On the Characters, or Marks, employed for classifying the Schizomycetes.

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THOSE who have contributed to the bringing about of the existing state of chaos in the classifying of the Schizomycetes have much to answer for, and the task of unravelling the tangled skein of records will be no less honoured than onerous, which is saying a good deal. Without implying any competition for that honour, it may be of some little use to try and show how the chaos has come about, and to discover a way out of it, or at least to discover one or two paths which might be put together to make a way out of it.

I take it that two chief sets of causes have been at work, in different directions, to bring about the deadlock; on the one hand, the botanists of the past decade have confined their attention too exclusively to the morphological characters of the various species they have created, while, on the other, the bacteriologists—using the word simply in its technical sense—have directed their attention too exclusively to the behaviour of *their* species on or in certain media, especially on gelatine. It is not implied, intentionally at any rate, that either class of observers has wilfully neglected the observations of the other; but it needs no pointing out that each

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has unconsciously heaped up an immense store of trouble for the new type of bacteriologist which the needs of the times are bringing forward. The trouble has arisen quite naturally, owing to the two sets of observers having had their backs turned to one another, and their attention concentrated along different avenues of research. Let us look for a moment at the kinds of characters that each has brought into the foreground, and then try to make out from the work of the few who are now turning round as they work (so to speak) and looking at each other's efforts with scientifically sympathetic eyes. Since it is not my object to write a history of bacteriology, I pass over the work of the earlier observers Leeuwenhoek, Will, O. F. Müller, Bory de St. Vincent, Spallanzani, Ehrenberg, Dujardin, Pertz, Hallier, Burdon-Sanderson, Pasteur, and others, simply reminding my readers that many very interesting facts had been recorded, and even classifications of micro-organisms constructed, long before Cohn's time.

The school which culminated in the brilliant efforts of Cohn was almost entirely concerned with the preparation of the ground on which the subsequent struggles were fought, and from which the new departures were taken.

Ehrenberg, Kützing, Rabenhorst, Schröter, Warming, Cohn, and others had recorded, prior to 1880, a considerable number of forms of Bacteria of various kinds. For the most part these records were records of 'finds': that is to say, each observer overhauled the contents of the ponds, aquaria, macerating-troughs, and so on, at his disposal, and faithfully delineated the forms of the organisms found therein, named them, and added the habitat, &c. The method was the usual one of an exploring botanist in a new country, and quite properly so. The results began to take a modern shape under the hands of Cohn, who, in 1872 and 1875, brought forward his long celebrated system of classification of these organisms, based almost entirely on their forms as found and recorded; though, at the same time, I think Cohn was more alive to the imperfections and tentative character of his

proposed system than is always admitted. Cohn's great merit, in fact, was in pointing out that there is a *relative* consistency in the recurring forms met with, sufficient to enable us to describe them more or less definitely: he did not insist on the absolute persistence of these forms to the extent he has been supposed to have done.

Two double sets of dissentients to the Cohn-Ehrenberg school, of the decade prior to 1881, seem to have arisen about this period, and I shall briefly sketch the peculiarities (as I understand them) of each of these camps, or schools, or whatever we choose to term them, merely reminding the reader that each touches the period just referred to in very different ways and at different points.

First, there was a double set of botanists. One of these sets may be best referred to as the systematists, who seem to have directed their attentions almost entirely to the getting hold of every new form of Schizomycete, as soon as it was published, and no matter by whom, and giving it a name, implying that the form recorded is a species. This set of workers, of very unequal merit, has culminated in the unquestionably brilliant leaders, Winter, the deplored compiler of the celebrated Pilz-Flora of Winter and Rabenhorst, and Trevisan and De-Toni, the splendidly talented and industrious compilers of the volume on Schizomycetes of Saccardo's monumental Sylloge Fungorum, and now *the* authority on European systematic mycology.

The second of this set may be termed the morphologists, and their distinguishing feature—the one which binds them together as a band of workers—has been the investigation of the *development* as well as the forms of the Schizomycetes. Influenced throughout more or less by the two masters of microscopic methods, De Bary and Brefeld, and also, it should be mentioned, by Cohn himself, who was an exceedingly able investigator, quite alive to the morphology of the subject he tried to set in order, this assiduous band of observers, comprising Cienkowski, Prazmowsky, Billroth, Lankester, Dallinger, Eidam, Hueppe, Klebs, Klein, Kurth,

Van Tieghem, and others, has culminated in the modern partial school of Zopf. It should be noted, however, that this series of observers has already undergone an important differentiation along two more or less diverging lines of thought: some of them, influenced by the writings of Naegeli, Billroth, and Zopf especially, have declared emphatically against the systematists, in so far that they have either denied the existence of species altogether among Schizomycetes, or have at least claimed that as the polymorphy of these organisms is now shown to be so marked—and it must be admitted that polymorphy, as measured by the Cohn-Ehrenberg standard, *is* very marked—species cannot be defined by the morphological and developmental characters of the Schizomycetes.

The others, comprising Beyerinck, Kurth, Cienkowski, Winogradsky, Klein, &c., remaining more faithful to the cautious utterances of Cohn and De Bary on this point of polymorphy, have satisfied themselves with declaring, or implying, more or less clearly, that, while polymorphy cannot be denied and should not be under-estimated, the difficulty of finding diagnostic morphological characters is after all a relative one, largely due to the minuteness of the organisms, and the few and simple differential features that they possess; or at least that they exhibit under our microscopes.

This double set, constituting a school, if we so choose to put it, of botanists, have undoubtedly done wonders during the last decade. It is only necessary to mention De Bary's study of *Bacillus Megaterium*, Prazmowsky's and Van Tieghem's of *Clostridium butyricum* and *Leuconostoc*, Klein's on the spores of Bacilli, Brefeld's work on *Bacillus subtilis*, Kurth's on *Bacterium Zopfii*, and *Photobacteria*, Winogradsky's on Sulphur-bacteria and *Nitromonas*, Lankester's and Zopf's on *Cohnia* (*Clathrocystis*) *roseo-persicina*, Billet's on *Cladothrix*, Beyerinck's on *Bacillus cyano-fuscus*, and very many others, to see what enormous strides have been made in our knowledge of the forms and evolution of Schizomycetes during the period referred to, and what a weighty mass

of evidence is accumulating which must have, and is having, its effect in checking mere recording of forms, on the one hand, and wild speculations on the other.

The second double set of observers, which we may call the non-botanists—without implying the slightest want of respect for the magnificent edifice of knowledge which they have erected—may, it seems to me, be said to have taken their origin from two sources. One of these sets, which has culminated in the grand school now centred in the Pasteur Institute in Paris, sprung quite naturally from the epoch-making work of Pasteur on fermentations[1], and its leading characteristics are unquestionably derived from the teachings and writings of the illustrious master who still directs the school. We may, I think fitly, denominate it the school of Pasteur and Duclaux. Its leading feature, and the one which binds it together as a very compact body, is the concentrated attention to the processes of zymotic energy displayed by micro-organisms. It has concerned itself very little with questions of morphology, and still less with the interests of the systematists, towards whom, in fact, its attitude seems occasionally somewhat supercilious.

Since I am committed to the invidious task of reminding my readers of some of the leading work of each set of investigators, it is only necessary to point to the following as examples of the magnificent achievements of the Pasteur-Duclaux school: the works of Downes and Blunt, Arloing, Duclaux and Roux on the action of light on the spores of *Bacillus anthracis*; Schloesing and Müntz, Warrington, Frankland and Winogradsky on nitrification; Metschnikoff on phagocytes; Tyndall on dust; Hansen on yeasts; and many others influenced by the author of the classical works, Études sur le Vin, Études sur la Bière, and the director of the great experiments on hydrophobia now being carried out. It is unnecessary to go into the details of Pasteur's labours on anthrax, fowl-cholera, vaccination, immunity,

[1] It is therefore pre-Cohnian in many respects, though it touches Cohn's work, and that of his contemporaries, at many important points.

&c.; they are known to all. The great link between this school and the one to be taken next is the specialisation of the labours of the Pasteur-Duclaux school in the direction of pathology, and the ferment-theory of disease.

The other branch of the non-botanical workers started more directly from the Cohn-Ehrenberg school of bacteriologists, under the direct leadership of Koch. It originated distinctly, I think, with Koch's path-breaking work on *Bacillus anthracis*, and the foundation of the Mittheilungen aus dem kaiserlichen Gesundheitsamte in 1881, and has been carried on ever since, under the banner of the great German doctor, by men like Flügge, Fraenkel, Gaffky, Eberth, Briegen, Pfeiffer, Woodhead, and a whole army of pathologists. On the whole it has kept more in touch with the systematists, especially of the Cohn-Ehrenberg school as shown by the works of Flügge, Migula, Cornil, and Babès, &c., though its special work is marked throughout as pathological in nature.

The contributions to our knowledge of anthrax, cholera, and tuberculosis made by Koch, Gaffky, Klein, Hankin, and others, suffice to show this; and it may be remarked in passing that such work on the part of the pathologists of the German and French schools at once explains their departure from the older traditions of bacteriology. Another remarkable feature of the Koch-Flügge school, if we may thus term it, has been their extraordinary fertility in the devising of methods of culture and of staining. The same has been true of the Pasteur-Duclaux school, and it is indeed a very invidious task to compare and contrast the two in respect of their achievements in any part of the general domain they have opened up; but I am not attempting to detract in the slightest from the high honours of either by trying to select what seem to be the distinguishing peculiarities in their special lines of development of the science.

It seems to me that while the German school has paid particular attention to the methods of gelatine-culture and of staining by means of aniline-dyes, the French school has rather developed the methods of culture in liquid media, and

the examination of the products of action of the Schizomycetes. This would seem to explain why the Koch-Flügge school has given us several special modes of staining:—e.g. Kühne's methylene-blue method, the Ziehl-Neelson carbol-fuchsin method, the Gram-Weigert, and Koch's various ingenious methods of preparing, staining, and mounting Bacteria; why the various developments of culture on solid media have come from Germany; and why the characteristic forms, colours, and liquefying powers of the colonies of Schizomycetes have received so much attention at the hands of the Koch-Flügge school.

The above way of looking at the history would also seem to explain some peculiarities of the Paris school. The perfection to which they have carried the method of dilution-cultures, as exemplified by Miquel's results at the Montsouris laboratories; their recent triumph, the Chamberland filter; the successful pursuit of anaërobic Bacteria at a time when such anomalous organisms were looked at with suspicion; and last, but by no means least, their remarkable persistence and success in the employment of virus-material which they treat as if it contained Schizomycetes, although no one can demonstrate the presence of organisms in it—I refer of course to the hydrophobia-virus—reminds one of the methods of the chemist and physicist with their assumptions of atoms and molecules which no man has ever seen.

Each of these two schools has imparted much information to the other, and, naturally, their mutual reactions tend to eliminate their differences as schools in some respects, and to emphasize them in others. I think, however, that, taken as a whole, each has its special peculiarities much on the lines sketched above. Each of the schools, moreover, has given evidence of its fertility in the branching out of more special little bands of workers, whose particular object is to apply the results of bacteriology in certain directions. The hygienic institutions of various countries may be cited as examples, and nothing better illustrates the truth of the preceding remarks than the persistent difference in methods of culture

between the Montsouris Observatory in Paris, and the various German institutes of hygiene: the former severely criticises the gelatine-plate method as untrustworthy, the latter employ it almost exclusively.

Enough has been said to show how it has come about that various bands of observers have been traversing and mapping out the enormous domain of bacteriology, each with little or no regard for the presence or work of the other. The result may be compared to a number of maps, begun by various parties of surveyors, each starting along a different route and with no pre-arranged plans as to scales, comparative surveys, or intercommunication of any particular kind. Moreover, one set of explorers has confined its attention chiefly to contours, while another has recorded climate, and another artistic features, and so on, whence the difficulties of comparing the results and compiling a map up to date are very great.

All are more or less conscious of the need of a good systematic account of these organisms, however; and I now propose to try and set forth in some detail what kinds of characters are being used by those who wish to inform others how given 'species' may be distinguished. I shall of course confine my remarks entirely to modern work.

In order to remind the reader of the scheme propounded by Cohn, I append his system in a tabular form (Table I) as put forward in 1875.

TABLE I.—*Cohn*, 1875.

Tribe I. Gloeogenae.

Cells free, or connected by intercellular substance into slimy colonies (zoogloeae).

A. Cells free, or grouped in pairs or fours.

Chroococcus (Naeg.). Cells globular.

Synechococcus (Naeg.). Cells cylindrical.

B. Cells, in the resting state, gathered into amorphous zoogloea-masses.

(*a*) Cell-membrane passing imperceptibly into the intercellular substance.

(i) Cells devoid of phycochrome, very small.
Micrococcus (Hall. emend.). Cells globular.
Bacterium (Duj.). Cells cylindrical.
(ii) Cells containing phycochrome, larger.
Aphanocapsa (Naeg.). Cells globular.
Aphanothece (Naeg.). Cells cylindrical.

(β) Intercellular substance stratified concentrically into shells.
Gloeocapsa (Kg., Naeg.). Cells globular.
Gloeothece (Naeg.). Cells cylindrical.

C. Cells united into definitely circumscribed zoogloea-masses.

(α) Families in flat layers, arranged in one plane.
(i) Cells in fours, arranged in one plane.
Merismopedia (Meyen).
(ii) Cells irregularly arranged on the periphery of a sphere.
Clathrocystis (Henfr.). Families clathrate. Cells spherical.
Coelosphaerium (Naeg.). Cells cylindroid-wedge-shaped: families forked.

(β) Colonies aggregated into spheroidal, many-layered masses.
(i) Numbers of cells definite.
Sarcina (Goods.). Cells in fours, globoid, colourless.
Gomphosphaeria (Kg.). Cells cylindroid-wedge-shaped, irregularly disposed, containing phycochrome.
(ii) Numbers of cells large and indefinite.
Ascococcus (Billr. emend.). Cells colourless, very small.
Polycystis (Kg.).
Coccochloris (Spr.).
Polycoccus (Kg.), &c.
} Cells larger, and containing phycochrome.

Tribe II. Nematogenae (Rab.).

Cells arranged in filaments.

A. Filaments always unbranched.

(α) Filaments free or matted together.
(i) Filaments cylindrical, colourless, and obscurely segmented.
Bacillus (Cohn). Filaments very thin and short.

Leptothrix (Kg. emend.). Filaments very thin and long.

Beggiatoa (Trev.). Filaments thicker, and long.

(ii) Filaments cylindrical, containing phycochrome, evidently segmented. Reproductive cells unknown.

Hypheothrix (Kg.).

Oscillaria (Bosc.), &c.

(iii) Filaments cylindrical, segmented, and forming gonidia.

Crenothrix (Cohn). Colourless.

Chamaesiphon, &c. Containing phycochrome.

(iv) Filaments spirally twisted.

* Devoid of phycochrome.

Vibrio (Ehr. emend.). Filaments short, slightly undulated.

Spirillum (Ehr.). Filaments short, spiral, rigid.

Spirochaete (Ehr.). Filaments long, spiral, flexile.

** Containing phycochrome.

Spirulina (Link). Filaments long, spiral, flexile.

(v) Filaments moniliform.

Streptococcus (Billr.). Without phycochrome.

Anabaena (Bory). } Containing phycochrome.
Spermosira (Kg.), &c. }

(vi) Filaments tapering to the apex, like riding-whips.

Mastigothrix, &c.

(β) Filaments joined into zoogloea-masses by intercellular substance.

Myconostoc (Cohn). Filaments cylindrical, colourless.

Chthonoblastus } (Kg.), &c. Filaments cylindrical,
Limnochlide } and containing phycochrome.

Nostoc, *Hormosiphon*, &c. Filaments moniliform, and with phycochrome.

Rivularia (Roth). } Filaments tapering, like riding-whips, and containing phycochrome.
Zonotrichia (Ag.), &c. }

B. Filaments branched falsely.

(α) Without phycochrome.

Cladothrix (Cohn). } Filaments cylindrical.
Streptothrix (Cohn). }

(β) Containing phycochrome.

Calothrix (Ag.). *Scytonema* (Ag.), &c.	Filaments cylindrical.
Merizomyria (Kg.). *Mastigocladus* (Cohn).	Filaments moniliform.
Schizosiphon (Kg.). *Geocyclus* (Kg.), &c.	Filaments tapering, like riding-whips.

It will be remembered that Cohn was attempting a scheme to embrace the whole of the Schizophyta, and not merely the Schizomycetes; and although we now exclude the forms containing 'phycochrome,' as relegated to their proper position among the lower Algae, it seemed advisable to retain them in the above scheme, as Cohn did in his classical memoir. It will be obvious to all who are acquainted with the subject that Cohn's chief divisions have always afforded important bases for subsequent systems of classification of these organisms; though it was soon shown, by Koch, Prazmowsky, and others, that the zoogloea cannot be employed as a distinguishing mark in the sense Cohn employed it, and other characters had to be sought for the primary divisions. I now propose to set forth some of the best known of these systems, which will at the same time mark the main points of progress attained since Cohn's time.

In 1881, Winter published his system, designed for his edition of Rabenhorst, and I append his tabular *résumé*—or key—in its original form, as it best illustrates the author's attempt to make a definite Flora for the group.

TABLE II.—*Winter*, 1881.

1.	Cells spherical or ovoid	2
	Cells cylindrical—short or long . . .	5
	Cells lanceolate, ribbon-like, spirally coiled .	*Spiromonas.*
2.	Cells isolated, or in chains, or grouped in amorphous slime	*Micrococcus.*
	Cells in large numbers, united into colonies with definite contour	3

3.	Colonies hollow, the cells in a single peripheral layer	*Cohnia.*
	Colonies solid throughout, and filled with cells	4
4.	Cells few, and joined in regular families, each with a definite number . . .	*Sarcina.*
	Cells in larger numbers, aggregated in irregular colonies, each with an indefinite number	*Ascococcus.*
5.	Cells shortly cylindrical, isolated, or two or more loosely joined	*Bacterium.*
	Cells as long cylinders, united into filaments	6
6.	Filaments isolated or matted together .	7
	Filaments in rounded gelatinous matrix .	*Myconostoc.*
7.	Filaments unbranched	8
	Filaments with false-branching . . .	*Cladothrix.*
8.	Filaments rectilinear	9
	Filaments spiral or curved	11
9.	Filaments short and distinctly segmented .	*Bacillus.*
	Filaments long, segments usually obscure .	10
10.	Filaments very slender	*Leptothrix.*
	Filaments thicker	*Beggiatoa.*
11.	Filaments short, spiral with a few turns, or merely curved: stiff	*Spirillum.*
	Filaments longer, and with numerous spiral turns, and flexile	*Spirochaeta.*

[Appendix—*Sphaerotilus* and *Crenothrix*.]

In the meantime the controversy had begun as to the meaning of 'species' among the Schizomycetes. Billroth, in 1874, had stated his conviction that all the forms are mere varieties of one fundamental species, and some experiments of Buchner's (1882) with *Bacillus anthracis* and *B. subtilis* (which Buchner thought he had proved to be convertible one with the other) seemed to support the idea. Naegeli, with whom Buchner was associated, took up a similar view, and thus arose the split, already referred to, between the extremists who regarded the polymorphism of the Schizomy-

cetes as universal, and those who committed the error of paying too little attention to the existence of polymorphism in the group.

As usually happens in such cases, the truth lies somewhere between the extremes, and the reader unacquainted with the literature cannot do better than consult De Bary's beautiful fourth lecture on this subject, where the evidence for and against is weighed with the fairness and thoroughness so characteristic of that gifted master of morphology.

Van Tieghem, in 1884, proposed to take into account the planes of division of the cells as furnishing the chief bases for dividing the Schizomycetes into three primary groups, thus :—

TABLE III.—*Van Tieghem*, 1884.

I. Divisions in one plane only. Thallus filamentous, or forming aggregates of segments.
  A. Simple forms.
    (*a*) Non-sheathed.
      (i) Of minute spheroidal cells, in gelatinous matrix or free. May be more or less seriate.
        Micrococcus.
      (ii) Elongated in one plane, and free.
        * Rodlets short and at once free.
          Bacterium.
        * * Rodlets longer, and may remain for a time in series.
          Bacillus.
        * * * Filaments.
          Leptothrix.
      (iii) Elongated in spiral form.
        * Short comma-like twisted rodlets.
          Vibrio.
        * * Longer and helicoid.
          Spirillum.
        * * * Longer still, and with numerous turns.
          Spirochaete.

(β) Sheathed forms.
(i) Unbranched.
Crenothrix.
(ii) With false ramifications.
Cladothrix.

B. Colonial or aggregated forms.
(α) Non-sheathed.
(i) Micrococcus-like cells.
Punctula.
(ii) Rod-like cells.
Polybacteria.
(β) Sheathed.
(i) Micrococcus-like cells.
Ascococcus.
(ii) Rod-like cells.
Ascobacteria.
(iii) With spiral segments.
Myconostoc.

II. The planes of division run in two directions, and the membrane-like surfaces break up into groups of quadrates.
Merista.

III. There are three planes of division, resulting in the development of solid cuboidal masses.
Sarcina.

It may be regarded as an objection to Van Tieghem's system that the three chief divisions are so very unequal, and that some of the characters employed for subdividing the first primary group, which contains nearly all the forms, are of more importance than those used for separating the three main divisions. This criticism seems well-founded if we remember that planes of division only affect the vegetative stages. Van Tieghem himself points out that the division-planes in the second and third groups do not always follow equally rapidly, and in their proper order: a young *Merista* may be uniseriate, and a young *Sarcina* meristate.

It should be stated that Van Tieghem does not himself draw up a detailed table, possibly because he recognised how

difficult it was to put these three groups on the same footing.

The further subdivision of the larger genera was based on the behaviour of the Schizomycetes towards the substratum—chromogenes, zymogenes, and pathogenes respectively—an idea started by Schröter and Cohn, and already partly employed by Winter and others, and one which has gained ground since.

Van Tieghem seems to have relegated the characters derived from the method of spore-formation to quite a subordinate position, whereas De Bary, it will be remembered, elevates this into a diagnostic character of the highest importance.

On the whole, we may regard Van Tieghem's contributions to the classification of the Schizomycetes as consisting in the recognition of the importance of the mode of division and the behaviour towards the substratum. In no other way can it be considered as an advance on Cohn and Ehrenberg's system.

Flügge, who has exerted considerable influence on the pathologists, especially in Germany, arranged the Schizomycetes in groups, much after the method of Cohn. I give his system in Table IV.

TABLE IV.—*Flügge*, 1886.

I. Cells spherical or ovoid.

A. Cells isolated, or merely seriate, or in amorphous aggregates.
Micrococcus.

B. Cells forming colonies more or less definitely circumscribed.

(*a*) Colonies solid and entirely filled with the cells.

(i) Colonies large, irregular, and numbers indefinite.
Ascococcus.

(ii) Colonies small, regular, and numbers definite.
Sarcina.

(*β*) Colonies excavated, with simple layers of cells at the periphery.
Cohnia.

II. Cells cylindrical.

A. Cells as short rodlets; isolated, or aggregated into loosely united or gelatinous families.

Bacterium.

B. Cells several or many times longer than broad, and united into filaments.

(*a*) Filaments isolated, or matted together, or in fasciculi.

(i) Filaments not branching.

* Filaments straight.

† Filaments short and distinctly segmented.

Bacillus.

† † Filaments long and segments indistinct.

Very thin.

Leptothrix.

Thicker.

Beggiatoa.

* * Filaments undulate or spiral.

† Short and rigid.

Spirillum (*Vibrio*).

† † Long and flexile.

Spirochaete.

(ii) Filaments with false ramification.

Cladothrix (*Streptothrix*).

(*β*) Filaments enveloped in rounded gelatinous matrix.

Myconostoc.

The chief advance here, in addition to the expurgation of certain genera no longer admitted as Schizomycetes, is the greater clearness in definition of the forms, gained partly by the fusion of trivial genera, and partly by the expression of the diagnostic characters. Nevertheless, Flügge's modification of Cohn's system suffers from the same defects as Van Tieghem's and the other older schemes, namely, that the forms selected as types are often only form-genera, and we undoubtedly meet with transient phases of one and the same filamentous genus which would be placed in two or more genera if such a system were rigidly followed.

Hueppe, whose book on methods, especially, has deservedly attained a world-wide reputation, has proposed a scheme

which brings into the foreground De Bary's suggestion that the distinction between endosporous and arthrosporous forms is a real one, and should be insisted upon: Hueppe, however, does not make the distinction so fundamental as De Bary proposed, but employs it as a subsidiary character, as Table V will show.

TABLE V.—*Hueppe*, 1886, and modified later.

I. Vegetative stage Coccoid.
  A. Cocci seriate in single chains.
    (α) In zoogloea-masses of medium size.
      (i) With endospores.
        Endo-streptococcus.
      (ii) Without endospores.
        Arthro-streptococcus.
    (β) In pronounced zoogloea-masses.
      Leuconostoc.
  B. Cocci in fours, or in short chains.
    Devoid of endospores (?), arthrospores only (?).
      Merista.
  C. Cocci in fours or eights, but not in chains. Endospores or not.
      Sarcina.
  D. Cocci in irregular masses of various kinds.
    (α) No definite arrangement.
      Micrococcus.
    (β) Grouped like bunches of grapes.
      Staphylococcus.
    (γ) In rounded zoogloea-masses.
      Ascococcus.

II. Vegetative stage rod-like.
  A. Forming filaments or single cells, flexile or rigid, more or less segmented or not, and with no distinction into base and apex.
    (α) Filaments straight or undulate, arthrosporous. No endospores.
      Bacterium.
    (β) Filaments straight or undulate or spiral. Arthrosporous only.
      Spirulina (*Proteus*).

(γ) Filaments straight or undulate, and with endospores.

(i) Rodlets not altered in shape during sporification.

Bacillus.

(ii) Rodlets fusiform, or undergo some changes in shape as spores form.

Clostridium.

B. No filaments, but spindle-shaped rods which undergo division in the *longitudinal* direction, and develope endospores.

Pasteuria.

C. Filaments, differentiated into base (usually fixed) and apex.

(α) Filaments not distinctly septate or divided, and without sheath.

(i) Devoid of sulphur granules.

Leptothrix.

(ii) Containing sulphur granules.

Beggiatoa.

(β) Filaments segmented and sheathed.

(i) Unbranched.

Crenothrix.

(ii) Branched (false branches).

Cladothrix.

III. Vegetative stage consisting of spiral filaments or segments, flexile or rigid.

(α) Arthrosporous only.

Spirochaete.

(β) Endosporous.

(i) No alteration in form of the sporogenous cells.

Spirillum.

(ii) The cell changes in shape as the spores are developed.

Vibrio.

The main advances in the De Bary-Hueppe scheme are, besides the distinct one of the employment of the spore-characters, the much clearer rendering of the diagnoses derived from the vegetative forms, and the embracing of the new types *Clostridium* of Prazmowsky, and (subsequently) *Pasteuria* of Metschnikoff. There is also a much more thorough analysis of the various forms allied to *Micrococcus*, though the diffi-

culties of this type are by no means overcome. Hueppe, among his numerous other contributions to bacteriology, has shown clearly how much the formation of zoogloea depends on circumstances, and is therefore a character to be employed very cautiously in distinguishing genera and species.

Zopf, in 1885, devised a scheme of classification which, in spite of the admitted difficulties in practical application, has the merit of being a very praiseworthy attempt at a scientific summary of our knowledge. It differs from the preceding especially in that the author tries to bring out the polymorphy of the Schizomycetes. Zopf divides these organisms into four main groups, as shown in Table VI.

TABLE VI.—*Zopf*, 1885.

I. Coccaceae.

Only cocci or serial chains or groups of cocci, so far as is known. No spores known. Divisions in 1, 2, or 3 planes.

A. Divisions in one plane only; the cocci in moniliform series, but separating later.

Streptococcus.

B. Divisions in two planes at right angles, leading to the formation of plates; the cells separating eventually.

Merismopedia.

C. Divisions in three planes, and therefore leading to the formation of packet-like colonies; the cells separating later.

Sarcina.

D. Divisions in one plane only, and the cocci separate at once, forming irregular or botryoid groups.

Micrococcus (with *Staphylococcus*).

E. Like *Micrococcus*, but the colonies immersed in dense gelatinous investment.

Ascococcus.

II. Bacteriaceae.

Usually presenting coccus—(may be absent),—rodlet,—and filamentous forms; the rodlets and filaments being spirally curved or straight, and presenting no difference between base and apex. Divisions in one plane only, so far as known. Spore-formation known in some: in others unknown and perhaps absent.

A. Cocci and rodlets, or only rodlets known, and arranged in linear series or filaments. No spores known.

Bacterium.

B. Filaments spiral: segmented into rodlets (long or short), or into rodlets and cocci. No spores known.

Spirillum.

C. Filaments spiral, and forming spores in the long or short segments.

Vibrio.

D. Develope cocci and rodlets, the former containing spores.

Leuconostoc.

E. Rodlets, or rodlets and cocci, in linear or spiral filaments. Spores developed in the rodlets or in cocci.

Bacillus.

F. As *Bacillus*, excepting that the spŏrogenous rodlets are peculiarly swollen.

Clostridium.

III. Leptotricheae.

Filaments, which are differentiated into base and apex, and may be linear or spirally curved, segmented into rodlets and cocci. Spores unknown.

A. Filaments sheathed. Cells without sulphur-granules. Aquatic forms.

Crenothrix.

B. Filaments not sheathed. The segments with sulphur-granules. Aquatic.

Beggiatoa.

C. Filaments not sheathed, and much divided up by numerous successive septa. No sulphur granules. Aquatic.

Phragmidothrix.

D. Filaments sheathed or not; segmentation not remarkable. Cells devoid of sulphur granules.

Leptothrix.

IV. Cladotricheae.

Filaments falsely branched. Breaking up into cocci, rodlets, and straight and coiled filamentous segments. No spores[1] known.

Cladothrix.

[1] This is no longer the case according to Billet.

Zopf's classification, admirable as it is in many respects, is difficult to work in practice, because it is necessary to have all the stages of development before we can decide on the position of a species: at the same time it should be noted, that in this very respect it is as far ahead of the merely tabular classifications, used for hurriedly determining the name of a form, as a good Flora is ahead of a mere museum catalogue of plants.

It is, in fact, just in respect of this particular attention to all the facts in the *development* of the species that Zopf's classification is scientifically so far in advance of his predecessors. Unquestionably it renders the problems more difficult, because it insists on the working out of all the phenomena before a species is accepted; but, since such a scheme must embrace all the merely diagnostic *form-characters* used by the Cohn-school, it must be admitted to be superior to their system. The matter of difficulty of application, in such a connection, cannot be urged as a reason for desisting from obtaining and recording all that can be discovered regarding an organism.

The only really valid objection to a purely scientific classification is the old objection of the purely utilitarian 'practical man,' and even then the validity of the objection is relative. This leads me to bring out the point that the bacteriologists, in the widest sense of the word, are really looking at the question of classification from at least two very different points of view. On the one hand we have the botanists, who direct their attention to the organism, the Schizomycete, itself, as a biological phenomenon to be examined and reported upon as thoroughly as possible: for them, no classification is complete which does not record, or (what amounts to the same thing) imply in its records, all the life-phenomena of the organism, including its pedigree.

On the other hand we have the pathologists, hygienists, brewers, chemists, &c., who regard the organism simply as an object to be named for convenience in reference, because it brings about certain changes in the tissues, waters, and other

media which they are more especially concerned with. They do not care, and naturally so, what vagaries the organism exhibits, so long as they can recognise it when they meet with it.

As matter of experience, however, it is just these vagaries that bring about the sources of error which beset them on all hands, and hence they are equally interested with the botanist in having them cleared up, and explained.

It must not be overlooked, moreover, that many of them are alive to the dangers referred to, as witness Cassededebat's industrious and able investigation of the differences between the true typhoid bacillus, and the various false ones which simulate it: also the numerous researches which have been made on the distinguishing characters between *Bacillus subtilis* and *B. anthracis*, and so on.

Whence we come to the conclusion that, whatever may be believed to the contrary, the real interests of 'bacteriologists' of all kinds are identical. Exactly the same kind of discussions, and apparent difference of interests, arise in the relations of Forestry, Agriculture, Horticulture, &c., to Botany; but in these cases also the broadest thinkers all recognise the true state of affairs.

At the same time, botanists must concede that the big special problem of working out these life-histories, and of compiling the ideal classification, still a long way ahead of us, devolves upon themselves. It is useless to merely criticise the imperfect tabular classifications of the pathologists and hygienists and others: the only thing to do is to take the organisms in hand and expose their vagaries by cultivating them under the microscope, and subjecting them to the tests devised by modern morphologists and physiologists.

The most recent and the most thorough classification of the Schizomycetes extant, is that of De-Toni and Trevisan, published in 1889 in Saccardo's 'Sylloge Fungorum.' It embraces the description of more than 650 species, and may be taken as the most complete account of the Schizomycetes, from the systematists' point of view, that has ever been

attempted. When we reflect that Winter, even so lately as 1881, only described sixty-nine species, we obtain some idea of the extraordinary activity which has been displayed within the last ten years. I append Trevisan and De-Toni's scheme in tabular form.

TABLE VII.—*De-Toni and Trevisan*, 1889.

I. Trichogenae.

Presenting three vegetative stages—filaments, rodlets and cocci. The filament is the typical individual, sheathed or not, and is usually differentiated into apex and base, the plants being fixed by the latter and radiating from a central point. Some have no distinction between base and apex. Rodlets and cocci enclosed in the filaments.

A. Spores (arthrospores) developed in special sections of filaments (pseudo-sporangia) (**Crenotricheae**).

Crenothrix. Filaments simple, sheathed.

B. Spores (arthrospores) in the normal filaments.

(*a*) Filaments falsely branched (**Cladotricheae**).

(i) Sheathed.

Sphaerotilus. Filaments uniform in diameter from base to apex. Arthrospores very numerous. Divisions in three planes.

Cladothrix. Filaments widening upwards. Arthrospores developed in pairs in individual rodlets.

(ii) Filaments devoid of sheaths.

Nocardia. Arthrospores produced by the transformation of cocci.

(*β*) Filaments simple (**Kurthieae**).

(i) Arthrospores 4–5 in individual rodlets.

Detoniella.

(ii) Arthrospores consisting of transformed cocci.

Rasmussenia. Filaments fixed below.

Kurthia. Filaments equal throughout and free.

C. Spores absent, or unknown. Filaments simple (**Leptotricheae**).

(*a*) Filaments sheathed and differentiated into base and broader apex, fixed.

Leptotrichia. Reproduced by rod-shaped gonidia.

(β) No sheaths, equal in diameter throughout. No rod-like gonidia.

Phragmidothrix. Fixed. Reproduced by numerous cell-divisions in two planes, longitudinal and transverse.

Beggiatoa. Free. Divisions transverse only.

[Appendix *Agonium.*]

II. Baculogenae.

Presenting three stages, as before, filaments, rodlets, and cocci; but here the rodlet is the typical individual, and gives rise to the filaments and cocci. Filaments transitory, free, not sheathed, and with no distinction into base and apex: merely due to the prolongation of rodlets as yet imperfectly segmented.

A. Rodlets and cocci nude—i.e. with no special investment or 'capsule' (**Bacilleae**).

(α) Endosporous.

(i) Rodlets dividing by repeated *longitudinal* divisions (**Pasteurieae**).

Pasteuria. Rodlets inaequipolar. Spores.

(ii) Rodlets dividing by repeated *transverse* divisions.

* Rodlets connected into a network (**Thiodictyeae**).

Thiodictyon. Rodlets aequipolar.

* * No reticulated coenobium.

† Rodlets straight or curved, but never *spirally* twisted (**Eubacilleae**).

§ Spores not larger than the major transverse diameter of their mother-cells.

ℙ Spores developed in normal and unaltered rodlets.

1. Contents of rodlets homogeneously diffused.

Mantegazzaea. Rodlets fusiform.

Bacillus. Rodlets cylindrical.

2. Contents bipolar.

Pasteurella.

ℙ ℙ Spores developed in specially swollen ellipsoidal or fusiform rodlets.

Clostridium. Contents uniform.

§ § Spores with diameter greater than the transverse diameter of mother-cells.

Cornilia. Spores in normal rodlets of which the median part swells.

Vibrio. Spores in special rodlets with a swollen apex.

† † Rodlets spirally coiled (**Spirilleae**).

Spirillum. Rodlets cylindrical. Spores smaller than mother-cells.

Spiromonas. Rodlets compressed. Spores unknown.

(β) Arthrosporous.

Pacinia. Rods cylindrical, straight or curved. Filaments often undulous-flexuose or with irregular false spirals.

Bacterium. Rodlets ellipsoid, straight. Filaments never with false spirals.

B. Rodlets and cocci invested with a special membrano-gelatinous 'capsule' (**Klebsielleae**).

(α) Rodlets straight or curved, never spirally twisted (**Eu-Klebsielleae**).

(i) Capsule repeatedly branched.

Winogradskya.

(ii) Capsule simple, never branched.

Klebsiella. Contents uniformly diffused in rodlets.

Dicoccia. Contents of rodlets bipolar.

(β) Rodlets spirally twisted.

Myconostoc.

[Appendix *Cystobacter*—see *Winogradskya* (?).]

III. Coccogenae.

Exhibiting one condition only—i. e. cocci.

A. **Ascococceae.**

Cocci associated in colonies and surrounded by a firm gelatinous investment, or cyst.

(α) Cocci segregated in the mucous matrix.

(i) Cocci destitute of special cysts, but gathered together in families invested by the universal cyst (**Eu-Ascococceae**).

* Cocci very numerous and grouped in large families.

† Cysts homogeneous, not lamellated.

Lamprocystis. Families solid, and then hollow, and eventually irregularly clathrate. Cocci dividing at first in three, and then in two planes.

Ascococcus. Families solid at all ages. Cocci dividing in one plane.

†† Cysts lamellated.

Bollingera. Families solid in all stages. Cocci dividing in three planes.

* * Cocci not very numerous. Families small.

† Cysts pluri-lamellose.

Leucocystis. Cocci dividing in three planes.

†† Cysts homogeneous, not lamellated.

Cenomesia. Cysts very large and dense. Cocci grouped at the periphery, in families which eventually become hollow. Divisions at first in all planes, then two only.

Thiothece. Cysts rather large and dense, persistent. Cocci sparse and remote. Divisions in one plane.

Thiocystis. Cysts large, subdeliquescent. Cocci in small crowded families. Divisions in three planes.

(ii) Cocci surrounded by special cysts: no universal cysts. (**Gaffkyeae**).

Chlamydatomus. Cysts firm, persistent, numerous, in dense groups, solid throughout.

Gaffkyea. Cysts tenuous, eventually diffluent, solitary, never in dense groups.

(β) Cocci joined loosely into filamentous series in the mucous matrix. Universal cysts tenuous, and soon deliquescing. No special cysts. (**Amoebacterieae**).

Amoebobacter. Cocci dividing in one plane.

B. **Sarcineae.**

Cocci in strata, one or more deep, and surrounded by more or less evident mucous matrix. No cysts. Endospores smaller than the mother-cells—cocci—which produce them.

(α) Cocci closely packed in firm cartilaginous-mucous matrix.

Thiopolycoccus. Cocci densely grouped without order, in irregular compact families. Divisions in one plane.

Sarcina. Cocci in regular, cubical, closely packed families of eight. Divisions in three planes.

(β) Cocci loosely aggregated in a flattened mucous matrix.

Lampropedia. Cocci loosely grouped in fours, regularly distributed in a firm mucus in flat tables, like parallelograms. Cocci dividing in two planes.

Thiocapsa. Cocci few, in irregular families, in a firm flat mucous membrane, without definite outlines, loosely associated without order. Divisions in three planes.

Pediococcus. Cocci in fours, the small regular families loosely connected in one stratum, enveloped by an amorphous, thin, hardly conspicuous deliquescent mucus. Divisions in two planes.

C. **Streptococcaceae.**

Cocci in moniliform chains. Arthrospores, larger than the mother-cells, developed in or at end of chains.

(α) Filaments (chains) in membranous gelatinous capsules.

Leuconostoc. Capsule large, dense, lamellose.

Schuetzia. Capsules compressed, thin, not lamellated.

(β) Filaments (chains) in cylindrical sheaths.

Perroncitoa. Sheath membrano-gelatinous.

(γ) Filaments (chains) devoid of capsule or sheath.

Babesia. Filaments falsely dichotomous, with arthrospores at apex.

Streptococcus. Filaments simple, with scattered arthrospores.

D. **Micrococceae.**

Cocci devoid of either cysts, capsules, or definite sheaths of

any kind, and not arranged in chains. Endospores in cocci, and smaller than they are.

Neisseria. Cocci paired.

Staphylococcus. Cocci in botryoidal groups.

Micrococcus. Cocci solitary, or scattered without order in amorphous zoogloea-masses.

Unquestionably a large number of the species are 'bad'; that is to say they are so imperfectly described that one cannot forthwith recognise a given form as belonging to a species recorded in the monumental volume under review; but it is by no means the least valuable function of a work like this to show in what directions more remains to be done, and this alone would have justified the publication of the one hundred and sixty odd pages of closely packed, and industriously compiled, information in this book.

But the treatise in question does much more than that. It shows what great advances are being made in the discovery of new *types* of Schizomycetes, as a glance at the table will show, and how (as a natural consequence) new ideas as to the relative value of characters have to be entertained.

This brings me to another phase of the subject in general. The real difficulty in classifying organisms like Schizomycetes is not so much that they are so small, especially in these days of homogeneous immersions and improved staining and illuminating methods, as that (largely consequent on their minuteness, it may be admitted) they exhibit so few morphological characters. A Fungus, like *Mucor* or *Penicillium*, has organs and differentiated parts which can be described very definitely; but when one deals with minute structures like *Micrococcus* or *Bacterium* the case is different.

Now the researches of the last fifteen years or so have brought to light numerous points which can be made use of in classifying these tiny specks of living matter, quite apart from their shapes and sizes, and those of their spores, capsules, zoogloeae, &c., and Trevisan and De-Toni have made considerable use of these accessory characters, which, by the bye, we

owe very largely to the efforts of the non-botanists as well as to those of the botanists.

Some of these characters had already been drawn into use, e. g. the chromogenic, zymogenic, or pathogenic powers, but there are others which are coming more and more into use as the subject progresses. Such are the shapes, colours, and mode of extension of the colonies in the mass, when grown on certain solid media, and especially gelatine and agar-agar: the powers of the colonies to liquefy the gelatine, by peptonising it, and the shapes and mode of progress of the excavations made. We owe nearly all these characters, and especially the systematisation of them, to the non-botanists of the Koch-Flügge school.

Then, again, more attention is being paid to the temperatures at which the cultures flourish—the optimum-temperatures as Sachs has it. There are forms which will grow at temperatures as low as 0° C., and there are others which will grow, not merely live but *grow*, at 60° to 70° C. and even slightly beyond, e. g. Miquel's *Bacillus thermophilus*; and a whole host of species are known which flourish below 20° C., as contrasted with species which require 30° or 40° C., and something has been done towards utilising these characters for classifying the Schizomycetes.

Every one now knows that, as Pasteur first discovered, some Bacteria are anaerobic, while others are aerobic, facultative or obligate in each case as may be, and these peculiarities have been pressed into the service.

Miquel has, only this last year, proposed to employ such characters as the above for drawing up a 'bacterial flora,' for the use of specialists who are engaged in the analysis of water. As it is both interesting and instructive—I shall criticise some of the points later on—I have appended the outline in Table VIII.

TABLE VIII.—*Miquel*, 1891.

Miquel first separates the aerobian from the anaerobian forms, subdividing according to the temperatures, as follows:—

I.	II.
Aerobian, growing	*Anaerobian*, growing
at 20° C. . . = Section A	at 20° C. . . = Section D
only above 20° C. = „ B	only above 20° C. = „ E
only above 40° C. = „ C	only above 40° C. = „ F

He then proposes to break up the 'Sections' into 'Tribes,' as follows:—

Section A—i. e. Aerobian species which grow at 20° C.

		TRIBE
(α) Cells = *Cocci*	Pathogenous . . . =	I
	Zymogenous . . . =	II
	Saprogenous . . . =	III
(β) Cells in *Filaments*	Pathogenous . . . =	IV
	Zymogenous . . . =	V
	Saprogenous . . . =	VI
(γ) Cells = *Spirilla*	Pathogenous . . . =	VII
	Zymogenous . . . =	VIII
	Saprogenous . . . =	IX
(δ) Cells of other forms	Pathogenous . . . =	X
	Zymogenous . . . =	XI
	Saprogenous . . . =	XII

Section B is then divided up in similar fashion.

The following tabular statement shows how Miquel then proceeds to further subdivide each 'Tribe' of each 'Section' into 'Groups,' according as the forms will or will not grow on nutrient gelatine, the colour and other peculiarities of the colonies, and so on.

Aerobian forms.

Developing at 20° C.

As cocci, which are pathogenous = Tribe I.

* Growing on ordinary nutrient gelatine.

† Colonies white or grey.

§ Liquefying the gelatine . . = Group 1

§ § Non-liquefying . . . = „ 2

† † Colonies yellow or yellow-greenish.
§ Liquefying . . . . = Group 3
§ § Non-liquefying . . . = „ 4
† † † Colonies red or reddish.
§ Liquefying . . . . = „ 5
§ § Non-liquefying . . . = „ 6
* * Will not grow on ordinary nutrient gelatine.
† Grow in alkaline gelatine.
§ Colonies whitish, &c.
(i) Liquefying . . . = „ 7
(ii) Non-liquefying . . = „ 8
§ § Colonies yellowish, &c.
(i) Liquefying . . . = „ 9
(ii) Non-liquefying . . = „ 10
§ § § Colonies reddish, &c.
(i) Liquefying . . . = „ 11
(ii) Non-liquefying . . = „ 12
† † Grow in acid gelatine.
§ Colonies whitish, &c.
(i) Liquefying . . . = „ 13
(ii) Non-liquefying . . = „ 14
§ § Colonies yellowish, &c.
(i) Liquefying . . . = „ 15
(ii) Non-liquefying . . = „ 16
§ § § Colonies reddish, &c.
(i) Liquefying . . . = „ 17
(ii) Non-liquefying . . = „ 18
† † † Grow on blood-serum . . . = „ 19
† † † † Grow in broth.
§ Producing turbidity . . . = „ 20
§ § Forming deposits . . . = „ 21
§ § § „ films at surface . . = „ 22
† † † † † Grow in *animal* juices sterilised *without heating*.
§ Producing turbidity . . . = „ 23
§ § Forming deposits . . . = „ 24
§ § § „ films at surface . . = „ 25
† † † † † † Grow in *vegetable* juices sterilised *without heating*.
§ Producing turbidity . . . = „ 26

§ § Forming deposits . . . = Group 27
§ § § „ films at surface . . = „ 28
† † † † † † † Grow in *mineral* solutions.
§ Producing turbidity . . . = „ 29
§ § Forming deposits . . . = „ 30
§ § § „ films at surface . . = „ 31

Tribes II, III, &c. to XII are then broken up in the same way, and then each group—1 to 31—is subdivided according to its microscopic characters as follows. I have put it into tabular form.

I. Aerobian forms.
Section A, growing at 20° Centigrade.
(*a*) The organism is a coccus.
Tribe I. Pathogenous forms.
* Capable of growth on ordinary nutritive gelatine.
† Colonies white or grey.
§ Liquefying the medium.
ℙ **Monococcus.**
Colonies white.
Colonies grey.
Colonies iridescent.
ℙ ℙ **Diplococcus.**
Colonies spherical.
Colonies discoid.
Colonies lamelliform.
ℙ ℙ ℙ **Streptococcus.**
Colonies mamillated.
Colonies with prolongations.
Colonies irregular.
ℙ ℙ ℙ ℙ **Tetracoccus.**
Colonies radiating.
Colonies motile.
Colonies amoeboid.
ℙ ℙ ℙ ℙ ℙ **Sarcinae.**
Colonies very opaque.
Colonies translucent.
Colonies concentrically zoned.

And so on with each of the other groups.

Miquel's scheme, by no means the only one of the kind, it

should be stated, is only suggested as a possible way out of a well-known and much felt difficulty, namely, the very natural one that obtrudes itself on the non-botanical bacteriologists, who meet with numerous forms of Schizomycetes in their records, of rapidly identifying these forms and learning whether the same have been met with before. I am scarcely concerned here with the question whether such knowledge is worth anything or nothing: personally, I feel that all conscientious comparative records are valuable, however much we may deplore the fact that these forms are usually merely recorded, and not studied further.

The first character employed by Miquel is that of aerobism. Now it is in some cases extremely difficult to determine whether a Schizomycete is aerobian or not, but of course the question is more easily answered if the organisms are always cultivated on or in the same medium. There is evidence to show, however, that an organism may be anaerobian in saccharine solutions, but aerobian on gelatine, whence difficulties may arise to those who neglect such facts.

Miquel's second character is the temperature. This is a relatively easy point to make out in some cases, but it presents undoubted difficulties where the optimum-temperature lies close to the demarcation point (20° C.) selected, and it is by no means clear how we are to get over these difficulties. In any case the character ceases to be useful where the optimum-temperature is 18–22° C.

Miquel's third diagnostic character is the form of the organism. Obviously this is subject to all the criticism that has been accorded to the morphological systems referred to ; but I may now point out a truth which is frequently overlooked by those who criticise too severely the attempts of the systematists, namely, that if we have two aerobian Schizomycetes, capable of growth at the *same* temperature on the *same* medium, then if one of them persists in developing as a *Micrococcus* and the other as a *Bacillus*, we are justified in regarding them as distinct species. True, the converse does not follow, if both grow as *Micrococci* or as *Bacilli* they may

or may not be distinct; but we must be thankful for small mercies where Schizomycetes are concerned, and a great point is gained when we have, as here, good grounds for a safe conclusion. It does not affect the truth of the above statement if the *Micrococcus*-like form gives rise to a *Bacillus*-like form, or the *Bacilli* to *Micrococci* on *different media*, or under different conditions: the only fairly comparable cases are those where the forms are growing under like circumstances. This has now been recognised for some time by many of the workers in both the French and German schools of bacteriology, as reference to the works of Flügge, Hueppe, Eisenberg, Miquel, Macé, and others abundantly testify.

Miquel then proceeds to employ a character very difficult of application in this country, because the question whether a Schizomycete is pathogenic, zymogenic, or chromogenic is not answered forthwith by the circumstance of finding the given form in the tissues, or in a fermenting medium, and so on. It can only be determined by experiment, and I need not refer to the difficulties set up in this country owing to the clamour and activity of a possibly well-meaning, but certainly ill-informed, faction of sentimentalists.

The next character employed by Miquel is an exceedingly useful one in general. If we take a sample of water, containing several forms of Schizomycetes, as almost all waters do, and distribute it equally in nutrient gelatine, in beef-broth, and in solutions containing sugar, the resulting growths are certain to differ, and often differ enormously. I assume that the conditions as to temperature, access of air and light, &c. are the same.

The question then arises, are the differences due to the fact that the initial sample of water contained a number of aerobian species, capable of growing at the chosen temperature, equal to the aggregate number of forms found in the three media? This question is a perfectly pertinent one, and we could put another, namely, are the different forms met with in the three different media mere adaptation-forms to these media?

The fact which militates most distinctly against the latter view is that there is no evident correspondence between the numbers of the forms in the three different media. But, on the other hand, there is experimental evidence to show that a form which grows like an ordinary *Bacillus* in a saccharine medium may look very different if cultivated in beef-broth, and so on. Such facts should make us very circumspect in dealing with such cases as mixed cultures.

The case is different, however, when we are deciding as to the identity or distinctness of two pure cultures. If we find that, other circumstances being equal, one of the forms will grow readily on gelatine, but the other will not, then the conclusion is justified that they are distinct: the converse is not true, however. It may be remarked here that a close examination of the literature shows abundantly that many bad records are due to negligence of these, now obvious, precautions that all the circumstances of comparison should be equal, including even the apparently trivial, but really important one, that the nutritive gelatine, broth, or other medium, should be of the same stock and make.

Having once 'run a form down' to this point, it is pretty clear that Miquel's further characters—the importance of which has been recognised more and more since cultivation on solid media was introduced by Brefeld and Koch—are both distinctive and, on the whole, easy of application. The colour and shape of the colonies, the liquefaction of the gelatine, the formation of scums, production of pigments, the shape of the cells and their mode of aggregation, and so forth, are all points comparatively easy to observe, and their utility needs no comment.

It is pretty clear then that a scheme like this of Miquel's, if properly and consistently applied, is calculated to perform two great functions in advancing our knowledge of Schizomycetes.

In the first place, it satisfies the subsidiary requirements of the specialist who merely wants to 'spot' a given form, and, as said, we are not concerned in criticising the desirability of that object.

In the second place, it records and classifies a number of facts of great value to the systematist and to the physiologist. True, it leaves him the trouble of putting the facts into his schemes, but I see no valid objection to that, as it is naturally part of his work.

The only objection to such schemes as the one just criticised seems to be that they obviously lead to the creation of 'multiple species'; because, since the pathologist tabulates one set of forms, the water-analyst another, the sewage-examiner another, the agricultural expert another, and so on, we have the difficulty of unravelling these various records.

Unfortunately this last criticism is at present the more cogent because no one scheme has as yet been decided upon, and every book on the subject propounds a different scheme.

I will simply illustrate the last remark by the following table taken, in outline, from Woodhead's recent little book, Bacteria and their Products, since it shows the application of a similar scheme to pathological forms—not entirely, but chiefly. I only select a few of the species to illustrate each group.

TABLE IX.—*Woodhead*, 1891.

1. The organism is a **Micrococcus.**
   - I. Grows on gelatine, but does not liquefy it.
     - A. The colonies are white.
       - (*a*) Colonies small, not confluent, slow-growing.
         - *Streptococcus pyogenes.*
         - *S. erysipelatosus.*
         - *S. pyogenes malignus*, &c., &c.
       - (*β*) Colonies confluent, and grow luxuriantly.
         - (i) Cocci arranged irregularly.
           - *Micrococcus candicans.*
           - *M. ureae.*
           - *Staphylococcus cereus albus.*
         - (ii) Cocci arranged like a dumb-bell—diplococci.
           - *Diplococcus lacteus faviformis.*
           - *D. albicans amplus*, &c.
         - (iii) Cocci arranged as *Sarcinae.*
           - *Micrococcus tetragenus.*

B. The colonies are yellow.

(*a*) The colonies form raised drops.

Staphylococcus cereus flavus.

Sarcina lutea, &c.

(*β*) The colonies form flat deposit-like masses.

Micrococcus versicolor.

C. The colonies are red.

Micrococcus cinnabareus.

M. roseus, &c.

D. The colonies are black.

Black 'torula' (not a Schizomycete).

II. The gelatine is liquefied.

A. The colonies are white.

Staphylococcus pyogenes albus.

Micrococcus ureae liquefaciens.

Sarcina alba, &c.

B. The colonies are yellow.

(*a*) The liquefaction proceeds slowly and imperfectly.

Micrococcus flavus desidens, &c.

(*β*) The gelatine becomes completely fluid.

(i) Colonies confined to the centre of the liquefying area.

Staphylococcus pyogenes aureus.

(ii) Colonies both in centre and at periphery of liquefying area.

Micrococcus radiatus.

M. flavus liquefaciens, &c.

III. There is no obvious growth on gelatine at 22° C.

Diplococcus intracellularis meningitidis.

Micrococcus pyogenes tenuis, &c.

2. The organism is a **Bacillus**.

I. The nutrient gelatine is not liquefied.

A. Colonies white, no staining of the gelatine near the growth.

(*a*) Colonies as minute translucent drops on plates—as delicate growths in streak- or puncture-cultures.

Bacillus cholerae-gallinarum.

B. septicus agrigenus, &c.

(β) Colonies colourless, forming thin films on plates, &c.

(i) Odourless.

Bacillus acidi-lactici.

B. typhosus (Eberth).

Bacterium coli commune, &c.

(ii) Distinctly odorous.

Bacillus ureae.

B. pyogenes foetidus, &c.

(γ) Colonies form white 'nail-head projections' on plates, &c.

(i) Colonies microscopically small, with granular margins.

Bacterium pneumoniae, &c.

(ii) Colonies with smooth borders.

Bacterium lactis aerogenes, &c.

(δ) Colonies branched irregularly, not circumscribed.

Bacterium Zopfii.

B. Colonies colourless, but the gelatine near is stained.

(α) Staining greenish.

Bacillus erythrosporus, &c.

(β) Staining blue or greyish brown.

Bacillus cyanogenus.

(γ) Staining violet.

Bacillus janthinus.

C. Colonies cream-coloured.

Bacillus of septic pneumonia.

D. Colonies yellow.

Bacillus luteus.

B. fuscus, &c.

II. The nutrient gelatine is liquefied.

A. Colonies white; nutrient substratum not coloured.

(α) Colonies branched, or with processes.

(i) Colonies not motile.

Bacillus anthracis.

B. ramosus liquefaciens.

B. subtilis, &c.

(ii) Colonies motile and swarming, rapidly liquefying the gelatine.

Proteus vulgaris, &c.

(β) Colonies circumscribed, without branches.

(i) Bacilli large—2·5 μ broad.

Bacillus megaterium.

(ii) Bacilli not more than 1 μ broad.

* Developing *Clostridium* forms before sporification.

Clostridium butyricum, &c.

* No *Clostridium* forms.

Bacillus mesentericus vulgatus, &c.

B. Colonies or substratum coloured.

(α) Colouring-matter red.

Bacillus prodigiosus, &c.

(β) Colouring-matter green.

Bacillus fluorescens-liquefaciens, &c.

(γ) Colouring-matter violet.

Bacillus violaceus.

III. The organisms will not grow on nutrient gelatine, and only on other media at higher temperatures, and in the presence of air.

Bacillus tuberculosis.

B. mallei, &c.

IV. Organisms anaerobic—i. e. will not grow in presence of air.

Bacillus tetani.

B. butyricus, &c.

V. Organisms described in the tissues, but will not grow under ordinary conditions in cultures outside the body.

Bacillus Leprae, &c.

3. The organism is a **Spirillum.**

(i) Gelatine liquefied.

Spirillum cholerae-asiaticae, &c.

(ii) Gelatine not liquefied.

Spirillum rubrum, &c.

(iii) Not yet cultivated on artificial media.

Spirillum Obermeieri, &c.

This leads me to the enunciation of a suggestion which I think might occupy the attention of experts at the next Hygienic Congress, and might, it seems to me, guide us to a

path out of the profound wilderness now obscurely darkening our maps under the name of bacteriology. The suggestion is that botanical bacteriologists and the bacteriologists engaged in pathological, hygienic, and other departments of science, meet and attempt to determine some international scheme for recording the peculiarities of the Schizomycetes they meet with, and see if some common ground of agreement cannot be attained.

In conclusion, it is important that all who are interested in the study of Bacteria should try to obtain answers to as many as possible of the following questions before they publish a 'new species.' These questions have been formulated gradually from the experience of numerous workers since Cohn's time, and I have already shown how the answers to them lend themselves to what systems of classification we possess. Obviously a complete description of a species requires an answer to all of them, and possibly others.

1. *Habitat*:—

This should be carefully recorded, under such headings as Air, Soil, Water (Fresh, Stagnant, Sea, Thermal, Mineral, &c.), Milk, Food, Faeces, Dead or living Animals, Plants, &c.

2. *Nutrient medium*:—

The best pabulum should then be sought for—the organism having of course been separated by suitable methods, and obtained as pure cultures. It should be stated clearly whether it will grow on gelatine, agar, or potatoes, or in broth, saccharine liquids, mineral solutions, &c., and its further behaviour traced on or in that which suits it best. In deciding this point it should be clearly observed whether the medium serves best when neutral, or slightly acid or alkaline.

3. *Gaseous environment*:—

It is important to determine whether the Schizomycete is aerobian or anaerobian, as many forms which will not grow on or in the above or other media in air, will do so when the free access of oxygen is suppressed, partially or entirely. It should also be noted whether carbon-dioxide, hydrogen,

or nitrogen affect this matter; and experiments *in vacuo*, or under pressure may give further information.

4. *Temperature*:—

The range of temperature within which growth and other functions are carried on should be clearly recorded; and the *optimum-temperature* is even more important than the *maximum* and *minimum* cardinal points. It is best to determine these most in detail with the organism growing on or in the best nutrient medium; and it must be remembered that the cardinal points are not necessarily the same for all media.

5. *Morphology* and *life-history*:—

It seems advisable to defer the working out of the biological details until the best conditions of growth, &c. have been determined on pure mass-cultures. The prevailing forms of cells will of course be recorded, and cultures (examined from time to time, or, better, continuously observed under the microscope), must be made to determine the morphological changes. The shapes, sizes, mode of union, and sequence of division in the growth-forms; development of zoogloeae; aggregation into colonies, presence of sheaths, capsules, cysts, matrix, &c.; the development of spores—endospores or arthrospores: motile forms, cilia; flexibility or rigidity of filaments; involution forms, &c. all come under this head.

6. *Special behaviour*:—

If spores are obtainable, the further peculiarities should present fewer difficulties, except in abnormal cases—which exist, however. The growth on gelatine should give characters of the following kind; but it must be noted that these characters may vary if the conditions are varied, and precautions taken accordingly. Answers should be obtained to at least the following questions:—Does the organism peptonise and liquefy the gelatine? If so, is the liquefaction complete? If incomplete, what is the shape and course of the liquefying area, funnel-shaped, tunnelled, general, &c.? What are the sizes, colours, and shapes—lumpy or flat, circular, radiately branched, &c.—of the colonies?

If it only grows in fluids, are skin-like pellicles formed, or

precipitates, or merely a turbidity? What colour-changes, if any?

In all cases, the development of gas-bubbles, odours, and so on should be carefully noted. The products of fermentation or putrefaction may be left for special enquiry; a remark which is by no means to be taken as undervaluing the enormous and ever-growing importance of such enquiry, but simply because the subject lies outside my present theme, and we must put a limit to the discussion.

7. Finally, wherever possible it should be determined clearly whether the Schizomycete is pathogenic or not; whether it induces special fermentations, or nitrification, or reductions; whether sulphur-granules are deposited in its cells, or compounds of iron in its walls; whether it can alter starch, cellulose, &c.; and whether it can live in ordinary waters and so on. The resistance of its spores to desiccation, high temperatures, isolation, the action of anti-septics, and so on, may also be mentioned as subjects for investigation.

If we had answers to all these questions, with respect to the 650 odd 'species' of Saccardo's Sylloge, it is pretty certain that some changes of importance would result, for no one can doubt that *the* great cause of multiple species has been growth under different conditions. If we could have *every* 'species' that will grow on a normal gelatine at 20° C., compared on that medium and at that temperature, under like conditions, the advantage would be enormous; and similarly with all 'species' which will only flourish in bouillon at 35° C., and so on.

Bacteriology is, after all, a sort of microscopic horticulture; and what we want is a kind of bacteriological congress to decide on the best standard methods of comparison and growth. When a form is once isolated, and growing under the best conditions, the morphologist can then take it in hand and work out the details. I see no other way of emerging from the chaos the subject is now in.