water, and acidified by dilute sulphuric acid, usually, in twelve hours, gave vesicles, which elongate rapidly, forming a long, branching, jointed vegetable, of which drawings are given in the different stages of its development; albumen and acid are necessary for its growth: if, therefore, this vegetation is met with in the urine, we may immediately conclude that albumen exists in solution, and heat and nitric acid will certainly confirm the truth of your opinion."

The above observations comprise nearly all the information contained in the writings of English authors in reference to torulæ in the urine.

It appears then, from these extracts, that Drs. Griffith, G. O. Rees, and G. Bird, on the one side, regard the development of fungi in urine as affording a very valuable and decided test of the presence of sugar, the latter observer even considering it to be so delicate as to be capable of detecting such small portions of sugar as are too little even to affect the specific gravity of the urine materially, or to cause it to assume a diabetic character.

On the other hand, Dr. Jones states, that torulæ are by

[1] On Animal Chemistry, p. 121. [2] Loc. cit., pp. 109-10.

no means diagnostic of saccharine urine, although they form very soon and very plentifully in diabetic urine. In making this statement, however, Dr. Jones does not adduce the reasons which have led him to adopt this view; the opinion, however, as we shall see hereafter, follows as a necessary consequence from the inquiries of MM. Andral and Gavarret, on the development of penicilium glaucum in acid albuminous fluids.

Dr. Jones likewise states that, "If this vegetation is met with in the urine, we may immediately conclude that albumen exists in solution, and that heat and nitric acid will certainly confirm the truth of your opinion."

There is therefore a very considerable discrepancy of opinion as to the value of the torulæ test as an indication of sugar in the urine.

I will now proceed to record the results of my own observations on the Development of Torulæ in Urine.

I set aside, in the first place, at different periods, a considerable number of samples of non-saccharine urine of all kinds; some of these were acid, others alkaline, a few contained albumen, but the majority were non-albuminous. The changes which ensued in the several samples were observed and registered from day to day. In a large proportion of the samples torulæ quickly became developed, while in others they did not appear at all; they presented many distinct appearances and conditions of development, all of which, after a time, however, were ascertained to belong to, and to be characteristic of, one and the same species of fungus, of which the following is a description. Three distinct stages in the development of this plant may be recognised, each of which we shall describe separately under the heads of sporules, thallus, and aërial fructification.

Sporules.—Of these two kinds exist.

The *first* make their appearance in urine at an early period, usually in the course of a few hours, the precise time is determined, however, by the nature of the urine and the temperature of the weather; they are first visible as innumerable minute vesicles or cells, of a perfectly globular

form, reflecting, when seen with an object-glass of one fourth of an inch focus, bright centres and dark outlines, and presenting a tolerably uniform size; when viewed, however, with a glass of one eighth of an inch focus, the shaded outlines nearly disappear, and the sporules are then observed to present considerable differences of size, from the $\frac{1}{6778}$th of an inch in diameter to the $\frac{1}{11222}$d of an inch; the larger all include a vesicular nucleus, sometimes placed in the centre of each sporule, at others it is eccentric; but the smaller sporules are not nucleated, and resemble in size and appearance the nuclei of the larger sporules; the cavities of all are occupied by a fluid containing granules. (Pl. 1, fig. 1.)

In a short space of time, generally in a few hours, the sporules multiply to such an extent as to form, first, distinct circular patches and afterwards a continuous scum on the surface of the urine, as contained in a bottle or glass. In this scum the sporules are not heaped up over each other, but form a delicate stratum, constituted of a single layer of sporules, which, while they evidently repel the water, yet adhere to each other. Sometimes the patches, although small, cease to grow; in other cases they extend, until they touch each other, and from being circular become angular from mutual compression, the several patches yet remaining distinct; very frequently, as soon as they touch each other, they run together and form a continuous stratum, as noticed above: this may remain, as indeed it often does, without undergoing further change, or it may become wrinkled, or thrown up in a waved manner; both these appearances arise from the extension of the single layer of sporules, which having covered the entire surface of the urine, and being unable to spread itself out further, becomes variously folded or plaited. In general, the patches, whether small or large, are thin, delicate, transparent, and film-like; but in some cases they appear dry and white, resembling flour; upon what this difference depends I am not clear, but I believe it is connected with the amount of phosphates present. It is in this latter state that the surface of the urine presents the powdery aspect considered by so many

observers as characteristic of saccharine urine. The smaller patches are usually, but not always, composed of the rounded sporules above described; sometimes the sporules have lost their spherical form, and this is almost constantly the case in the larger patches, and elongating slightly, become oval. (Pl. I, fig. 2.)

The elongation of the sporules is not constant, for sometimes the development of the fungus ceases with the formation of the globular sporules. This change of form occurs when the condition of the urine is such as to favour the growth of the fungus; under such condition the sporules quickly extend themselves, and become three or four times longer than broad, when they resemble short threads of nearly equal diameter (Pl. I, fig. 3), having rounded extremities; after a time, the sporules becoming still further elongated, pass into separate filaments, which consist of cells placed end to end, and all enclosed in a common transparent membrane; the threads are more or less curved, and increase in length sometimes by the extension of both extremities, but usually only of one, which is distinguished by its smaller diameter.

From this condition, which is frequently attained on the second or third day, the fungus passes into the higher state of *thallus*. (Pl. IV, fig. 1.)

The *second* kind of sporules, which for the sake of distinction I shall call *vesicles*, are many times larger than the ordinary sporules; their surface is frequently hirsute, like the pollen granules of the compositæ; they are globular, and from each proceed one, two, or three buds or shoots, which gradually extend into filaments, at first simple and afterwards branched, thus forming, as the sporules did in the previous case, the thallus.

Now while the ordinary sporules during growth are merged entirely into and lost in the filaments, the vesicles remain as prominent swellings or enlargements on the threads, not unfrequently increasing in size with the growth of the threads themselves. When the vesicles give origin to but a single filament, they are seen as terminal inflations; when to two

or more, they of course are situated in the midst of the ramifications which have emanated from them. (Pl. 1, fig. 4.)

The number of vesicles present varies greatly in different cases: in general, the ordinary sporules alone are met with; in others a few vesicles only occur mixed up with the common sporules; in others, the number of vesicles has been considerable; and again in a few rare cases, I have detected vesicles only in a state of germination.

Of the sporules and vesicles we shall shortly have to speak again.

Now in some urines the growth of the fungus goes no further than to produce the sporules and vesicles; at this point, and even at any stage, all development not unfrequently ceases. The cause of this singular circumstance will be explained hereafter.

We will now pass on to the description of the thallus.

Thallus.—The thallus, then, it appears, takes its origin either in the sporules or vesicles. The filaments or threads, at first simple, which proceed from these, afterwards become branched, and the myriads of threads developed interlace together. It is therefore a compound structure, made up of innumerable perfectly distinct plants, which are held together simply by the interlacement of the filaments.

Like the sporules, it forms a layer upon the surface of the urine often of considerable thickness, several days being usually required for its complete formation. The growth of the thallus takes place principally from the extremities of the filaments; these mostly lie the deepest in the fluid, and it is near the extremities also that the branchings are most numerous, and therefore best seen.

The filaments forming the thallus are comparable to the roots of higher plants, and they extend themselves for some distance through the fluid in which they are developed in search of the nourishment by which the fungus is sustained.

The cavities of the jointed and branched filaments, like those of the vesicles and sporules, are filled with granular and vesicular material.

Now the thallus is met with in urine in two states,—it either forms patches on the surface, or one continuous stratum, these states depending upon the number and distribution of the sporules which precede it. As is the case with the sporules, the development of the thallus may be arrested at any stage of its growth.

When this happens, it soon breaks up and dies; before the breaking-up of the threads occurs, however, I have frequently observed the granular and vesicular contents of the filaments to collect into little rounded or oval masses, which escaping through the common investing sheath of the threads, become so many sporules. When in a mass of thallus, some filaments are seen transparent and destitute of contents, while others contain little rounded or oval bodies; and when a large number of sporules are lying about intermixed with the threads, we know that this elimination of sporules has occurred.

Every perfect fungus developed in a fluid consists of two parts, an aquatic and an aërial. The thallus represents the aquatic portion, and the filaments of which it is formed readily imbibe the fluid in which they are immersed; on the other hand, the stems and sporules which form the aërial portion of the fungus, repel the water and manifest an affinity for the air.

The last stage, then, in the development of the fungus, is that of aërial fructification:

Aërial Fructification.—After the lapse of a still further time, a mouldiness appears on the surface of the already-formed thallus. This follows exactly the distribution of the thallus itself; if it be in patches, then the mouldiness will appear only in places on the surface of the urine; but if the thallus form a continuous stratum, the mould or mildew will do the same.

The mould or aërial fructification presents the following structural peculiarities:—

If the surface of the thallus be carefully examined sometime previous to the appearance of the mould or fructification, a number of short upright stems or threads will be observed.

Each vertical stem having attained a certain height, divides into one or two branches, each of which becomes subdivided into several other very short and slightly moniliform branches,—thus a tuft or head is formed; at the extremity of the several branches rows or strings of circular bodies appear; these, on the slightest movement, become detached from the head, and fall either on the thallus or into the water (Pl. IV, fig. 2); now these circular bodies are identical with the sporules first described, and each represents a separate plant. It is in these sporules that the glaucous green colour so characteristic of this fungus in its perfect state of development is located; the colour varies, however, greatly in different cases; sometimes the patches or stratum of the fungus possess scarcely a tinge of green, at others they are not in the least green, but of a fawn colour; lastly, in some cases in which the spore-bearing heads are not formed at all, the vertical threads, upon which when present they are supported, become considerably elongated, and then the patches resemble pieces of white wool.

Such is a short sketch of the development of this fungus in its different stages; it is to be observed, however, that the several conditions described do not always keep separate from each other; thus, frequently, the sporules and thallus coexist, and in some cases we find sporules, thallus, and fructification, all more or less mixed up together; in certain urines, successive generations even of sporules may be seen passing through the several phases of their development.

It has already been stated that the growth of this plant, from a cause to be mentioned presently, is frequently arrested at any one stage of its development; but this is not all, for soon afterwards it begins to decay, and finally disappears from the urine; the only trace of its presence remaining is a deposit of sporules, circular in form, but irregular in size, and situated at the bottom of the urine. After the plant has once attained its full development, however, many days must elapse before its total destruction and disappearance.

We will now conclude this description by a few remarks on the propagation of this fungus.

Its perpetuation appears to be provided for in several ways.

First.—By the sporules thrown or pinched off, as it were, from the reproductive tufts, and which therefore appear to be nothing more than definite and minute portions of the mother plant, each being endowed with independent vitality and capability of reproduction.

Second.—By the generation of secondary sporules within the cavities of the primary or first-formed sporules.

Third.—By the granular and vesicular matter which occupies the cavities of the filaments and cells forming the thallus; in these cavities circular bodies resembling somewhat sporules, but much smaller, may be seen, and it is probable that it is by their means that the species is perpetuated in those cases, of very frequent occurrence, in which the plant has been destroyed before attaining its full development.

Fourth.—By vesicles; these I regard as sporangia, analogous to the vesicular bodies, met with in the tribe of algæ, as parent-cells in fact, containing a number of germs.

The vesicles or sporangia are not confined to this one species, but are frequently to be met with in many other fungi, some belonging even to distinct genera.

Two other facts connected with the development of this fungus yet remain to be mentioned.

The first is, that it will develope itself with nearly similar facility, and in the same quantity, in urine passed directly into a new bottle and immediately corked, as in urine exposed to the air.

The second is, that sporules and even filaments may be detected in some urines almost immediately after they have been passed; from this it becomes probable that the development had commenced even in the bladder itself.

We have now to enter upon the consideration of the conditions necessary to the development of this fungus. The first step which I took with a view to determine what these conditions are, was to put aside in bottles, all corked,

a variety of urines, fixing the dates to each, and noting from time to time the changes which ensued; the results of this proceeding are given in the following table:—

1st SAMPLE:—*Aggravated Dyspepsia.* Urine very acid, non-albuminous, passed 24th August; on the 28th inst., a scum of circular sporules appeared; on the 2d of September, there was much of the fungus fully developed, with a few spherical sporules; on the 5th inst., the fungus was in the same state.

2d SAMPLE:—*Chlorosis,* with œdematous feet. Urine acid, passed on 17th of August; on the 20th inst., a scum of spherical sporules became visible; on the 2d and 8th of September, the sporules were in the same state.

3d SAMPLE:—*Dyspepsia.* Urine alkaline, non-albuminous, passed on the 22d of August; on the 24th inst., no sporules; on the 2d of September, no sporules.

4th SAMPLE:—*Disease of Liver,* Anasarca. Urine albuminous, alkaline, passed on the 19th of August; on the 23d inst., no trace of fungus; on the 2d of September, still no fungus.

5th SAMPLE:—*Dyspepsia.* Urine somewhat, but not strongly acid, non-albuminous, passed on the 17th of August; on the 20th inst., a few oval sporules; on the 2d of September, a scum of spherical sporules; on the 8th inst., sporules in same condition.

6th SAMPLE:—*Disease of Kidneys,* Anasarca. Urine albuminous, decidedly acid, passed on the 23d of August; on the 27th inst., a scum of elongated sporules and filaments; on the 2d of September, a thick and continuous stratum of the fungus fully developed.

7th SAMPLE:—*Disease of Kidneys.* Urine albuminous, feebly acid, passed on the 17th of August, examined on the same day, sporules with a few short filaments were visible; on the 20th inst., a few sporules, circular; on the 2d of September, sporules in the same state.

8th SAMPLE:—*Articular Rheumatism.* Urine somewhat acid, non-albuminous, passed on the 18th of August; on

the 20th inst., an incipient scum of spherical sporules; on the 2d of September, a very dense film of oval sporules; on the 8th inst., sporules in same condition.

9th SAMPLE:—*Chorea.* Urine passed on the 28th of August, not very acid, non-albuminous; on the 2d of September, a dense scum of spherical sporules; on the 14th inst., scum on surface of urine all gone, a few sporules irregular in size, at the bottom of the bottle.

10th SAMPLE:—*Phthisis.* Urine decidedly acid, slightly albuminous, passed on the 2d of September; on the 8th inst., spherical sporules abundant, with much fungus in a state of perfect fructification.

11th SAMPLE:—*Dyspepsia.* Urine neutral, non-albuminous, passed on the 2d of September; on the 8th inst., no sporules.

12th SAMPLE:—*Fever.* Urine feebly acid, non-albuminous, passed on the 19th of September; on the 20th inst., a few spherical sporules; on the 28th inst., the same; on the 2d of October, no increase in the number of sporules.

13th SAMPLE:—*Inflammation of Kidney.* Urine non-albuminous, acid, passed on the 21st of August; on the 26th inst., a scum of spherical sporules; on the 2d of Sept., sporules much elongated; on the 8th inst., fungus in same state.

14th SAMPLE:—*Disease of Kidneys,* Anasarca. Urine neutral; albuminous, passed on the 16th of August; on the 20th inst., no fungus; on the 2d of September, none.

15th SAMPLE:—*Typhus.* Urine alkaline, non-albuminous, passed on the 24th of August; on the 30th inst., no fungus; on the 2d of September, none.

16th SAMPLE:—*Fever.* Urine feebly acid, non-albuminous, passed on the 20th of August; on the 23d inst., powdery patches of circular sporules; on the 2d of Sept., a few sporules only.

17th SAMPLE:—*Aggravated Dyspepsia.* Urine somewhat acid, non-albuminous, passed on the 16th of August; on the 20th inst., a scum of spherical sporules; on the 2d of

September, a dense film of sporules; on the 8th inst., sporules in same state.

18th SAMPLE:—*Congestion of Liver.* Urine decidedly acid, non-albuminous, passed on the 21st of August; on the 26th inst., a scarcely perceptible scum of spherical sporules; on the 2d of September, sporules still circular; on the 8th inst., a patch or two of thallus.

19th SAMPLE:—*Disease of Brain.* Urine alkaline, non-albuminous, passed on the 21st of August; on the 24th inst., no sporules visible; on the 2d of September, none.

20th SAMPLE:—*Dyspepsia.* Urine alkaline, non-albuminous, passed on the 17th of August; on the 20th inst., no fungus; on the 2d of September, none.

21st SAMPLE:—*Fever.* Urine alkaline, non-albuminous, passed on the 28th of August; on the 30th inst., no fungus; on the 2d of September, none.

22d SAMPLE:—*Typhus.* Urine alkaline, non-albuminous, passed on the 19th of August; on the 23d inst., no fungus; on the 2d of September, none.

23d SAMPLE:—Urine acid, non-albuminous, passed on the 16th of August, at night; on the morning of the 18th inst., sporules appeared, some oval, others elongated; on the 20th inst., a dense scum of oval sporules and filaments; on the 2d of September, fungus in same state; on the 8th inst., still in the same condition.

24th SAMPLE:—*Phthisis.* Urine very acid, non-albuminous, passed on the 21st August; on the 2d of September, a scum of oval sporules; on the 8th inst., fungus fully developed.

25th SAMPLE:—Urine strongly acid, non-albuminous, passed on the 28th of August; on the 30th inst., an abundance of spherical sporules; on the 8th of September, a continuous scum of fully-developed fungus.

26th SAMPLE:—Urine acid, non-albuminous, passed on the 17th of August; on the 22d inst., spherical and oval sporules; on the 2d of September, a dense scum of circular sporules; on the 8th inst., sporules in the same state.

27th SAMPLE:—Urine acid, non-albuminous, passed on

the 19th of August; on the 22d inst., a thick scum of spherical sporules; on the 2d of September, sporules in the same state; on the 8th inst., sporules less numerous, many having sunk to the bottom.

28th SAMPLE :—*Typhus.* Urine feebly acid, non-albuminous, passed on the 18th of August; on the 20th inst., a few somewhat oval sporules; on the 2d of September, sporules in the same state.

29th SAMPLE :—Urine strongly acid, non-albuminous, passed on the 4th of September; on the 8th inst., circular sporules, with patches of fully-developed fungus.

30th SAMPLE :—Urine strongly acid, non-albuminous, passed on the 4th of September; on the 8th inst., sporules and filaments of fungus; on the 14th inst., fungus in perfect fructification.

31st SAMPLE :—*Phthisis.* Urine acid, non-albuminous, passed on the 30th of August; on the 8th of September, a scum of spherical sporules; on the 12th inst., no further development of the fungus.

32d SAMPLE :—Urine acid, non-albuminous, passed on the 30th of August; on the 8th of September, a scum of spherical sporules; on the 14th inst., sporules in the same state.

From an analysis of the above table it appears therefore—

1st. That the fungus was developed in twenty-four out of the thirty-two urines submitted to examination.

2d. That in thirteen samples the development did not proceed beyond the sporule-stage.

3d. That in two it was arrested when in the condition of thallus.

4th. That in the remaining nine urines, it attained its perfect state, viz., that of aerial fructification.

5th. That those urines in which the fungus made its appearance, were invariably more or less acid, the degree of development varying with the acidity.

6th. That those urines in which it failed to make its appearance, were either neutral or alkaline, or though acid when passed, very quickly became first neutral and then alkaline.

7th. That the fungus appeared alike in albuminous and non-albuminous urines, provided these were sufficiently acid.

The frequency of the presence of this fungus in the urine is thus clearly established. One of the conditions necessary to its development, as we have seen, is an acid state of the urine; the degree of acidity and the length of time during which the urine remains acid, regulating to a considerable extent the growth of the plant. When the acidity is great, and of some days' duration, other causes being favorable, the fungus is enabled to pass through all the stages of its development, and to reach the state of mould or perfect fructification; when, on the other hand, the acidity is but feeble, the growth proceeds but slowly, and ceases entirely at whatever stage it may happen to have attained on the passing of the urine from an acid to an alkaline condition, and which, as appears from the Table above given, very frequently occurs when the fungus is still in the first stage of its development, that of circular sporules.

As is also shown by the Table, for some days after the urine has ceased to be acid and has become alkaline, the fungus does not appear to undergo any material alteration, but at length it begins to decay, and finally disappears.

The condition of development of this fungus in any urine is therefore, to some extent, an indication of the degree of acidity once possessed by that urine; it must be remembered, however, that although this plant is never developed in alkaline urine, it is yet sometimes present in it, the urine having been in the first instance acid, and having become alkaline subsequently.

We have now to seek for other conditions necessary to the development of this fungus.

When it is remembered that fungi contain nitrogen in their composition, and when their constant association with dead or diseased organic matter is called to mind, the idea that the presence of the species under consideration in the urine is closely connected with the animal matter contained in that fluid will at once appear as highly probable.

Now, animal matter, and even albumen, as in mucus and epithelium, are constantly present in the urine in greater or less amount, the albumen being contained in the epithelial scales and mucous cells; and hence this fluid ordinarily supplies another of the conditions requisite for the growth of this fungus.

With the view to ascertain whether the notion just referred to was correct, I procured a number of urines. I divided each sample into two portions, one was carefully filtered so as to remove at least part of the animal matter, the other was allowed to remain just as it was passed; the whole of the samples were set aside in corked bottles, and examined from time to time. The results obtained by this proceeding were as follow:—

1st Sample:—Urine passed on the 13th of September; on the 20th inst. there was a very decided scum of sporules upon the surface of the unfiltered urine, but none upon the filtered; and on the 22d inst., still no appearance of fungus upon the surface of the latter.

2d Sample:—Urine passed on the 13th of September; on the 17th inst. a thick scum of sporules upon the unfiltered, but none upon the filtered urine; on the 22d inst. fungi on both, but the layer thickest on the unfiltered portion.

3d Sample:—Urine voided on the 9th of September; on the 14th inst. a scum upon the unfiltered, but none upon the filtered urine; and on the 17th inst. the scum was upon both, but much less thick upon the filtered portion.

4th Sample:—Urine voided on the 9th of September; on the 17th inst. no scum upon the filtered, but a slight one upon the unfiltered urine.

5th Sample:—Urine passed on the 10th of September; on the 17th inst. no scum on the filtered, but a very decided one upon the unfiltered.

6th Sample:—Urine voided on the 14th of September; on the 20th inst. a very decided scum upon the unfiltered, but none upon the filtered urine; on the 22d inst. still no scum upon the filtered portion.

It is thus evident that the removal of even a portion of the animal matter contained in urine, exercises a very marked influence over the development of the fungus, and there is no doubt that if it were more completely separated the results would be still more obvious.

The separation of the whole of the nitrogenised matters almost invariably present in urine, can seldom be effected by filtration; nor do I know of any unobjectionable means by which, without altering the chemical condition of the fluid, it may be removed; were it in any case completely abstracted, it is certain that no development of the fungus would take place.

The condition, then, of the development of the fungus is likewise, to some extent, an indication of the amount of animal matter, especially albumen, contained in the urine; if the fungus be in patches only, then it is certain that the quantity of nitrogenous matter is but small, but if the fungus form a continuous stratum over the whole surface, then it may be inferred that the amount is considerable, sufficient indeed to excite a *suspicion* of the presence of albumen.

The necessity for the presence of animal matter is shown by the fact, that when a little albumen is added to any slightly acidulated solution, the same fungus as that ordinarily met with in the urine makes its appearance in the course of a few hours; without such addition the solution might be kept for any length of time, and no development of the fungus would occur.

A second condition necessary to the growth of this fungus is therefore the presence of animal matter.

But there are still probably other conditions requisite. The abundant growth of this fungus in bottles nearly filled with urine and corked, would appear, at first sight, to show that atmospheric air was not necessary, and from this fact it certainly appears that a very free or large supply of air is not required.

That some portion of air is, however, indispensable, is shown by the following circumstances:—1st. If the bottle containing the urine be well corked, and filled within a

very short distance of the neck, the development of the fungus will be retarded, and sometimes altogether prevented. 2d. Occasionally it has happened to me to notice that, after the removal of the cork for a minute only and the admission of air, the plant, which had previously been in a stationary condition, has grown surprisingly. 3d. The fungus will not grow in an atmosphere of carbonic acid, an experiment which may be easily tried by means of a bell jar filled with urine, inverted, and into which a small quantity of carbonic acid has been passed.

A third condition, then, is the presence of a certain amount of atmospheric air, or, rather, of the oxygen of which the air is in part constituted.

We have next to inquire, is the fungus so frequently found in urine a new species, or is it identical with one already known and described? From a careful comparison of this plant, in the several stages of its growth, with the well known *Penicilium glaucum*, it becomes evident that the fungus common to the urine is that species. *Penicilium glaucum* is a very common fungus, and is that which imparts the mildewed appearance so frequently presented by a variety of decaying vegetable and animal substances.

It is now proper to mention, that some of the particulars above referred to in the account given of the conditions necessary to the development of penicilium glaucum, are not altogether new.

That an acid fluid and albumen were conditions essential to the growth of this fungus was first made known by Dutrochet,[1] who recognised the plant, however, only in its filamentous state. These conditions have subsequently been further elucidated by MM. Andral and Gavarret,[2] who also have given a much more complete account of the development of penicilium glaucum, than existed up to the

[1] Mémoire pour servir à l'Histoire Anatomique et Physiologique des Végéteaux et des Animaux, t. ii.

[2] Recherches sur le développement de Penicilium glaucum sous l'Influence de l'Acidification dans les liquides Albumineux Normaux et Pathologiques, 'Annales de Chimie,' t. lxxxiii, p. 385.

time of the publication of their memoir. These well-known observers were likewise the first to show that the presence of atmospheric air, or rather oxygen, was necessary to its growth; this they did by replacing the air over the surface of the fluid with carbonic acid; the development of the plant was entirely arrested for ten days, when the air being readmitted or oxygen supplied, the growth proceeded as before.

The fluids experimented upon by MM. Andral and Gavarret, were the serum of the blood, white of egg, the serosity from the peritonæum, from a hydrocele, and from blisters, also pus; the urine, the most important and interesting in a pathological point of view of all the animal fluids, being so entirely overlooked as not once to be alluded to even in the whole course of their investigations; lastly, one of the conditions laid down by these observers as essential to the development of penicilium glaucum, is really not so, since the fungus makes its appearance and grows in acid solutions containing animal matter which is not albuminous; as, for example, the aqueous humour of the eye diffused through water, a substance which is not coagulable by heat.

From a review, then, of the whole of the facts and observations above recorded, the following conclusions may be deduced:

1st.—That there is very frequently developed in urine a species of fungus known by the name of penicilium glaucum.

2d.—That this fungus ordinarily passes through three stages of development, any one of which is characteristic of the species; it exists first as sporules, these pass into thallus, and from this proceeds the perfect or aërial fructification.

3d.—That the conditions necessary for the development of this plant are, animal matter, especially but not exclusively albumen, an acid solution and oxygen, its growth being likewise much influenced by temperature.

4th.—That it may be developed at will in a variety of

other animal solutions besides the urine in which the above conditions are fulfilled, as in solution of white of egg, acidified with acetic, phosphoric, or any other acid.

5th.—That one of these conditions, viz. the presence of albumen, exists in almost all urines, whether neutral, alkaline, or acid.

6th.—That inasmuch as one of the requisite conditions is wanting in neutral and alkaline urines, the fungus never makes its appearance in these, no matter how much albumen they contain.

7th.—That the plant may, however, be developed at will, in even neutral and alkaline urines, simply by rendering such urines acid by means of phosphoric or any other acid.

8th.—That its presence may be regarded as, to some extent, an indication of the degree of acidity of the urine.

9th.—That it is not characteristic, as has been supposed, of the presence of an abnormal quantity of albumen in acid urines, since it is frequently developed in many urines which contain only a normal amount of epithelium and mucus, and in which also not a trace of albumen can be detected by means of heat and nitric acid.

10th.—That, nevertheless, it affords some indication of the amount of animal matter contained in acid urines; for where this is large, the fungus is usually developed in considerable quantity, and in all such cases it is proper that the urine should be tested for albumen.

11th.—That this fungus is no indication whatever of the presence of sugar in the urine, since the observations above recorded were all made upon non-saccharine urines, and since the fungus may be developed at will in solutions which it is certain do not contain a particle of sugar.

I have now to remark, in bringing the *first part* of this communication to a conclusion, that the observations detailed on the development of penicilium glaucum in the urine under different conditions, were made principally in the summer and autumn of the year 1849.

Part II.

I come in the next place to the consideration of the second division of the subject—viz. the development and growth of Torulæ in saccharine urine.

From the quotations given in the first part of this communication, it appears that, up to the present time, great difference of opinion prevails as to the value of the torula-test as an indication of sugar in the urine—some asserting that it affords positive evidence; others denying altogether that it is a reliable test. From the facts and observations already advanced, it is at least certain that torulæ in urine are not, in all cases, indicative of the presence of sugar. It has yet to be determined, however, whether the torulæ contained in saccharine urine are not characterised by such peculiarities as constitute a satisfactory test for sugar when present in that excretion.

For the determination of this point, a number of samples of diabetic urine were placed in distinct vessels, the changes which ensued being observed from day to day. Each sample was divided into two, and sometimes three, portions.

The first was placed in a glass, and freely exposed to the air; the second, in a bottle, air being admitted to a limited extent only through an aperture in the cork; and the third was enclosed in a tightly-corked bottle.

The following changes were observed by the eye alone to occur in that portion of the urine which was exposed to the air:—

In the course usually of two or three hours after being voided, the urine began to lose its transparency, and to present a milky appearance.

At the end of from 24 to 36 hours, cloudy, gelatinous-looking masses appeared suspended in the urine just beneath the surface, but extending some depth into the fluid. Although visible on the surface, the form and size of the masses were best seen from a side view. These masses, being exceedingly soft and delicate, quickly broke into

pieces on the least disturbance of the glass, and slowly subsided to the bottom; the same thing happened occasionally when the masses had attained considerable size—an inch or more in diameter—even when the urine remained undisturbed. Thus, after a few hours, the exact time varying according to temperature, there was an accumulation of these gelatinous-looking masses, not only on the surface of the urine, but also at the bottom of the glass, forming a cloudy sediment, the turbidity of the whole urine now having become very considerable.

Entangled in the masses, particularly those near the surface, were numerous bubbles of gas; these, separating from time to time, escaped into the air. Many bubbles were also thrown off from the masses at the bottom of the vessel, rising slowly to the surface; occasionally a number of globules became developed in these masses, which ascending, carried with them the masses in which they were included.

This elimination of gas was continued for some days, and was so great as clearly to indicate an active fermentation in the fluid. The gas generated was ascertained, by the following simple proceeding, to be carbonic acid gas:

Two or three ounces of the urine were placed in a wide-mouthed glass jar. In this a second vessel filled with lime-water was suspended, the mouth of the jar being well secured. The lime-water was soon observed to become turbid, and at the end of two days a considerable deposition of a white powder had taken place in the inner vessel. This precipitate effervesced on the addition of acetic acid, showing that the gas which had escaped from the urine and combined with the lime was the carbonic acid gas. A modification of this experiment was performed by means of a *Woolfe's* apparatus.

One of the flasks was partly filled with the urine; the other, with lime-water. A bent glass tube adapted connected the two, one end dipping into the lime-water. The gas, as it was evolved from the urine, passed through the tube, rendering the lime-water turbid, and producing a

precipitate, which effervesced as before on the addition of an acid.

For the next two or three days, reckoning from the end of the first 36 hours, the urine continued to present nearly the same character, except that the masses increased in size and number, became whiter, and acquired greater consistency; the globules of gas eliminated also becoming larger and more numerous.

At the end of about the fifth or sixth day the gelatinous masses had disappeared, some having subsided to the bottom, while others had gradually merged into and formed a continuous stratum of a fawn colour, having, to a certain extent, the consistence and characters of beer-yeast.

This stratum, from day to day, acquired increased firmness; so that, at the end, usually of seven or eight days,—sometimes earlier,—it might be removed as a distinct and coherent layer. By degrees its texture became altered, and it soon presented a woolly and filamentous appearance. Lastly, a crop of delicate transparent threads sprang up from the surface, bearing on their summits minute spherical heads of a black colour, barely visible with the naked eye.

In the course of a few days, the stratum, now of considerable thickness, gradually altered in colour—became brownish—and, after a further time, soft and brittle, ultimately breaking up and sinking to the bottom of the glass.

These changes, visible with the eye alone, are so marked and peculiar, that when once carefully noted, they cannot be mistaken. But there are still other more important changes and peculiarities corresponding with the several outward changes above described, and for the determination of which the microscope is necessary.

Examined with that instrument, the cloud-like masses were found to consist of the minute sporules of a fungus, imbedded in a mucoid base. These sporules were very irregular in size; some, when viewed with an object-glass of $\frac{1}{8}$th of an inch focus, being visible as mere black points, while the largest did not exceed the $\frac{1}{6750}$th of an inch in

diameter. These masses, composed of the minute sporules, constitute *the first sub-stage* of the development of the fungus. (Pl. II, figs. 1, 2.)

The soft, fawn-coloured scum is composed, for the most part, of circular sporules many times larger than the former. These, although usually separate, are occasionally feebly united in rows formed of two, three, or even more sporules; sometimes the sporules collect together in groups, the smaller surrounding the larger, or parent-cells. Intermixed with the sporules are also a few jointed and beaded threads.

These sporules, like the former, vary considerably in size, the smallest being scarcely the $\frac{1}{1900}$th of an inch, whilst the largest are as much as the $\frac{1}{628}$th of an inch in diameter, the medium size being the $\frac{1}{922}$d of an inch. (Pl. II, figs. 3, 4. Pl. III, fig. 1.)

Between these sporules and those first described, it will be observed a considerable difference of size exists; this, for a time I was at a loss to explain; the explanation is furnished, however, by a consideration of the manner in which the sporules are developed.

The sporules are multiplied by the constant escape, from the interior of the larger sporules, of other and smaller cells, these, on their escape, appear on the surface as buds, and are usually included in a pouch-like protrusion of the parent-cell wall, which with its contained nucleus, is finally thrown off, becoming a new and independent sporule.

This evolution of sporules at the early period of the development of the fungus, is so rapid and continuous as not to allow any of the sporules to attain a large size. Subsequently, however, as the quantity of sugar becomes diminished, the evolution is less rapid, and time is afforded for a large proportion of the sporules to acquire the size characteristic of the fungus in *the second sub-stage* of its development.

The more consistent stratum is made up of branched and jointed threads, intermixed with a few separate circular sporules. These threads are frequently beaded, the beaded

cells being sometimes placed in the course, but more frequently forming the termination of the threads. In the latter case, the beaded extremities are often raised above the surface of the urine, and project a short way into the atmosphere. (Pl. v, fig. 1. Pl. III, fig. 2.)

Not unfrequently single cells several times larger than the others are observed; these are placed in the course of the beaded portion of the threads; but sometimes they are seen lying loose; these cells appear to be of the nature of vesicles. (Pl. III, fig. 3.) This forms the second stage of the development of the fungus, that of thallus.

The stratum presenting a woolly structure is divisible into two parts; the one, rests upon and is immersed in the urine; the other, projecting into the air, may be termed aërial. The first consists principally of the thallus above described, while the second is made up of the slender, transparent, jointless, and occasionally branched stems which here bear the globular heads.

The state and appearance of the heads vary with the development. At first they present a smooth outline from being covered with a delicate membrane. (Pl. v, fig. 2.) This afterwards bursting and becoming retracted, a rounded mass of circular sporules of a brownish colour is disclosed to view. The sporules falling off, leave the dilated extremities of the threads or filaments exposed.

These changes constitute the third or perfect stage of development of the fungus, that of aërial fructification.

The rapidity with which the fungus is developed is dependent, to a great extent, on temperature; heat, as the warmth of summer, greatly accelerates, while cold retards the growth to an equal degree. So much is this the case, that it is doubtful whether the sugar fungus would be developed at all in mid-winter, and when the thermometer was below the freezing point.

The observations upon the development of the diabetic fungus, above recorded, were made during the summer months; the periods given are those which were found to correspond to the several stages of the growth of the fungus at

that season of the year. It must be remembered, however, that the development is influenced considerably by variations of temperature, even in summer.

Although the appearances above described were all noticed in the first sample of saccharine urine subjected to observation, yet a variety of other samples, which were afterwards submitted to similar investigation, furnished results in all essential respects identical.

Such is a brief description of the changes which ensued in samples of saccharine urine exposed to the atmosphere. We have, in the next place, to notice those changes which occurred in the two other portions of the first urine, to one of which air was admitted to a limited extent, and from the other entirely excluded.

The portion of urine partially excluded from contact with the air quickly became, like the first, whitish and opaque; the cloud-like masses appeared as before, broke up on the slightest motion, and subsiding, formed a copious sediment. Many globules of carbonic acid gas arose from all parts of the liquid, and after accumulating on the surface escaped into the atmosphere. The masses were, however, fewer and smaller than in the sample freely exposed to the air, and the globules of gas were much less numerous, and their evolution ceased at an earlier period.

Examined with the microscope, the masses were ascertained to consist, as in the first portion, of myriads of minute sporules.

With the formation of the masses, the development of the fungus ceased; the only ulterior changes being, that the masses gradually became whiter, and more consistent.

The sporules, no matter how long the urine was kept, never attained the large size which distinguishes them in a more advanced condition of development.

The urine contained in the closed vessel was turbid when introduced; this turbidity afterwards increased somewhat, and bubbles of carbonic acid gas became evolved here and there. At the end of a few hours, however, the weather being extremely warm, the bottle burst with a loud ex-

plosion, breaking into many pieces, which were scattered far and near; the liberated urine effervescing on its escape, as though it were so much ginger beer. The same occurred in a second sample; but in other trials, this result was obviated by employing a Woolfe's apparatus. One of the flasks was partly filled with lime water, which, by absorbing the gas as quickly as generated, removed the pressure, and so prevented the bursting.

The changes which took place could now be readily noted: the urine, slightly turbid at first, soon became more opaque, and some carbonic acid gas was evolved, yet its opacity was soon lost; the elimination of gas ceased, and ultimately it became perfectly transparent. The few minute sporules which were originally diffused throughout the liquid fell to the bottom, forming a slight sediment, and for whatever period the urine was kept, no gelatinous masses were developed in it, nor was any stratum of fungus formed.

From the great differences observed in the fungus in the several portions of urine, it is very evident that free exposure to the air is a condition indispensable to its perfect development; deprived of this, its growth is quickly arrested.

It is also very evident from the description and illustrations now given, that the fungus developed in saccharine urine is a species very different from that treated of, in the first part of this communication, viz., *Penicilium glaucum*.

Further, a comparison of the diabetic fungus with the yeast plant, shows that the two are identical; a point of very considerable interest. The figures which accompany this communication contrasted with those of the yeast plant, published in the 'Lancet,' vol. i, 1850, are in themselves sufficient to establish this identity.

Up to a very recent period, great uncertainty, and even mystery, hung over the development of the yeast plant; the efforts made by able observers, to trace it through all the phases of its development, having for the most part completely failed.

In the communication referred to,[1] I gave a description of the yeast plant; and traced it through several stages of development; I followed the transformation of the sporules into branched threads, or thallus; detected the beaded threads and the large sporangia-like cells; and at that time thought I had really traced it, step by step, to its final condition. I have since ascertained, however, that under favorable circumstances, perfect aërial fructification is produced, precisely similar to that described as constituting the last and perfect stage in the growth of the diabetic fungus.

Now the changes described, as occurring in the three portions of the same sample of diabetic urine placed under such opposite circumstances, were with slight differences repeated in a variety of other samples, some obtained from patients labouring under diabetes in different degrees. So there is no doubt, but that these changes, under similar conditions, are constant, and therefore they afford valuable and unmistakeable evidences of the presence of sugar in the urine.

It is not to be understood, that the whole of the changes described as occurring in diabetic urine, were fully appreciated from the observation of a single specimen, and that the first submitted to examination. It was necessary in order to arrive at all the results above recorded, to watch the changes which ensued in a variety of samples; but these changes having once been clearly ascertained, the whole of them were readily afterwards followed out in even single specimens.

I will now proceed to give the results, recorded from day to day, derived from the observation, as well as chemical and microscopical examination, of several samples of diabetic urine, in order that the precise and positive character of the facts upon which the description contained in the foregoing pages is founded, may be the more clearly comprehended.

[1] Bread and its Adulterations, 'Lancet,' April, 1850.

Results recorded from day to day, of the Examination of Samples of Diabetic Urine.

1st SAMPLE:—This urine was passed on the morning of the *7th* of *June*, 1852, but did not come into my possession until the 11th inst., it having been kept in a corked phial; it was very acid, had a specific gravity of 1037, and examined with the microscope there were detected in it numerous octohedra of oxalate of lime; it was divided into two portions.

1st portion in open vessel.—Examined on the 11th inst. There were observed near the surface of the urine a few cloud-like gelatinous masses composed of myriads of minute sporules imbedded in a mucoid base. Sporules of *Penicilium glaucum*, some round, but the majority of an oval form, were likewise noticed resting on the urine.

Examined on the 13th inst. There were seen on the surface with the naked eye a few small circular patches of Penicilium glaucum, composed of sporules, some round, but the greater number oval, while at the bottom of the glass was an abundance of sporules, both small and large, of the saccharine torulæ, as well as a few filaments of the same, some with bearded cells.

Examined on the 19th inst. The Penicilium was still in the same state, but a thick white woolly stratum of the diabetic fungus had become developed, forming a ring round the whole margin of the glass; this consisted principally of the thallus; that is, of the root-like portion of the plant, which is made up of branched and bearded threads; intermixed with the filaments were, however, numerous large sporules, and from the upper surface of the stratum a considerable number of straight filaments shot up.

Examined *June* 23*d*. The woolly stratum now extends nearly over the whole surface of the urine; and the vertical threads are seen by the eye alone to bear on their summits the minute spherical and black heads which are cha-

racteristic of the fungus in its perfect state. (See Pl. v, fig. 2.)

Examined *July* 3*d*. The globular heads have lost the smooth outline which they at first presented, and they now consist of masses of sporules of a rounded form and deep brown colour, supported on the extremities of the vertical filaments; in some cases, the sporules have fallen off, the dilated extremities of the filaments then coming into view. The stratum breaks up readily; and on replacing it in the glass from which it had been removed for a few minutes, it sank to the bottom.

Examined *July* 19*th*. Stratum risen again, and spread over the whole surface of the glass; patches of Penicilium glaucum in perfect fructification have appeared; the diabetic torula now seen is chiefly the results of a second development, which, like the first, has passed through all the stages, even the last, that of aërial fructification.

The urine is now pale, but thick and turbid, as though mixed with flour; and there is a copious deposit, consisting principally of the sporules of the diabetic fungus: it is alkaline, contains an abundance of triple phosphate, and the potash and copper tests furnish no results, showing that the sugar has at length disappeared.

2*d portion in closed vessel.*—Although passed on the 7*th* of *June*, this portion was not placed in the closed vessel until the 11th inst., the saccharine torula had therefore become developed to some extent previous to the exclusion of the atmosphere.

Examined on the 13*th* of *June*. Urine in the same condition, and containing the same structures as were detected in the portion exposed to the air at the same period, the only difference being that there was very much less of the saccharine torula.

Examined 19*th June*. No increase in the quantity of saccharine torula, and none present at the top of the urine, the pellicle of Penicilium on surface in the state of oval sporules.

Examined 3*d July*. Saccharine torula in the same state, no scum of Penicilium on surface.

Examined 18*th July*. Urine pale, perfectly clear, and possessing a strong acid reaction; still contains traces of sugar.

Examined 16*th September*. Urine bright, clear, and still very acid; the sugar has now disappeared entirely.

2d SAMPLE:—*Placed in partially closed vessel.* Passed 22*d May*, but did not come into my possession until some time afterwards, it having been kept in a corked phial. When examined with the microscope, there were detected in it at the bottom a few sporules, both large and small, of the saccharine fungus, hexagonal crystals of uric acid, and octohedra of oxalate of lime, (see Pl. III, fig. 3.) It was placed in a partially closed vessel on the 14*th June*.

Examined 19*th June*. There was an abundant gelatinous frothy scum on surface, consisting of the small sporules of the diabetic torula, and of numerous bubbles of carbonic acid: there was also a considerable deposit of the small sporules, intermixed with a few of the large ones at the bottom of the vessel.

Examined 3*d July*. Gelatinous and frothy scum nearly all subsided to the bottom, the sediment consisting, as before, of the minute sporules, with a few large ones; urine still acid.

Examined 18*th July*. The thick frothy gelatinous scum had reappeared on the surface, but on shaking the urine it again fell; there is now a very considerable deposit divisible into two layers, the lower of a fawn colour, consisting of the small and large sporules of the saccharine torula, and the upper of the small sporules only: the smell of the urine is sour and acetous, but the reaction slightly alkaline; sugar gone entirely.

Examined 8*th August*. The crystals of uric acid have disappeared, and their place is supplied by numerous globules of some urate: small sporules of the diabetic fungus may still be detected. It will be observed that neither in this nor the previous specimen did the saccharine torula attain its full development.

3d SAMPLE:—*In open vessel.* This urine did not reach

me until some time after it had been passed; it was when received, however, of highly specific gravity, acid, and contained a considerable quantity of sugar. Exposed to the air for some days, the surface became covered with the thick woolly stratum, which, on examination, was found to consist of the diabetic torula in its perfect condition.

4th SAMPLE:—*1st portion in open vessel.* Urine passed *23d July*, 1852; specific gravity 1033, acid.

Examined *July 24th.* Copious gelatinous-looking flocculi, with many bubbles of carbonic acid imbedded in and surrounding them, have appeared near the surface of the urine; these consist of vast numbers of minute circular and oval sporules, immersed in a mucus-like base. The urine has a milky or floury appearance, which is occasioned by the great numbers of sporules diffused throughout.

Examined *25th July.* Flocculi increased in size, and many have fallen to the bottom, bubbles of carbonic acid gas are seen rising from all parts of the urine to the surface.

Examined on the *27th July.* Nearly in the same state.

Examined *30th July.* Surface covered, particularly at the edges, with a thin plicated scum of Penicilium, which consists of oval sporules, some extending into short threads. No large sporules of the saccharine torula have as yet appeared; the urine is still very acid, and has a specific gravity of 1024.

Examined *8th August.* The plicated thin scum, consisting of the sporules and threads of Penicilium, is still seen at the sides; and in the centre a large mass raised above the surface, and also extending much beneath it, having the consistence and colour of yeast: this mass consists principally of the large sporules of the saccharine torula, and which are not distinguishable under the microscope from those which form the yeast plant; the urine is thick, as though flour were diffused through it, very acid, and still contains sugar, but a small quantity, judging from the action of the potash test.

Examined *8th August.* The perfect fructification of the

Penicilium has now become developed, forming a green circle round the yeast mass. The further changes which ensued in this sample were not followed.

2d portion in partially closed vessel.—Examined on the *24th July*. This urine is in the same condition as the specimen in the open vessel at the same date.

Examined *25th July*. Same state as the previous specimen.

Examined *8th August*. A thin pellicle of Penicilium on surface, consisting of sporules intermixed with a few short threads; gelatinous masses both on the surface and at the bottom of the vessel, a very few diabetic sporules of large size being detected in the latter situation: specific gravity 1022, very acid, and contains more sugar than the urine exposed to the air.

Examined *17th September*. The masses near the surface have become whiter and more consistent, and there is a very considerable deposit of the same. With the microscope the masses were found to consist of the sporules of the saccharine fungus, both large and small, but chiefly the latter, mixed with a few broken filaments: resting on the flocculi near the surface were many fine crystals of oxalic acid. This urine has a smell like that of sour milk, is very acid, and still contains a little sugar.

3d portion placed in closed vessel.—Urine a little thick and white, as though mixed with flour; bubbles of gas rising from all parts to the surface, showing that it is on the work: bottle burst the same day with a loud explosion, the fragments being scattered here and there, and the urine effervescing like so much ginger beer.

5th SAMPLE:—*1st portion placed in open vessel.* Passed *2d August*: the urine became milky almost as soon as voided, from suspended sporules of the saccharine torula; it also quickly threw up a large quantity of carbonic acid gas.

Examined *8th August*. Scum of torula on surface composed, in part, of the oval sporules and branched threads or thallus of Penicilium glaucum, and in part of the small sporules of the saccharine fungus: at the bottom of the

vessel there was a considerable deposit formed by the small diabetic sporules only. At this date the urine was very acid, and still contained sugar, although it only had a specific gravity of 1006: the density of the urine when first passed was not ascertained; it was most probably of low specific gravity, however.

Examined 14*th August*. Diabetic torula in nearly the same condition; sugar all disappeared. The fungus in this instance did not pass through all the stages of its development, in consequence of the early and rapid transformation and disappearance of the sugar.

2*d portion in partially closed vessel*.—Examined *August* 8*th*. Very acid, specific gravity 1004: thin scum of torula on surface formed of the sporules of the saccharine fungus and of Penicilium glaucum intermixed; a considerable deposit of the same.

Examined 17*th August*. Saccharine fungus in the same state; urine smells very sour, and is strongly acid.

Examined 17*th September*. A thin brownish scum of torula on surface, composed chiefly of the small sporules of the saccharine fungus: urine gelatinous-looking, very acid, and of a sour smell; does not now contain sugar.

3*d portion in closed vessel*.—The urine was placed in the closed vessel the day on which it was voided; the next morning it was milky, and many bubbles of gas had collected on the surface: in the course of the day the vessel burst with a loud noise, the urine effervescing briskly from the large quantity of carbonic acid gas set free.

6th Sample:—1*st portion placed in open vessel*. Urine passed 7*th August*, of specific gravity 1028, became somewhat milky shortly after being voided.

Examined 8*th August*. Several gelatinous-looking masses had formed on the surface, where also large numbers of bubbles of carbonic acid gas had collected; the subsequent changes were the same as in the other samples freely exposed to the air; the fungus continued to grow until it reached its complete development.

The changes which occurred in the other two portions of

the same urine, the one partially exposed to the air, the other excluded from it, were so nearly similar, that it is unnecessary to describe them. Sufficient details have now been given to show the precise character of the alterations which ensue in specimens of saccharine urine placed under different conditions.

But it may be said that there are already tests sufficient of the presence of sugar in the urine; and, therefore, although the torula-test is very satisfactory, yet that it is not needed. To this objection I next reply—

There is no doubt but that in cases of confirmed diabetes, where the quantity of sugar in the urine is very considerable, the potash and copper tests afford positive indications; but do they in slight and incipient cases of that disorder?

The physician is not unfrequently consulted in supposed cases of diabetes, the symptoms being—loss of health, emaciation, but particularly an elimination of an increased quantity of urine; and yet, failing when he comes to test the urines by the ordinary reagents to discover the presence of sugar, he generally pronounces these cases not to be diabetic. Does he, in this way, always arrive at a correct conclusion?

The detection of diabetes in an early stage, where sugar is present in the urine, either occasionally only, or in small quantities, is of the highest importance; for it is then chiefly that the physician may entertain the hope of treating the disorder successfully.

In an article published in the 'Lancet,'[1] I showed that diabetic sugar might be introduced in quantities by no means inconsiderable into many different urines, and yet not be discovered afterwards by the most skilful application of the ordinary tests.

Now, this fact confirms in a remarkable manner the suspicion entertained by many that urines may contain small quantities of sugar, and yet that this shall not be detected by any of the methods ordinarily in use.

[1] On the Tests for Sugar in the Urine, 'Lancet,' 1851.

I have now ascertained that this is not unfrequently the case.

Several specimens of urine voided in a supposed case of incipient diabetes were set aside for observation, they having previously been carefully tested for sugar, but none having been discovered.

In some of these specimens, somewhat to my surprise, although such a result was not of course wholly unanticipated, the gelatinous masses previously described appeared, bubbles of carbonic acid were eliminated, and the diabetic torula or fungus was traced through all the stages of its development—even the last, that of perfect aërial fructification.

The only differences observed in the development of the fungus in these specimens contrasted with its growth in samples of urine containing large quantities of sugar, were in the size and number of the masses, which were fewer and smaller, in the thinness of the yeast-like stratum formed, and in the circumstance that this, as well as the perfect fructification which sprang from it, did not cover the whole surface of the liquid, but extended over part only, forming one or more patches.

In other specimens development entirely ceased at the end of the first stage, the urine became turbid, the gelatinous masses were formed, and carbonic acid evolved; but here the growth stopped—the masses broke up, and after a time disappeared.

Lastly, in other specimens, the diabetic torula did not make its appearance at all.

It was particularly noticed that those specimens in which the fungus went through all the stages of development were more than usually acid.

That those urines in which the development ceased quickly were but feebly acid when passed, the acidity soon being entirely lost.

Finally, that the urines in which the fungus did not make its appearance at all were frequently either alkaline when voided or very quickly became so.

It appears, then, that in the diabetic fungus we have a most valuable, and, indeed, the only certain and available, test of the presence of sugar in urine in small, but not inconsiderable quantities.

It has been remarked that it was only in the more acid samples that the fungus became fully developed. This may be readily accounted for.

When describing Penicilium glaucum, I stated that the conditions necessary for its development were free exposure to the atmosphere, albumen to act as a ferment, and an acid liquid. Now, the same conditions are requisite for the growth of the diabetic fungus, with the addition of a fourth —the presence of grape or diabetic sugar.

In the feebly acid or alkaline urines one of these conditions is not fulfilled, and, therefore, the fungus is not developed.

It may be said, however, that urines which contain sugar are always acid, and therefore, that the fungus should be developed in all cases where this is present. Where the quantity of sugar is very considerable the urine no doubt is constantly acid; but whether it is always so, where the amount is small, is less certain. With a view to determine this point, I adopted the following proceeding:

Several samples of the feebly acid or alkaline urine passed by the patient the subject of incipient diabetes were obtained; to these was added sufficient phosphoric or acetic acid to impart the decided acidity necessary for the development of the fungus should sugar be present. The specimens were watched from day to day, and as any lost their acidity, as sometimes happened, further quantities of acid were added. This proceeding furnished the following results:—In the whole of the samples the circular patches of Penicilium glaucum quickly made their appearance, ultimately passing through all the stages of their development. In one of the samples only was there any formation of the sugar fungus, and in this the growth did not proceed beyond the stage of large circular sporules. One would, therefore, be disposed to conclude as the result of this

experiment that sugar is not ordinarily contained in slightly acid, neutral, or alkaline urines.

In suspected cases of diabetes, then, should the fungus not appear in the first specimens of urine examined, it must not be concluded that sugar is not present, even although the urines possess some degree of acidity. We must ascertain whether they are sufficiently acid, and, if necessary, must increase that acidity; neither must we decide against the presence of sugar in those instances in which some of the samples of urine examined are alkaline, for, as is also shown above, sugar may be present in some and absent in other specimens. In the case of incipient diabetes, which I have made the subject of special observation, I have particularly noticed that sugar is most liable to occur in the urine voided after error and excess in diet.

In this place the observations of Dr. Basham, 'On the Cholera Sporules,'[1] may be referred to. While searching the urine of a dyspeptic patient for crystals of oxalate of lime "the appearance of some annular-formed cells attracted attention, some with minute nuclei. The field of the microscope presented these sporules amongst many crystals of the oxalate and some epithelium and mucous globules." Again, in examining, in another case, some urine which was strongly acid, abounded in stellated crystals of uric acid, and was of specific gravity 1018, Dr. Basham observed some sporules which he thus describes: "They are somewhat like the torula of diabetic urine; but they want the true confervoid character. They are oval cells, arranged by their long diameter in a bead-like form, with minute granules or cellules developing themselves from the surface and junction of the parent-cells."

Dr. Basham made pen-and-ink sketches of the appearances observed at the time. These are published in the third edition of Dr. Golding Bird's work, and from an examination of fig. 58, which represents the character of the fungus detected in the second sample of urine examined, I entertain no doubt whatever but that this drawing

[1] Medical Gazette, 1849, vol. xliv, p. 686.

exhibits a condition of the sugar fungus. The urine, therefore, although not of high specific gravity, contained a small quantity of sugar, to which the presence of the torula was doubtless due. This case affords further evidence of the value of the torula-test for sugar in the urine.

From the facts which I am now about to adduce, it becomes extremely probable that sugar frequently makes its appearance in the urine in connection with a more or less alkaline condition of that fluid. It is at all events certain that it sometimes does so.

For some years before, and up to the period of the discovery of sugar, the urine in the case of diabetes, so often referred to, was when first passed occasionally alkaline or neutral, but most frequently feebly acid. On becoming cold, and even while still acid, it usually threw up an abundant iridescent pellicle of phosphate of magnesia; and when cold, it deposited large quantities of triple phosphate. One of the consequences of this want of acidity was that Penicilium glaucum, one of the best tests of acidity, but seldom became developed in it, and, when it did appear, it still more rarely passed through all the stages of its growth.

But the most remarkable character of this urine was, and still is, that it frequently contains very large quantities of phosphate of lime in a crystallized state. Now, this earthy salt occurs but very rarely in the urine in this condition, and of it, so far as I am aware, no accurate or detailed description has yet been given. I have myself met with crystals of phosphate of lime in several different cases; and in 1850 I published in the 'Lancet' a short notice of the form and composition of certain modifications of the crystals of this phosphate.[1]

Although this salt has not yet been fully described, we yet find in works on organic chemistry one or two brief references relating to it.

Thus, in vol. ii, p. 133, of Simon's 'Animal Chemistry,'[2]

[1] On certain Important Points in the Chemistry and Pathology of the Urine, 'Lancet,' January 19, 1850.

[2] Translation by Dr. Day.

the following observation occurs: "The phosphate of lime may be recognised under the microscope as an amorphous mass. Sometimes, but rarely, it occurs in a crystalline form. Both varieties are figured in fig. 26." In this figure a granular powder, as well as certain foliaceous masses, are represented. The form of these is so irregular that it is impossible to refer them, with anything like certainty, to the crystals I am about to describe; while, appended to the explanation of the figure, a remark is added to this effect — the foliaceous bodies are most probably urates. It thus appears that Simon was himself in some doubt respecting the composition of the irregular bodies which fell under his observation.

Again, Dr. Griffith, in his little work, copies Simon's figure, and adds, "I have specimens of this."[1]

Lastly, I find crystals of phosphate of lime described and figured under the name of "*Penniform Crystals of the Neutral Salt,*" by Dr. Golding Bird. The description is as follows: "This very elegant variety of the neutral magnesian phosphate has only lately fallen under my notice, and has occurred in a very few cases. It presents the appearance of striated feather-like crystals, two being generally connected, so as to cause them to resemble a pair of wings. (Fig. 35.) I cannot give any satisfactory explanation of the causes of this curious and elegant variety, or whether they differ in any way chemically from the prismatic form. The few specimens I have met with occurred in acid urine."[2]

I found my opinion that the crystals thus described by Dr. Bird are not composed of the neutral triple phosphate, but of phosphate of lime, upon an examination of one of Dr. Bird's original preparations, kindly lent me, along with others, for the purpose of having figures made from them.

Since the occurrence of these crystals is of much importance, in more respects than one, but particularly in relation to the presence of sugar in the urine, I will describe the forms which they assume, and especially the

[1] Practical Manual. [2] Urinary Deposits, 2d ed., p. 212.

method of analysis adopted, so that no room may be left for supposing that a correct conclusion with respect to the composition of these crystals has not been arrived at.

First, I would observe the crystals have presented themselves to my notice in the urine for at least the last three or four years. Although generally present, especially when the health is more than usually affected, yet they are sometimes absent entirely,—it may be for days together,—or they may be absent from one specimen and present in the next. They vary also in number: sometimes there are but few; usually they occur in great abundance, particularly in the more acid samples, in which they are formed even while the urine retains a decided acid reaction, and long before the formation and deposition of the crystals of triple phosphate.

Viewed with a half or quarter inch object-glass, the crystals appear wedge-shaped—being broad at one extremity and narrow at the other; but when the $\frac{1}{8}$th inch glass is brought to bear upon the broad end of the crystal, which is the only completely-formed part, it is then seen that they are really six-sided prisms, with oblique, and sometimes dihedral, summits. Occasionally, but rarely, both ends of the crystal are perfect, and then the wedge-shaped appearance is lost, and both extremities are alike. Sometimes they occur singly, but the greater number usually form, by the union of several crystals by their narrow extremities, rosettes more or less perfect; in other cases, but this is very seldom, the crystals are compound, each breaking into numerous secondary crystals; when this occurs, both ends are generally of the same shape.

The crystals are formed first, and chiefly on the surface of the urine, but they are sometimes found in large quantities at the bottom of the glass, and even adhering to the sides; those on the surface are frequently imbedded in a crust of iridescent phosphate of lime.

The crystals were twice carefully analysed, being obtained for the purpose, in the following manner: after having been identified by means of the microscope, they were

skimmed off the surface of the urine, and repeatedly washed in distilled water, to free them, as far as possible, from impurity; it is rarely, however, that they can be procured in any quantity, entirely free from admixture with small quantities of either phosphate of magnesia, triple phosphate, or even both these.

In the first sample analysed, there were present a few crystals of triple phosphate, and a little phosphate of magnesia; the deposit thus contaminated exhibited the following characters: it was slowly dissolved by cold acetic acid, and very rapidly by hot; from this solution oxalic acid threw down a copious precipitate of oxalate of lime, when boiled with liquor potassæ ammonia was evolved; it was fusible with difficulty only before the blowpipe.

From the above reactions, it is evident that the crystals are composed principally, if not entirely, of phosphate of lime; the small quantities of magnesia and ammonia detected being derived from the triple phosphate and phosphate of magnesia, which were previously ascertained to be present; it is nevertheless possible, that the lime may be combined with a small quantity of ammonia.

The second sample was almost, if not entirely, free from the ammonio-magnesian phosphate, but it was admixed to a very small extent with phosphate of magnesia, animal matter in the form of vibriones, and perhaps with oxalate of lime.

On boiling a few of the crystals in a test tube with a little liquor potassæ, a small quantity of ammonia was evolved, which communicated a red stain, not permanent, to turmeric paper. After boiling for a quarter of an hour, the liquid was diluted and set aside; in a few hours, the clear supernatant liquid was poured off, then acidulated with acetic acid, and tested with lime for oxalic acid, on standing for two or three hours a faint precipitate of oxalate of lime formed. A little more of the deposit was then boiled with acetic acid, and the clear liquor tested for lime with oxalic acid, when an abundant precipitate was produced. After the precipitation of all the lime, the solution was super-

saturated with ammonia, and allowed to stand, when crystals of bibasic phosphate of magnesia and ammonia separated. Finally, heated before the blowpipe, the crystals melted with difficulty.

As the result of this analysis, it again appears that the crystals consist, for the most part, and in all probability entirely, of phosphate of lime. A trace of ammonia only was detected on this occasion, but very perceptible quantities of phosphate of magnesia and oxalate of lime, the former of these, and most probably the latter also, were present as impurities.

The question next arises, is there any connection between the crystals of phosphate of lime and sugar in the urine?

I have described these crystals as occurring most frequently and abundantly in the more acid samples of urine; now it is in precisely these that sugar most commonly makes its appearance.

Again, between sugar and lime there is a great and well-known affinity.

Lastly, lime is apt to occur in saccharine urine in another form, in combination with oxalic acid.

These considerations render it probable that there is some such connection; before, however, we shall be in a position to come to any definite conclusion on this point, further observations are required.

I will now give the results, as recorded from day to day, derived from the examination of specimens of the urine passed in the case of incipient diabetes.

Results of the Examination of Specimens of the Urine voided by the Patient the subject of Incipient Diabetes.

1st SAMPLE:—Urine passed 5th August, slightly acid, having a specific gravity of 1015, clear when voided, and of a pale brandy colour, but becoming cloudy and thick as it cooled; flocculi separated from it, which subsiding formed a deposit three fourths of an inch in depth in a twelve-ounce

bottle; while the urine was being passed a slight smarting sensation was felt.

Examined *6th August.* The urine has now become alkaline, the deposit white and granular.

Examined *8th Aug.* A scum or pellicle has now formed over the whole surface of the urine, this consists of vibriones, a very few crystals of the ammonio-magnesian phosphate, and an immense number of crystals of phosphate of lime, mostly in stellæ, but some also single. The first analysis of the crystals given at p. 65 was made from this sample.

As at the time no suspicion was entertained that the case was one of diabetes, no search was made for the sugar fungus.

2d SAMPLE:—Passed early in the morning, on the *5th* of *August.* Urine clear, very acid; specific gravity 1024, and of a very deep colour.

Examined *8th August.* Many small circular patches of a variety of Penicilium glaucum in all stages, some composed of sporules, others of thallus, and some fully developed, and of a dark olive-green or brown colour; interspersed amongst these were a few white woolly tufts of Penicilium of larger size.

Examined *27th Aug.* Tufts of Penicilium in much the same state, but faded somewhat, and now imbedded in a pellicle of phosphate of magnesia. While examining one of the tufts under the microscope, many large sporules, as well as some of the threads forming the thallus of the saccharine fungus, were somewhat unexpectedly discovered, and it was afterwards ascertained that a considerable quantity of this fungus in an advanced state of development was present, not only on the surface, forming the tufts in part, but also at the bottom of the vessel.

Examined *5th September.* Urine thick and turbid, alkaline, with much deposit at bottom of glass.

Examined *17th Sept.* Urine dark brown; on examination of the tufts with the microscope, numerous blue masses were seen, but there were no crystals of phosphate of lime; the deposits consisted of vibriones, a great many spherules of some urate, and a few crystals of triple phosphate.

The saccharine fungus in this specimen did not reach its complete development.

3d SAMPLE :—Examined *5th September*. Passed about ten days since, but no record kept of its characters at that time. A thick yellow scum has spread all over the surface, with here and there imbedded in it a patch of fawn-coloured penicilium in perfect fructification, one patch somewhat green in the centre; this scum is composed of vibriones, phosphate of magnesia, and an immense number of the crystals of phosphate of lime, some separate, others in stellæ; the urine is still slightly acid, thick, and of specific gravity 1015.

Examined *17th Sept.* Urine alkaline; the scum first formed was removed, and the crystals of phosphate of lime after being well washed were submitted to analysis; a second scum similar in appearance has now collected on the surface, this is composed entirely of phosphate of magnesia, much triple phosphate, and vibriones, very small and active. At the bottom, there was present much phosphate of lime, a very small quantity of triple phosphate, and many vibriones.

There were also detected a few sporules of fungus, most probably those of the sugar torula.

4th SAMPLE :—This sample was also passed about ten or eleven days since; it is somewhat acid, and the surface is covered with circular tufts, in perfect fructification, of an olive-green fungus, a variety of Penicilium glaucum. The tufts are small and interspersed with several white woolly tufts of larger size, and not in fructification; spreading over nearly one half the urine is another fungus in perfect fructification, the saccharine; this is recognised by the long filaments which spring up on all sides, bearing on their summits the minute spherical heads which are so characteristic of the species. On examination with the microscope, numerous large sporules and threads of the diabetic fungus, as also many stellæ of phosphate of lime, were discovered,

intermixed with the threads forming the thallus of the olive-green tufts of Penicilium glaucum; the thallus of the woolly tufts likewise contained an immense number of stellæ of phosphate of lime.

5th SAMPLE:—Urine passed after dinner, on the *5th* of *September*, clear, of the colour of pale brandy, decidedly acid; specific gravity 1019; colour somewhat deepened by boiling with potash; no result with the copper test.

Examined *7th September*. Still clear and acid, has deposited much mucus, and some oxalate of lime.

Examined *8th Sept.* Turbid, but still acid, a few mucus-like masses near surface.

Examined *11th Sept.* Still acid; a decided scum on surface, composed of the small sporules of the saccharine fungus, vibriones, and a few crystals of phosphate of lime.

Examined *17th Sept.* Alkaline; saccharine fungus fallen to bottom, and still in the state of minute sporules; scum on surface, composed of much phosphate of lime and triple phosphate, with many vibriones. At the bottom of the glass, groups of sporules imbedded in masses of vibriones were detected, also very much oxalate of lime, phosphate of lime, and triple phosphate; the same also adhering to the sides of the glass in large quantities.

6th SAMPLE:—Urine passed early in the morning of the *6th* of *September*, clear, of a pale brandy colour, acid; specific gravity 1022; colour more deepened than in Sample 5, by boiling with potash; the copper test does not afford any evidence of the presence of sugar.

Examined *7th September*. Very turbid, decidedly acid, contains great numbers of octohædra of oxalate of lime, and much vesical epithelium.

Examined *8th Sept.* Urine very thick; cloud-like masses of the sporules of the sugar fungus both on the surface and at the bottom, as also many bubbles of carbonic acid gas.

Examined *17th Sept.* Urine alkaline; colour of urine

not deepened by keeping; scum on the surface, composed of crystals of phosphate of lime, a pellicle of phosphate of magnesia, and many vibriones; at bottom of glass, very much phosphate of lime, numerous globules of some urate, a little triple phosphate, vibriones, and a few dark sporules of fungus, perhaps, those of the sugar plant.

7th SAMPLE :—Urine passed after dinner, on the *6th* of *September*, decidedly acid; specific gravity 1015; clear, and of a pale colour; colour deepened by boiling with potash; no evidence of the presence of sugar afforded by the copper test.

Examined *8th September*. Very turbid, contains much vesical epithelium, but no oxalate of lime.

Examined *10th Sept.* Feebly acid, smell a little offensive, very thick, with large masses of sporules near the surface, a considerable deposit of the same, and many globules of carbonic gas.

Examined *11th Sept.* Scum on surface composed of vibriones, and phosphate of lime; no triple phosphate; urine nearly neutral.

Examined *21st Sept.* No scum; crystals of phosphate of lime floating on surface, and adhering to sides, some compound, with both extremities perfect; triple phosphate, and a very few minute octohædra of oxalate of lime. At bottom, phosphate of lime, triple phosphate, and vibriones. No diabetic sporules of any size.

8th SAMPLE :—Passed after dinner, on the *6th September*, slightly acid; specific gravity 1019.

Examined *8th September*. Neutral, rather turbid.

Examined *10th Sept.* Alkaline, still more turbid; deposit of mucus with much triple phosphate; on surface, a scum consisting of vibriones, and much triple phosphate; no sporules of sugar fungus detected, and no phosphate of lime; the urine is now rather offensive, its colour was slightly deepened by boiling with potash; but the copper test gave no evidence of sugar.

Examined 21*st Sept.* Much urate in globules at bottom of glass, with triple of phosphate, but no phosphate of lime.

9th SAMPLE :—Passed *7th September*, at bedtime, nearly neutral; specific gravity, 1016.

Examined 10*th September.* Very thick, alkaline; scum on surface composed of vibriones, and triple phosphate, deposit formed of same; no sporules of sugar fungus detected, and no phosphate of lime.

10th SAMPLE :—Passed early on the *7th September*, decidedly acid; specific gravity 1016, clear pale straw colour; colour deepened by potash; copper test gave no evidence of sugar.

Examined *9th September.* A little turbid, contains much vesical epithelium, but no oxalate of lime.

Examined 10*th Sept.* Very thick; masses of sporules near the surface, falling to the bottom when the glass is moved; a few bubbles of carbonic gas; phosphatic scum on surface; urine smells rather offensively, but is still a little acid.

Examined 11*th Sept.* Scum more decided, composed chiefly of phosphate of lime, with a little triple phosphate and many vibriones; still faintly acid; bulky deposit formed of mucus; the small sporules of the sugar fungus and crystals of phosphate of lime.

Examined 16*th Sept.* Urine alkaline; no further development of the saccharine fungus.

Although the colour of most of the above urines was deepened by boiling with potash, yet this increase was not greater than is commonly observed in urines which do not contain sugar. It is usually stated that non-saccharine urine is bleached by boiling with potash. This is incorrect, as shown by me in a paper published in the 'Lancet,' March, 1851.[1] It almost invariably darkens every variety

[1] On the Tests for Sugar in the Urine, 'Lancet,' 1851.

of urine. Thus, in not one of the above samples did either the potash or copper tests betray the presence of sugar.

As scarcely any data exist tending to elucidate the early, and therefore the most important stages of diabetes, it may be useful to give a somewhat detailed description of the symptoms, by which for the last three or four years the case of incipient diabetes referred to was characterised.

Case.—William F—, æt. 35, of delicate organisation and nervous temperament, but free from organic disease, has for some years suffered considerably from chronic indigestion, as evidenced by frequently recurring attacks of headache and sickness; these were brought on by very slight causes, as any little error or excess in diet; the headaches were particularly distressing, and attended by giddiness and confusion of thought; the vomitings set in some hours after the commencement of the headaches, when these were unusually severe, and occurred as often as once or twice a week; each attack consisted of several successive fits of retching, and sometimes lasted as many as 10 or 12 hours, continuing long after the stomach had been well emptied. Within the last 8 months the headaches and sickness have nearly ceased, but occur still occasionally on any departure from the very temperate and regular method of living usually pursued.

The attacks were worst in summer, milder and less frequent in winter. For the last four or five years the patient has noticed that he passed his urine more frequently and in larger quantity than ordinary, his kidneys acting quickly on the slightest cause, as almost immediately after taking liquids of any kind, especially beer. The characters of the urine have already been described above. He has long also experienced a constant feeling of debility and exhaustion, both bodily and mental; as the appetite was generally good, he was unable to account for this extreme debility, and expressed a conviction that the large quantity of urine eliminated acted as a drain upon his system, the food and drink taken, by stimulating the kidneys, appearing rather to increase the exhaustion than to afford support.

Amongst the peculiar symptoms were the following:

1st. An occasional dry hacking cough without expectoration, and unaccompanied by symptoms of cold; this used to come on about noon, and was attended by slight febrile excitement; it was always removed for the time by food. 2d. Frequent dryness of the lips without positive thirst; this symptom attracted attention long previous to the discovery of sugar in the urine, and excited the suspicion that the case might possibly be one of incipient diabetes. 3d. Very great susceptibility to changes of temperature and weather; while rain, or the least dampness of the atmosphere, produced considerable depression; the heat of the sun seemed to inflame the blood, and to induce in it a state of fermentation.

The above symptoms, prior to the detection of the sugar, were set down to indigestion and the phosphatic condition of the urine; it is now clear, however, that they were mainly attributable to the sugar, the presence of which in the urine shows that the primary assimilative functions were very greatly at fault.

The patient attributed his bad health to excessive mental labour and long-continued anxiety. For some weeks past he has relaxed somewhat from study, has taken more exercise, the diet has been regulated, meat being allowed twice a day; as the result of all which, the health has considerably amended.

We have then occurring in the urine in different and very opposite states, two distinct species of fungus, the one being characteristic, to some extent, of the presence of albumen, and the other of sugar; but since the conditions necessary for the development of Penicilium glaucum all exist in saccharine urine, the only difference being the superaddition of sugar, we have next to ascertain whether the two species do not sometimes occur together in the same urine.

As might almost have been anticipated, the result of observation on this point is, that they not unfrequently occur together.

When the amount of sugar present is but small, the two fungi go on developing themselves almost in equal ratio,

each presenting its own distinctive characters, so that when they have attained their full development, part of the surface of the urine will be occupied with patches of the true saccharine torula, and part with those of Penicilium glaucum. In some cases, even the same tufts may be formed of the two species combined. (Pl. III, fig. 4.)

When, however, the quantity of sugar is considerable, the saccharine torula is developed with such rapidity and in such quantity as to outstrip the other species; and it is only when the fermentation has nearly come to an end, that Penicilium glaucum comes into view, and proceeds in its development.

We have, in the next place, to consider very briefly the chemical changes which ensue in saccharine urine placed under the three conditions already described, viz. free exposure to the air, partial exposure, and complete exclusion.

In all the specimens of saccharine urine freely exposed to the air, the following changes have ensued: the sugar has disappeared, carbonic acid has been evolved, and alcohol formed; of the alcohol part escapes into the air, diffusing a vinous odour, and part is converted into acetic acid. As the conversion of the sugar proceeds, the specific gravity of the urine becomes greatly lessened.

In the specimens partially exposed to the atmosphere, the urine, after a time, presented a gelatinous appearance, possessed a smell like sour milk, and was strongly acid; on analysis it was ascertained that the sugar had disappeared, that a small quantity of alcohol was present, and that the acidity was due to an abundance of acetic acid. In one or two of the samples, large quantities of oxalic acid in combination with lime were detected. As the saccharine fungus was imperfectly developed only, it is probable that in this case the greater part of the sugar passed directly into acetic acid.

In the specimens from which the air was excluded, as there was only a slight development of the sugar fungus, so was there scarcely any formation of alcohol; nevertheless, the sugar disappeared, and it was found on analysis to have

become converted into lactic acid, a small quantity of butyric acid, and what appeared to be aldehyde, from its smell and property of slightly reducing the oxide of silver, and giving a brownish-yellow coloration with liquor potassæ.

These several transformations of sugar are interesting, if not important; it has usually been considered that saccharine urine, when kept for any length of time, always undergoes the vinous fermentation; the lactic, acetic, butyric, and oxalic acids sometimes formed, as well as the circumstances which determine their formation, having been in general overlooked.

From a review, then, of the whole of the facts and observations above described, relating to the development of torulæ in saccharine urine, the following conclusions may be deduced:—

1st. That there is developed in saccharine urine, freely exposed to the air, a distinct species of fungus, which occurs in no other condition of that excretion.

2d. That this fungus is identical with the yeast plant.

3d. That it passes through three stages of development, any one of which is distinctive of the species.

4th. That since it is sometimes developed in urine in cases in which the potash and copper-tests fail to detect the presence of sugar, and in which, therefore, the quantity of sugar is not very considerable, it affords a most valuable and important test, and furnishes us with the means of detecting diabetes, even in its earliest stages.

5th. That the conditions necessary for its development are—free exposure to the air, an acid liquid, nitrogenous matter to act as a ferment, grape sugar or glucose, and a moderate temperature.

6th. That it may be developed at will in any sample of urine which is sufficiently acid, by the addition of a few grains of grape sugar.

7th. That when specimens of saccharine urine are imper-

fectly exposed to the air, the development of the fungus is incomplete only.

8th. That when the atmosphere is entirely excluded, no development of the fungus occurs.

9th. That in some few cases, where the quantity of sugar is very small, the fungus will cease to grow after having passed through the first stage only of its development, in consequence of the sugar, all having become converted into alcohol and carbonic acid.

10th. That sugar may be present in some very rare cases in small quantity, and yet the torulæ fail to be fully developed in consequence of the urine not possessing the necessary degree of acidity.

11th. That in such cases it is probable the development might be ensured by the addition of small quantities of phosphoric acid, or of a solution containing carbonic acid.

12th. That the presence of this fungus indicates the vinous fermentation, its development being accompanied by the disengagement of carbonic acid and the formation of alcohol.

13th. That the power of the fungus in aiding the transformation of the sugar is limited to the period when it is in the condition of sporules or yeast, the thallus and aërial fructification exerting no influence over the change.

14th. That in those cases in which the fungus is only partially developed, in consequence of imperfect exposure to the atmosphere, the sugar is converted chiefly into acetic acid, but a portion sometimes, also, into oxalic acid.

15th. That where the fungus is not developed at all, in consequence of the exclusion of the atmosphere, the sugar is transformed into lactic, acetic, and butyric acids, and also probably aldehyde.

16th. That since, in saccharine urine, the conditions requisite for the development of Penicilium glaucum exist, that species is likewise frequently met with in such urine.

17th. That in very many of the specimens of urine obtained from the patient labouring under diabetes, in a mild form, large quantities of crystallised phosphate of lime were detected.

POSTSCRIPT, *received January 11th*, 1853.—Since my paper on the Development of Torulæ in the Urine was read before the Royal Medical and Chirurgical Society, I have been so fortunate as to meet with another case, in which the urine threw down, on being allowed to stand for some time, an abundance of crystals of phosphate of lime, and which also contained some sugar, as shown by the development of the sugar fungus. This case affords, therefore, further and strong proof of the relation which I suggested might possibly be found to exist between crystals of phosphate of lime in the urine, and small quantities of sugar. The particulars of the case are as follows:—

CASE.—Mrs. T—, aged 32, of delicate constitution and nervous susceptible temperament, subject to dyspepsia, married, and has three children, the youngest only three months old. Attention was directed to the urine in consequence of the intense pain experienced on ceasing to micturate. The only sample of urine examined, and for which I am indebted to my brother, Dr. Hassall, of Richmond, was passed on the 19th of November; it was of specific gravity 1031, very acid, contained a large quantity of some pink urate, very many crystals of uric acid, and some octohædra of oxalate of lime; there was no albumen, nor could sugar be detected by means of Trommer's test.

Examined *22d November*.—The urine was still acid, and a cloud of vaginal epithelium had fallen to the bottom of the glass.

Examined *27th November*.—It was still acid, and the surface was covered all over with circular patches of Penicilium glaucum in the state of sporules. Under the microscope there was discovered, mixed up with the sporules, an abundance of crystals of phosphate of lime.

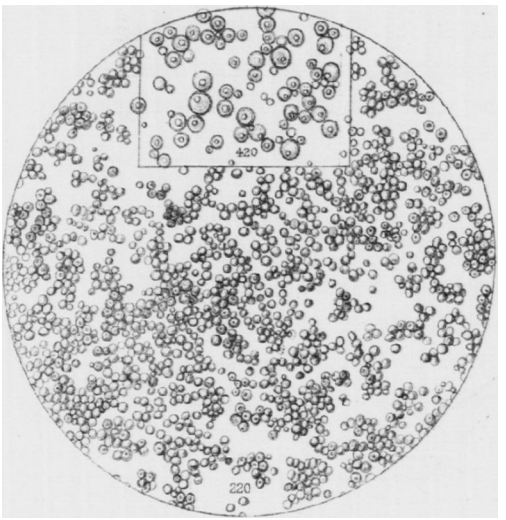
Examined *7th January*.—Urine alkaline; there was a scum of Penicilium in perfect fructification covering the whole surface of the urine, and mixed up with this were

immense numbers of globules of the urate and crystals of phosphate of lime, triple-phosphate, and oxalate of lime, together with some sporules and thallus of the sugar fungus.

POSTSCRIPT, *received August 27th*, 1853.—Since the above communication was presented to the Royal Medical and Chirurgical Society, I have on several occasions met with crystals of phosphate of lime in connection with the saccharine torula. The fact, therefore, that some close relation exists between these crystals and sugar in the urine may now be considered to be fully established.

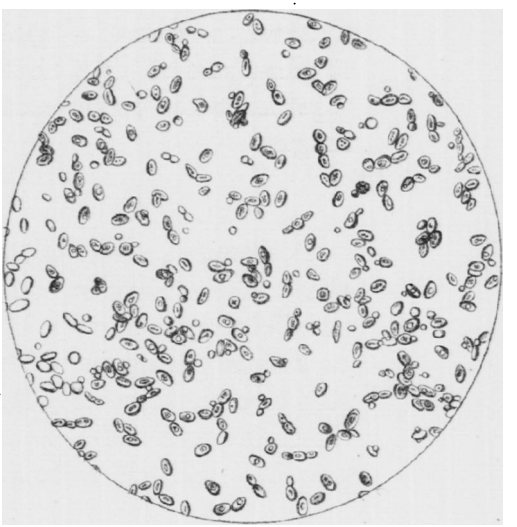
NOTE.—It may be well to state that the credit of establishing the real nature of yeast is due to Cogniard-Latour. In 1835 and 1836 he communicated to the *Société Philomathique* some researches on Ferments, which were afterwards published in a journal called 'L'Institut.' In 1837 he presented to the Academy of Sciences his "Memoire sur le Fermentation Vineuse," which was published in the 68th volume of the 'Annales de Chemie et de Physique,' 1838.

Fig 1.



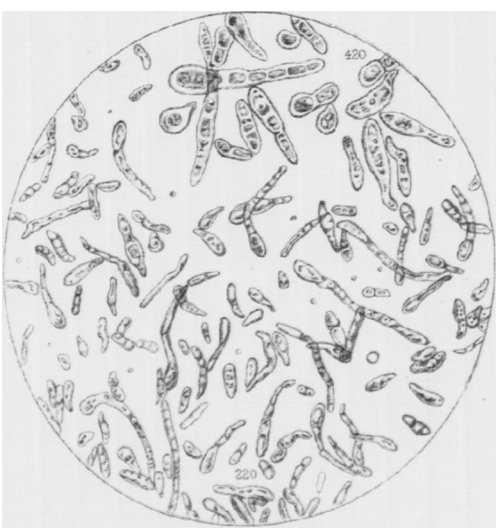
Circular Sporules of Penicilium glaucum

Fig 2



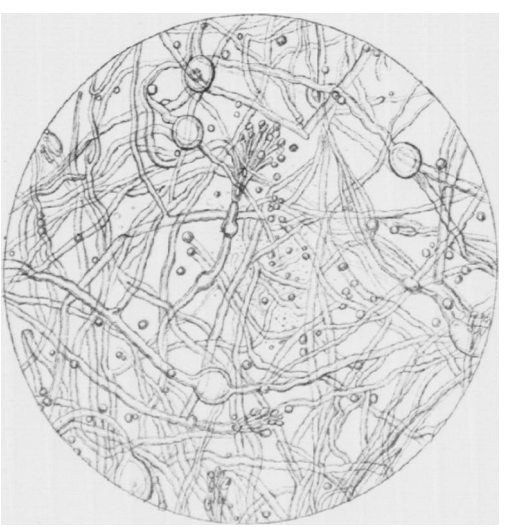
Oval Sporules of Penicilium glaucum 220 diam

Fig 3



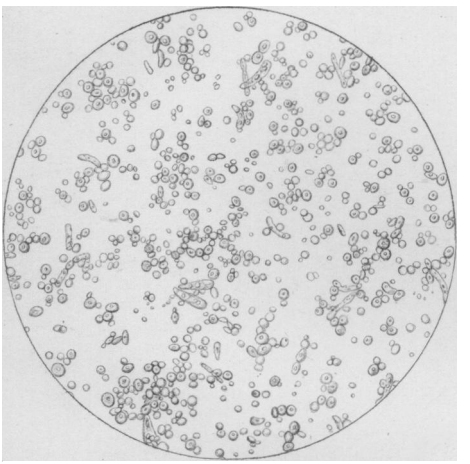
Sporules of Penicilium glaucum passing into filaments

Fig 4.



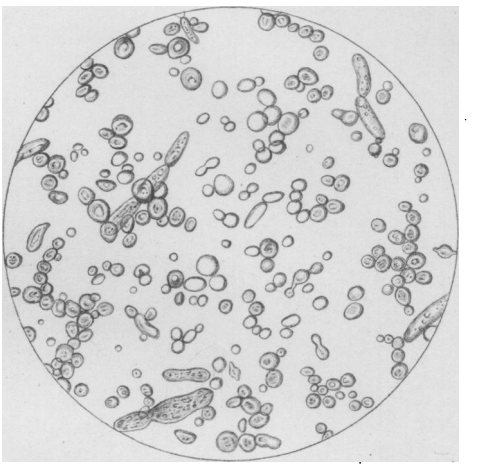
Thallus of Penicilium glaucum shewing the Vesicular Enlargements 220 diam

Fig 1.



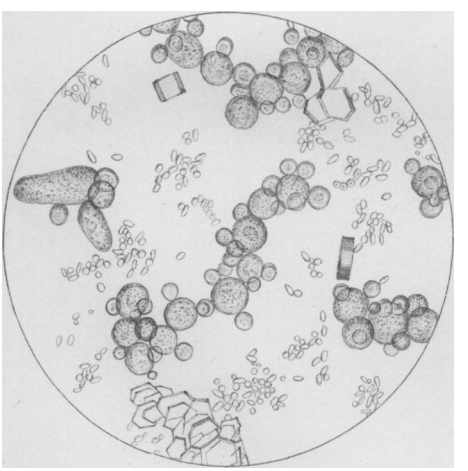
Sporules of Sugar Fungus in earliest stage of developement. 220 diam.

Fig 2.



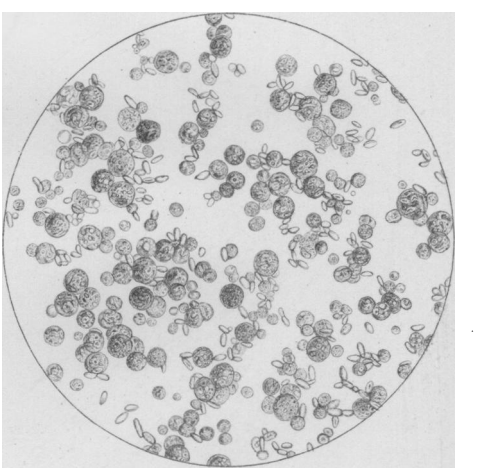
Sporules of Sugar Fungus in earliest stage of developement. 420 diam.

Fig. 3.



Sporules of Sugar Fungus also those of Penicilium glaucum with Crystals of Uric Acid 220 diam.

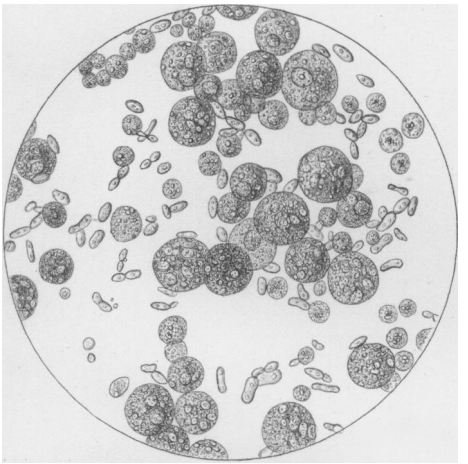
Fig 4.



Variety of Sporules of Sugar Fungus with those of Penicilium glaucum 220 diam.

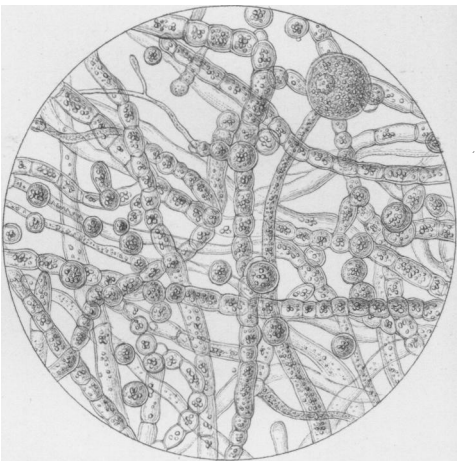
Ford & West. lith. 54 Hanson. Garden.

Fig 1.



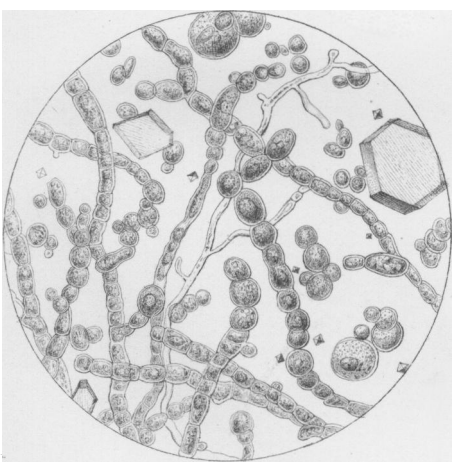
Variety of Sporules of Sugar Fungus with many of Penicilium glaucum. 420 diam.

Fig 2.



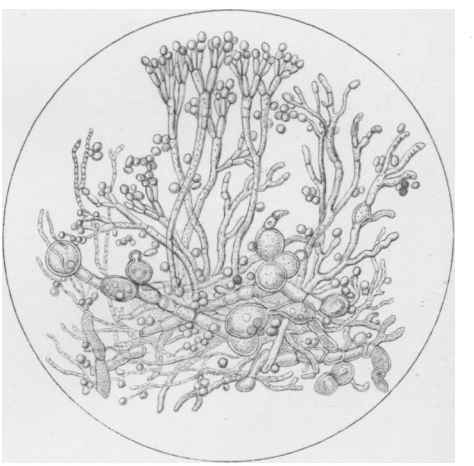
Thallus and Sporules of Sugar Fungus 220 diam.

Fig 3.



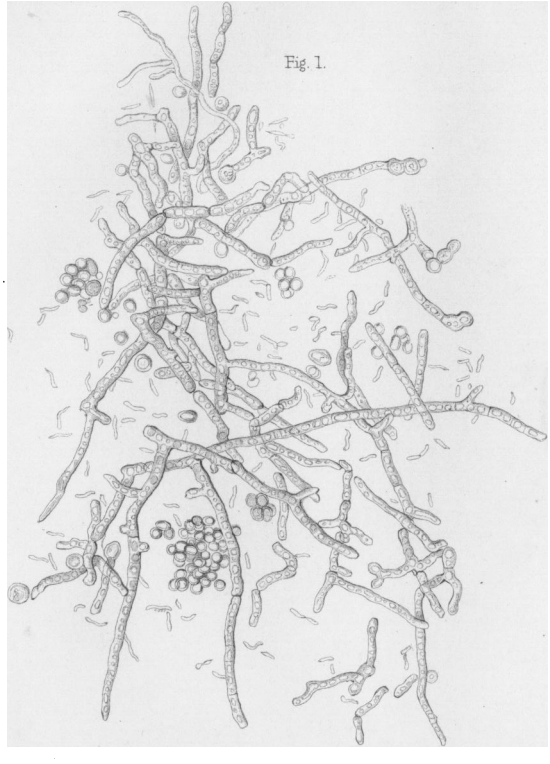
Thallus and Sporules of Sugar Fungus with crystals of oxalate of lime also Vesicles of the Same 220 d.

Fig 4.

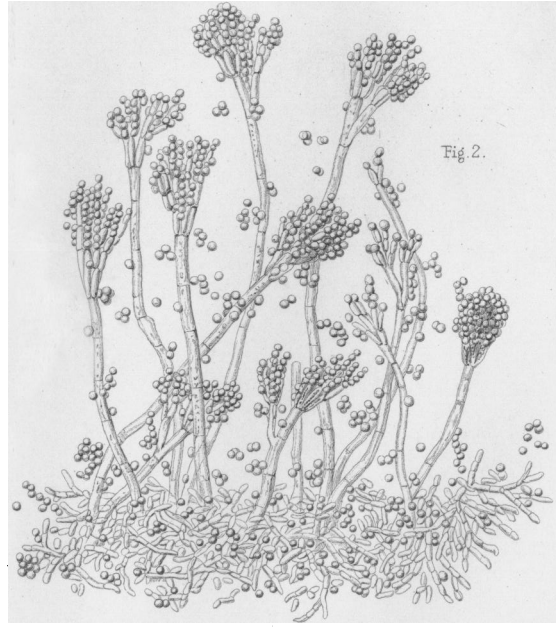


Penicilium glaucum in Perfect Fructification with Filaments and Sporules of Sugar Fungus. 220 diam.

Ford & West lith. 54 Heaton Garden.



Incipient Thallus of Penicilium glaucum with Groups of Sporules. 220 diam.



Penicilium glaucum. Perfect fructification. 220 diam.

Reid & West, lith. 54, Hatton Garden.

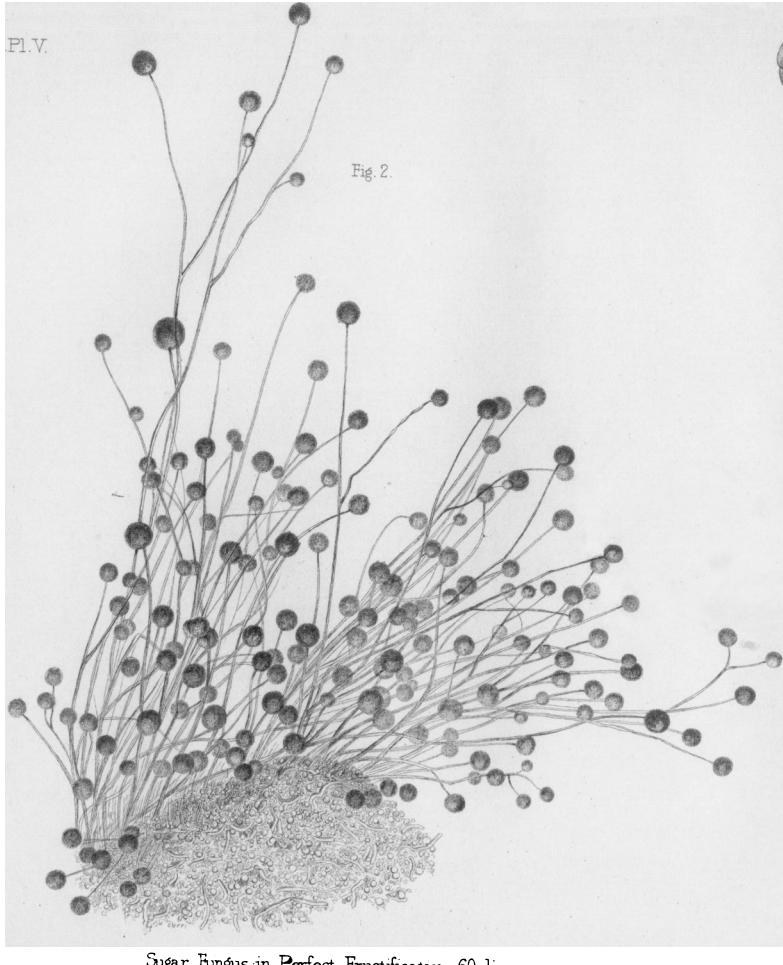


Fig. 2.

Sugar Fungus in Perfect Fructification. 60 diam.

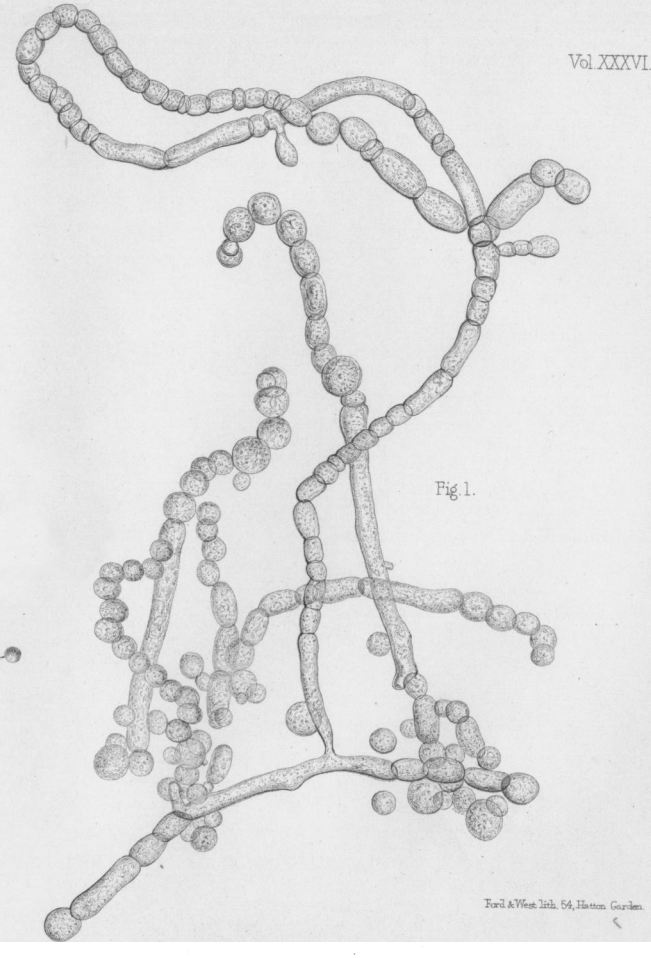


Fig. 1.

Ford & West lith. 54, Hatton Garden.

Thallus and Sporules of Sugar Fungus. 220 diam.