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THE KINDS OF BACTERIA FOUND IN RIVER WATER.

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IN the course of a bacteriological examination of river waters during the autumn of 1901, an opportunity occurred for studying an unusually large series of microorganisms. The material derived from comparing a considerable number of cultures—543—is so suggestive that, although the work is not yet complete, it may be of advantage to other workers if the general character of the results be indicated at this time.

The cultures were isolated from three different rivers, the Illinois, the Mississippi, and the Missouri. The nature of the collecting stations may be briefly described.

1. *The Illinois River at Averyville.* As is well known the Illinois River receives a large part of the sewage of Chicago, but by the time the flow reaches Averyville (130 miles below Lockport, the point where the Chicago drainage canal discharges into the Desplaines River[1]), oxidation of the organic matter is practically complete and the bacterial content is not high[2]. The mean monthly discharge of the river at this point was about 7000 cubic feet per second during Oct.—Dec., 1900[3].

2. *The Illinois River at Pekin.* Between Averyville and Pekin, a distance of about six miles, most of the sewage of Peoria (population 56,100, according to the U.S. Census 1900) enters the river, together with a great amount of stockyards waste, distillery slop, and refuse

[1] *Journal Experimental Med.*, 1900, v., p. 271; *Journal of Hygiene*, 1901, I., p. 295; see Map on p. 296.

[2] The average number of colonies per one c.c. during the investigation was 5800, the mean of 67 daily determinations.

[3] Illinois State Board of Health, *Report of the Sanitary Investigation of the Illinois River and its Tributaries*, 1901, p. 179.

from various manufactories. Pollution at this point is therefore both considerable in amount and recent in origin.

3. *The Illinois River at Grafton.* Between Pekin and the mouth at Grafton (143 miles), although several small towns and villages drain into the river, comparatively little additional sewage enters. The oxidation of the Peoria sewage is practically complete when the flow reaches Grafton.

4. *The Mississippi River at Grafton.* There is no considerable pollution for some distance above this point. The volume of water is much greater than that of the Illinois.

5. *The Missouri River at Fort Bellefontaine.* The collection of water was made about twenty-five miles above the mouth of the river and about seven miles above the town of St Charles (population U.S. Census, 1900, 7982).

6—9. *The Mississippi River at the St Louis Water-Works.* At this point, about three miles below the mouth of the Missouri, four samples were taken, nearly in line across the river: one near the Missouri bank, one at the inlet tower of the St Louis Water-Works, one in the channel, and one near the Illinois bank. The water of the Illinois River clings in part to the east (Illinois) shore[1] and that of the Missouri to the west (Missouri) shore, the intermediate body of water being an admixture in varying proportions of water from these two rivers and the Mississippi.

The exact point of collection in all cases corresponded to that chosen for the earlier observations (*op. cit.*). Three laboratories were installed for the work in 1901, one at Peoria, one at Grafton, and one at St Louis. It was found possible to plate the water within about an hour after its collection, except in the case of Pekin (2) and Fort Bellefontaine (5), where an interval of from two to three hours between collection and plating proved unavoidable.

The methods employed for the study of the bacterial cultures were directed toward securing uniform conditions. In the first place, immediately after isolation all the cultures were "rejuvenated" by incubating for three days at 20° C. in nutrient broth of 0·5 acid reaction. Gelatin plates were then made from the broth culture; if only a single species developed, agar tube-cultures were prepared and used as the stock-cultures of the organism. Full data regarding the source of material, date of isolation, etc., were preserved by a

[1] Cf. *Journ. Experimental Med.*, 1900, v., p. 271.

convenient card-catalogue system. In the second place, special efforts were made to insure a uniform composition of the culture media. The initial study of the cultures was pursued in the University laboratories by several different observers, but the culture media were all prepared by one person, and particular attention was devoted to the details of preparation[1], neutralization, etc.

To these two measures much of the uniformity in the results must be attributed. The method of preliminary broth cultivation in particular, as claimed by Fuller and Johnson[2], undoubtedly helps to place on a common biological level organisms that have been variously affected by aquatic life. The procedure is open to the objection that growth in a common medium may tend to cause convergence in organisms really distinct, and to create a uniformity of type that does not exist in nature. This objection must be admitted to have weight. Until, however, it becomes possible for bacteriologists to frame definite taxonomic rules, the practice of placing bacteria in "groups" rather than in "species" will be found expedient, and the method of rejuvenation, whatever else may be said for it, certainly facilitates such grouping.

The organisms studied in this work have been isolated from four different kinds of culture media: (1) from 48-hour-old gelatin plates incubated at 20° C.; (2) from 48-hour-old dextrose broth fermentation tubes incubated at 37°C.; (3) from litmus-lactose agar plates (35° C.) after passage through carbol broth at 35°C.; (4) from neutral red-broth at 37° C.[3]

It is apparent that in each case a selected flora is obtained. The limiting to two days the period of incubation of the gelatin plates leads, for example, to the isolation only of the more rapidly growing species, and the fermentation tube—when used in the way we have employed it—appears to yield *B. coli* more frequently than other gas-producing species (see Table I). For these reasons, the microorganisms

[1] The general methods of preparation employed in the work were those recommended by the Bacteriological Committee of the American Public Health Association (*Reports and Papers of the Amer. Public Health Association*, Vol. XXIII., p. 60, 1898); with slight modifications:

(*a*) The reaction of the sugar-broths used for the fermentation tests was neutral to phenolphthalein instead of 1·5 acid.

(*b*) The ordinary nutrient gelatin, agar and broth were 1·0 acid.

(*c*) The broth used for both the fermentation and the indol tests was always freed from sugar by Smith's method.

[2] *Journ. Experimental Med.*, 1899, IV.

[3] E. E. Irons, *Journal of Hygiene*, 1902, Vol. II., p. 314.

enumerated in this paper are not to be considered as representing the whole microbic flora of the river water, but must be looked upon as selected groups which have come to development under the conditions above specified. It must indeed be recognized that no single culture medium will permit the development of all the forms of bacteria actually present in water. The following data, therefore, relate only to those kinds of bacteria revealed by the particular methods that we have employed.

The characteristics of the different microbes were recorded in the first instance upon record sheets similar to those used by Fuller and Johnson (*op. cit.*), and in most cases the findings were afterwards verified independently by another observer. A definite time limit was set[1]. In this way 543 cultures were studied. The arrangement and classification of this large number of cultures presented peculiar difficulties, and I am especially indebted to Miss Mary Hefferan for assistance in this task. Provisional groupings were first made upon the basis of the characters tabulated on the record sheets (Table III), and a more thorough study was then made of each group, the whole work involving much detail.

The system of group arrangement of water bacteria has been employed by Marshall Ward[2], by Boyce and Hill[3], by Fuller and Johnson[4], and others. It presents many advantages, one of the most obvious being that the more salient biological characters of an organism receive in this way the greatest emphasis. This may not in all cases lead to the most "natural" classification, but it has at least the merit of avoiding much floundering among bewildering synonyms and incomplete descriptions. A more detailed study of each group would doubtless reveal a necessity for further subdivision; in the actual state of classification, however, the writer believes that more is to be gained by compression and unification than by a dispersive arrangement. The different groups considered in the present study may first be enumerated, and then treated separately in more detail.

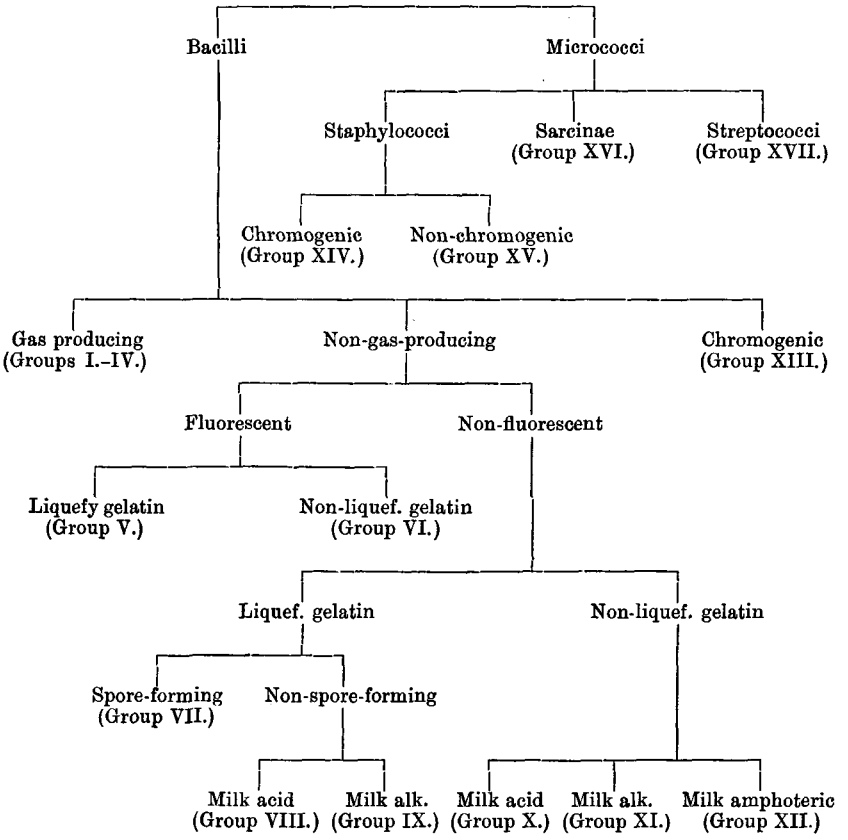
[1] Cultures not yielding positive results, *e.g.*, in curdling milk, *within ten days*, are recorded in the tables as negative, *i.e.* by the minus sign.

[2] *Proc. Roy. Soc.*, 1897, LXI. p. 415.

[3] *Journal of Pathol. and Bacteriol.*, 1899, VI. p. 32.

[4] *Journ. Experimental Med.*, 1899, IV. p. 609.

		TYPE.
Group	I.	*B. coli communis.*
Group	II.	*B. lactis aerogenes.*
Group	III.	*B. proteus.*
Group	IV.	*B. enteritidis.*
Group	V.	*B. fluorescens liquefaciens.*
Group	VI.	*B. fluorescens non-liquefaciens.*
Group	VII.	*B. subtilis.*
Group	VIII.	Non-gas-forming, non-fluorescent, non-spore-forming bacilli which liquefy gelatin and acidify milk.
Group	IX.	Similar to Group VIII., save that the milk is rendered alkaline.
Group	X.	Similar to Group VIII., save that gelatin is not liquefied.
Group	XI.	Similar to Group IX., save that gelatin is not liquefied.
Group	XII.	Similar to Group XI., save that the reaction of milk is not altered.
Group	XIII.	Chromogenic bacilli not included in the above groups.
Group	XIV.	Chromogenic *Staphylococci.*
Group	XV.	Non-chromogenic *Staphylococci.*
Group	XVI.	*Sarcinae.*
Group	XVII.	*Streptococci.*



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It is not supposed that these groups are of equivalent value or that the characters upon which they are based should have equivalent weight in a scheme of natural classification. It will be noticed both that the tabular key separates quite widely groups of organisms which are in reality closely allied (as Groups I. and X.), and also that certain groups are distinguished from one another on comparatively trivial grounds (as I. and II.), while others are more fundamentally dissimilar. The assignment of proper limits to a group of organisms is, however, a matter for future settlement; convenience of treatment rather than precise and balanced natural affinities must for some time to come be the guiding principle of such grouping. The gas-producing organisms, for example, owing to the prominence that has been accorded them in the sanitary investigations of water, are here given what might be regarded on purely taxonomic grounds a disproportionately detailed consideration. On the other hand large groups of microorganisms are here left in a relatively undifferentiated condition. All perhaps that need be said is that the grouping here used has proved a useful one in dealing with the microorganisms encountered in our work, and is presented simply as a framework upon which to arrange our results.

Group I. The organisms of which this group is composed possess the following characteristics: they are motile bacilli; curdling milk rapidly, with little or no subsequent solution of casein; fermenting dextrose and lactose, and in some instances sucrose also; always yielding gas in dextrose broth with excess of H (approximately $H : CO_2 :: 2 : 1$); showing no liquefaction of gelatin in ten days; usually, but not always, producing indol in marked amount. Type: *B. coli.* Two varieties: (*A*) fermenting dextrose, lactose and sucrose (variety α, Theobald Smith[1]; *B. coli communior*, Durham[2]), and (*B*) fermenting dextrose and lactose only (variety β, Smith[3]; *B. coli communis verus*, Durham[4]) were distinguished. Twenty-five cultures of variety (*A*) were encountered and twenty-one of variety (*B*). Durham[5] is inclined to think that variety (*A*) is a commoner inhabitant of the faeces than (*B*). Moore and Wright[6] in their observations upon *B. coli communis* from certain species of domesticated animals, studied in all forty-three cultures, of which twenty-one fall under variety (*A*) and twenty-two under variety (*B*).

[1] *American Journal of Medical Sciences*, Sept., 1895.
[2] *Journ. of Experimental Med.*, 1901, v. p. 368.
[3] *loc. cit.* [4] *loc. cit.* [5] *op. cit.* p. 372.
[6] *American Medicine*, 1902, III. p. 504.

Group II. One of the chief characters distinguishing this group from the preceding is the lack of motility. There are, however, other fairly constant correlated peculiarities. One of these is the more tardy action upon milk, the members of Group I. producing a firm clot within 48 hours, while those placed in Group II. do not as a rule curdle the milk until after three or four days, and in some cultures curdling does not appear until still later. All, however, curdle milk within 10 days. The appearance of the colonies upon a gelatin plate is another feature of differential value. The colonies are less spreading, more convex, fleshy, and with smooth well-defined margins. Type: *B. lactis aerogenes.* Possibly *B. coli immobilis* of some authors belongs here, but with few exceptions all the non-motile bacilli of this general class that have been examined possess together with their lack of motility the correlated characteristics above noted. As in Group I., a variety (*A*) (16 cultures) fermenting dextrose, lactose and sucrose and a variety (*B*) (11 cultures) fermenting dextrose and lactose only, have been found. Variety (*A*) gave a positive indol reaction in 10, a negative in 6 cultures; variety (*B*) a positive in 10, a negative in one.

Groups I. and II. (undifferentiated). A number of cultures were positively identified as belonging to Groups I. and II., but were not differentiated further. Of these, 17 belonged to variety (*A*) and 12 to variety (*B*); 13 cultures of variety (*A*) gave a positive indol reaction and one a negative (3 undetermined); 8 of variety (*B*) a positive and one a negative (3 undetermined).

In tabular form the relations of these groups appear as follows:

	No. of cultures	Indol +	Indol −
Group I.			
Var. (*A*)	25	16	9
Var. (*B*)	21	20	1
Group II.			
Var. (*A*)	16	10	6
Var. (*B*)	11	10	1
Groups I. and II. (undifferentiated)			
Var. (*A*)	17	13	1
Var. (*B*)	12	8	1

Cultures belonging to Var. (*A*), Groups I. and II., 57 %.
Cultures belonging to Var. (*B*), Groups I. and II., 43 %.
Group I., positive indol, 78 %.
Group II., positive indol, 74 %.
Var. (*A*), positive indol, 71 %.
Var. (*B*), positive indol, 92 %.

The fact that a large number of the organisms here placed in Group II. have yielded a positive result with the indol test may be taken to indicate either that grouping on the basis of non-motility is of questionable value or that inability to produce indol is not so constant a characteristic of the *B. lactis aerogenes* group as sometimes supposed. The cultivation of organisms related to this group under conditions favouring proteolytic activity has been shown by Miss Peckham[1] to enhance and even develop the indol-forming power. Possibly the rejuvenation method used in our work may have had a similar effect. In any case, if Groups I. and II. be considered as a whole, it is evident that there is no strict correlation between motility and power of indol production.

Group III. The members of this group ferment dextrose and sucrose, rarely lactose. They are for the most part vigorously proteolytic, rapidly liquefying gelatin and blood-serum, and precipitating and then dissolving casein. The organisms placed in this group fall conveniently into three subdivisions.

1. *Proteus vulgaris* type, always fermenting with gas production dextrose and sucrose, never lactose; liquefying gelatin, casein and blood-serum. Indol is almost always produced (20 positive cultures, 3 negative). Milk is almost always curdled with acid reaction, but the curdling is usually less rapid than in Groups I. and II. and the acidity less intense. All cultures are actively motile. The gas produced in dextrose broth always contains less CO_2 than H (residual gas), and the proportion of H is generally higher than in Groups I. and II.; in 2 cultures out of 23 there was no absorption with NaOH. The total amount of gas formed in the fermentation tube is as a rule somewhat less than that formed by the members of Groups I. and II., as has been noted by Smith[2], but there is considerable variation in this respect.

Widely divergent statements are found in the literature concerning the characters of the species designated as *Proteus vulgaris*[3]. Nearly all writers, for instance, state that milk is both curdled and rendered acid by the bacillus entitled to this name, but Ford[4] has used the term Proteus group for an assemblage of organisms that produce terminal alkalinity in milk and refuse to curdle. A number of varieties, with

[1] *Journ. Experimental Med.*, 1897, II. p. 549.
[2] The Fermentation Tube, *Wilder Quarter Century Book*, p. 212.
[3] *B. vulgare*, Lehmann and Neumann, *Bacteriology*, translation by Weaver, p. 295.
[4] *Journ. of Med. Research*, 1901, VI. p. 211.

different gas-producing properties, are included by Ford under this head. Fuller and Johnson[1] include two spore-bearing organisms under their Proteus type, although most writers state that *Proteus vulgaris* is not known to form spores. Marshall Ward[2], in the most thorough study of the group that has been made since Hauser, has described with great detail a number of forms both vigorous and attenuated, but leaves unmentioned the peculiarities of gas-production, although Smith[3] apparently regards the behaviour in the fermentation tube as one of the most conspicuous characters of the Proteus group.

In view of these marked discrepancies and discordant opinions, I have ventured here to limit the name *Proteus vulgaris* to those organisms possessing the gas-producing properties above indicated. The fact that when this is done the majority of the organisms so thrown together show a correlation in other important biological characters is the best evidence that this procedure is not so arbitrary as might appear, but that, in this case, arrangement according to gas-production brings into line organisms possessing a close affinity in other respects.

An interesting tendency to produce yellow pigment is manifested by many cultures belonging to this group. Marshall Ward[4] has already drawn attention to the occurrence of "Yellow Proteus" forms, and has pointed out their probable relationship to such "species" as *B. radiatus* and *B. ochraceus* (Zimmermann), and *B. arborescens* (Frankland). More than half of the cultures that I have placed in the Proteus group showed a more or less pronounced development of pigment on potato or agar, and nearly all imparted a distinct yellow or buff tinge to milk.

2. *Proteus varieties.* In addition to the 23 well-marked cultures placed in subdivision (1), a number of other cultures similar to the type, but differing in certain respects, have been separated from the type chiefly for the purpose of recording their frequency of occurrence. The most common departure from the type is in the direction of proteolytic power. It has been long recognised that the three Proteus varieties originally established by Hauser[5] upon the ground of their different behaviour towards gelatin were in reality most intimately related, and Hauser himself subsequently admitted that the liquefying varieties could be transformed into the non-liquefying[6]. In general,

[1] *Journ. Experimental Med.*, 1899, IV. p. 609.
[2] *Annals of Botany*, 1899, XIII. p. 197.
[3] *loc. cit.* [4] *op. cit.*
[5] *Ueber Fäulnissbakterien*, Leipzig, 1885.
[6] *Centralbl. f. Bakteriol.*, 1892, XII. p. 629.

observers of water and soil bacteria have encountered the liquefying member of this group more frequently, and have further noted that loss of liquefying power often followed prolonged cultivation under artificial conditions. Cultures with feeble proteolytic power are, however, occasionally met with among water bacteria. Marshall Ward[1] has carefully described a number of these organisms found in the river Thames, and presumably attenuated by aquatic life.

In the present study several of these aberrant or weakened forms were discovered: (1) essentially like the type, save that blood-serum was not liquefied (4 cultures); (2) like the type, save that blood-serum was not liquefied and casein was very slightly or not at all dissolved (2 cultures); (3) like the type save that casein was not dissolved (3 cultures); (4) like the type save that gelatin was liquefied only after 20—40 days (4 cultures); (5) like the type, save that gelatin was liquefied only after 20—40 days and casein and blood-serum were not dissolved at all (1 culture); (6) like the type save that milk was not curdled, although some acid was formed (2 cultures); (7) like the type save that milk was rendered slightly acid but was not curdled, and casein and blood-serum were not dissolved (1 culture).

3. *B. cloacae.* The organisms placed under this head are characterized by an "inverted gas formula," that is to say, an excess of CO_2 over H in the dextrose fermentation tube. In freshly isolated cultures the proportion of H to CO_2 may be as low as 1 to 5 or 6, but under cultivation there is a tendency for the proportion of H to increase until an approximately stable ratio of 1—2 is reached. I have had cultures under observation for upwards of 3 years, during which period the ratio has remained constant at 1—2. Sucrose is fermented by all cultures, and lactose, although often very slowly, by the majority (14 out of 21 cultures). Considerable variation is shown in the action upon gelatin: 2 cultures liquefying rapidly, 14 slowly, 4 only after 30—40 days, and one not at all. All are actively motile. Milk is curdled by all cultures with acid reaction and the casein is dissolved by 13 cultures. Only 8 cultures out of 21 have produced indol. Blood-serum was liquefied by 5 cultures out of 16 tested.

I have placed this group of organisms together with the Proteus forms, since it seems allied to the Proteus type through morphology, habitat, action upon sugars and proteolytic power. Lactose fermentation with gas production is absent in the Proteus group, and is either

[1] *op. cit.*

absent or feeble in most members of the *B. cloacae* group. On the other hand the forms included under the name of *B. cloacae* are as a class much less powerfully proteolytic than the Proteus type, and the less frequent occurrence of indol-producing cultures is perhaps to be correlated with this property. Both the Proteus and *B. cloacae* groups contain a number of varieties which in the future it will probably be possible and useful to differentiate fully. The individual organisms composing these groups are themselves quite variable, and the whole group seems to possess a less stable biological equilibrium than many other water bacteria.

Group IV. Bacilli closely related to the colon group, but fermenting dextrose only, never lactose or sucrose. Milk is rendered strongly alkaline and the casein is dissolved. All are actively motile. A compact group of 6 cultures. *B. enteritidis* type. Indol is produced in slight amounts by 5 of the cultures.

Groups V. and VI. Fluorescent Type. Bacteria producing fluorescence were frequently found in the water; in all 58 cultures were isolated and studied, of which 33 were able to liquefy gelatin, while 25 cultures did not possess this power. An interesting feature brought out by the study of this large series is the correlation between the behaviour in gelatin and that in milk. The power of liquefying gelatin was invariably associated with that of coagulating milk, accompanied by a more or less intense acid reaction and rapid peptonization of the casein. This certainly does not accord with the statement of Lehmann and Neumann (*op. cit.*) that *B. fluorescens liquefaciens* never coagulates milk. On the other hand, a strong alkaline reaction, without curdling, was produced in litmus milk by those cultures which did not liquefy gelatin. These correlated reactions, and the fact that all of the non-liquefying cultures were without the ability to grow either in the closed arm of the fermentation-tube or without oxygen (Wright's anaerobe method), while the liquefying forms varied in this respect, served to distinguish the two groups sufficiently. No indication was found of a reacquirement of the power of liquefaction upon subculture, such as was observed by Boyce and Hill[1].

In other characteristics the liquefying and non-liquefying forms were much alike, and the different cultures in each group varied so slightly as to suggest the essential identity of many of the fifty or more extant "species" of green fluorescing bacteria, some of which

[1] *Journ. of Pathol. and Bacteriol.*, 1899, Vol. VI. p. 32; *Thompson Yates Laboratories Reports*, 1898—99, p. 37.

appear to have been distinguished and named from characters not highly significant. The following are examples: *Bacillus scissus* Frankland[1] appears to differ from the original *Bacillus non-liquefaciens* Eisenberg[2] only in reduction of nitrates; *Bacillus putridus* Flügge[3] only in the production of a putrid odour; *Bacillus incognitus* Wright[4] has been distinguished from these because it grew at body temperature, while Wright's[5] *Bacillus fluorescens convexus* and *Bacillus fluorescens foliaceus* show no conspicuous differences. Again, three species described by Ravenel[6], *Bacillus fluorescens ovalis, Bacillus striatus viridis* and *Bacillus fluorescens undulatus*, seem to differ only in the size of the bacillus in each case. In the same manner, liquefying "species" have been distinguished on the basis of such uncertain reactions as the reduction of nitrates (cf. Schmolck's *Bacillus fluorescens nivalis*[7]), or by viscidity of growth (cf. *Bacillus viscosus* Frankland[8]).

All 25 cultures of *Bacillus fluorescens non-liquefaciens*, and all but three of the liquefying forms grew at 37° C., although a few developed only feebly, the existence of intermediate forms showing that this character is probably not a constant one. Inconstancy was also shown in the power of producing indol and reducing nitrates under ordinary conditions, some cultures reacting positively at two trials and negatively at the third, although grown under the same conditions and for the same length of time. Unrejuvenated cultures often failed to give a positive reaction, or gave it sporadically in one of a series of cultures. Lack of rejuvenation probably accounts for some of the differences in "species" noted above.

Nearly all varieties produced an unpleasant odour in bouillon, varying with the age and luxuriance of growth; some cultures showed viscidity.

Morphologically, all of the 58 cultures were small bacilli, which varied somewhat in length. All were motile; in fact, very few fluorescent organisms of this type have been described as non-motile, viz., Eisenberg's *B. non-liquefaciens*, Kruse's *B. fluorescens immobilis*[9], Lustig's *B. aquatilis fluorescens*[10]. Considering the difficulties often hedging the determination of motility, it would seem that observers

[1] *Zeitschr. f. Hygiene*, 1899, Vol. VI. p. 399.
[2] *loc. cit.* p. 145. [3] *loc. cit.* p. 292.
[4] *Memoirs, National Academy of Science*, 1895, Vol. VII. p. 436.
[5] *loc. cit.*, pp. 438, 439.
[6] *Memoirs, National Academy of Science*, 1896, Vol. VIII. pp. 9, 20, 22.
[7] *Centralbl. f. Bacteriologie*, 1888, Vol. IV. p. 544.
[8] *Zeitschr. f. Hygiene*, 1889, Vol. VI. p. 391.
[9] Flügge, *loc. cit.*, p. 294. [10] *loc. cit.*, p. 64.

meeting with a non-motile fluorescent form should scrutinize it with particular care in view of the evident rarity of such organisms.

All cultures were tested for the presence of pyocyanin by treatment with chloroform, but in no case was this pigment found.

Group VII. Spore-forming Bacilli: the Subtilis group[1]. Forty-six cultures were studied which were characterized by the formation of spores. Upon examination they fell into several subgroups: (1) the Subtilis type proper, comprising the majority of the cultures isolated (26). These showed a white and usually dull and wrinkled growth on agar slant, and the characteristic feathery or arborescent growth in the depth of an agar stab culture. All liquefied gelatin rapidly, curdled milk with acid reaction, and reduced nitrates. On potato the growth was dry, white, and later sometimes wrinkled. (2) Fourteen varieties seemed to belong to the mesentericus type. Three of these were distinctly *B. mesentericus fuscus*[2], very dry, yellow-brown, and wrinkled on agar, and showing a raised and exceedingly crumpled 24-hour growth on potato at 37°. Three others were dirty-white on agar, while on potato they produced a dry and rose-coloured growth. One of these even showed touches of deep red in an old, much wrinkled growth on the latter medium. These latter were probably varieties of *B. mesentericus ruber*[3]. Some of the eight remaining varieties of this subgroup would probably be classed with *B. mesentericus vulgatus*[4]. Their whole behaviour, however, points to the conclusion that no sharp line of demarcation can be drawn between the varieties of this latter type and those of *B. subtilis*. The growth of these eight forms on agar was intermediate between the thin, brown, wrinkled appearance of the typical *B. mesentericus fuscus* and the luxuriant, white, often moist growth of the typical *B. subtilis*. The indefiniteness in the affinity of these varieties is also increased by the fact that the occurrence of a wrinkled growth on potato cannot be relied upon to separate them from *B. subtilis*, although the character of such growth was distinctive for the three cultures mentioned above as *B. mesentericus*. As for the other reactions of these forms; all liquefied gelatin rapidly; the majority curdled milk, while others only acidified it; all reduced nitrates; and a few produced indol (like the culture of *B. mesentericus*

[1] Cohn, *Beiträge zur Biologie der Pflanzen*, Vol. I., Heft II. p. 175, 1875; also Flügge, *loc. cit.*, p. 196.

[2] Flügge, *loc. cit.*, p. 199.

[3] Globig, *Zeitschr. f. Hygiene*, 1888, Vol. III. p. 322.

[4] Flügge, *loc. cit.*, p. 198.

vulgatus isolated by Fuller and Johnson). Morphologically the bacilli were not unlike *B. subtilis*. (3) Four cultures were found of a spore-forming organism in which the vegetative form was a short, thick, non-motile bacillus, of a diameter greater than 1μ, tending to large, round, involution forms. On agar the growth was yellow and moist; on potato, soft, luxuriant and cream-coloured, later becoming somewhat wrinkled. They further differed from other spore-bearing forms in not reducing nitrates and in curdling milk with alkaline reaction. (4) One variety was isolated which was morphologically unlike the mesentericus-subtilis type, and conformed more closely to the descriptions of *B. megatherium*[1]. It was a large motile bacillus, "bogig gekrümmt" (Migula). It gave a smooth, moist growth on agar, liquefied gelatin, reduced nitrates, turned litmus milk slightly alkaline, and slowly decolorised it. (5) One variety of spore-forming bacillus was found which did not liquefy gelatin. It was a short, motile bacillus, with oval spores; the growth on agar was moist and smooth; milk was made alkaline.

Group VIII. Bacilli which liquefy gelatin and acidify milk. Ward notes in his Group XIV. some rapidly liquefying colourless bacilli, of which he studied 5 varieties "conforming to the type of *B. termo* as amended by Macé[2]." His series includes Eisenberg's *B. liquefaciens*[3], Frankland's *B. liquidus*[4], Zimmermann's *B. punctatus* and *B. devorans*[5]. He says: "The type is one of the commonest in the Thames and a pronounced putrefactive bacterium." Boyce and Hill entirely omit this group, which, like Ward, we have found one of the commonest in water. Our grouping, however, differs from that of the latter author in that the members of his Group XIV. are distributed between our Groups VIII. and IX.

Seventy-four varieties were isolated and their characters tabulated. Nearly all of these—62—coagulated milk in addition to acidifying it; these were further separated into subgroups on the basis of motility, 48 varieties showing independent motility, *i.e.*, possessing flagella. Twenty-four of these motile organisms produced indol, including 6 which also reduced nitrates; limited growth on potato characterized about one-half of these, while the others, in which the indol reaction was negative, were all luxuriant on potato. This series of 48 cultures was evidently allied to such organisms as *B. albus putridus* Maschek[6];

[1] Migula, *loc. cit.* p. 516. [2] *loc. cit.* p. 585. [3] *loc. cit.* p. 112.
[4] *Zeitschr. f. Hygiene*, 1899, Vol. VI. p. 382. [5] *loc. cit.* pp. 38, 48.
[6] Chester, *loc. cit.* p. 237.

B. circulans, B. hyalinus, and *B. delicatulus* Jordan[1]; probably also *B. pestifer* and *B. diffusus* Frankland[2]; and *B. sulcatus* Kruse[3].

The 14 non-motile varieties showed the same slight differences as those recorded above; six produced indol, three reduced nitrates, and several did not grow luxuriantly on potato. *Bact. flexuosum* Wright[4] and *B. radiatus* Zimmermann[5] are non-motile organisms of this description. The tan-coloured or yellowish growth which some of the varieties of this group show upon potato seems to connect them to the yellow forms of Group IX.

Aside from the varieties already noted, 12 were recorded which failed to precipitate the casein in milk, although they produced acidity of the medium. The majority of these were motile, and several produced indol or reduced nitrates. Their description coincides broadly with those of *B. superficialis* Jordan[6], *B. antennaeformis* Ravenel[7], *B. radiatum* Chester[8], and *B. inunctus* Pohl[9].

All of the 74 varieties of this group grew well at body temperature; the majority liquefied casein and blood-serum as well as gelatin, and were facultative anaerobes. They are probably closely allied putrefactive organisms. At some points they approximate the Proteus group.

Group IX. Bacilli which liquefy gelatin and produce alkalinity of milk.

Thirty cultures were included in this series. Five of these coagulated milk with alkaline reaction, the soft curd being later peptonized; of these five, two were motile and three non-motile, little difference existing otherwise. Among the remaining 25, were found eighteen motile organisms of the type exemplified by *B. formosus* Ravenel[10], *B. liquidus* Frankland[11], *B. punctatus* Zimmermann[12], *B. liquefaciens* Eisenberg[13], and *B. stoloniferus* Pohl[14]. None of these 18 varieties produced indol, and only 5 reduced nitrates, while nearly all grew at 37° C., liquefied casein and blood-serum, and were luxuriant on potato, upon which the growth was often yellowish or tan-colour. Two of them did not develop on potato, thus corresponding to Zimmermann's description of *B. devorans*[15].

[1] *Report of Mass. Board of Health*, 1890, pp. 831, 835, 837.

[2] *Zeitschr. f. Hygiene*, 1899, Vol. VI. pp. 381, 396. *Phil. Trans. Royal Soc.*, London, 1888, p. 277.

[3] Flügge, *loc. cit.* p. 318.

[4] *loc. cit.* p. 160. [5] *loc. cit.* p. 58. [6] *loc. cit.* p. 833.

[7] *loc. cit.* p. 25. [8] *loc. cit.* p. 162.

[9] *Centralbl. f. Bakteriol.*, 1892, Vol. XI. p. 143; Migula, *loc. cit.* p. 247.

[10] *loc. cit.* p. 12. [11, 12, 13] *loc. cit.* [14] *loc. cit.* p. 142. [15] *loc. cit.* p. 58.

Of the 7 non-motile organisms, two produced indol; in other characteristics they were all like those described above. *B. convolutum* Wright[1] and *B. ambiguum* Chester[2] are non-motile organisms of this type.

Group X. Bacilli which do not liquefy gelatin but acidify milk.

This and the two following groups comprise together some 91 cultures and correspond to the sixteen forms studied by Ward, and to the twenty studied by Boyce and Hill, and included by these investigators under their Group V. or Coli type. Ward remarks that these forms were common in the river, especially in summer, and states that they showed variation in such characters as production of gas, coagulation of milk, and pathogenicity. Only about half of the cultures examined by Boyce and Hill were capable of producing gas-bubbles in glucose gelatine, and only one formed indol.

That these forms were also found by us to be very abundant is shown by the number isolated. They are, however, separated from the Coli group in our classification by their entire lack of gas production in sugar bouillon; otherwise they showed similar characteristics to those described by Ward.

Of the 32 cultures which are placed in Group VI., only 13 were typically coli-like in their behaviour towards milk. Seven of these, moreover, were non-motile, and of them all only two produced indol, and two produced nitrates. If these, then, are varieties of *B. coli* there is an apparent rough correlation in the absence of four characteristics; the power of causing the free evolution of gas, of reduction of nitrates, of formation of indol, and finally the lack of motility. The absence of indol formation and of motility points perhaps to affinity with *B. lactis aerogenes.* Another series, of which 19 cultures were studied, produced only acidity and not coagulation of milk. Much the greater number (13) of these were non-motile; but on the other hand, a larger number (6) than of the series above, formed indol. (A few of the 19 were not tested for indol.) There is probably no material difference between this series and the foregoing, except that in the latter the amount of acid produced in milk was not sufficient to precipitate the casein.

The motile and non-motile cultures of this type probably contain many forms which have been isolated and named as distinct species. The limited descriptions of most of these forms, especially of the older

[1] *loc. cit.* p. 460. [2] *loc. cit.* p. 105.

and the unfamiliar ones, make it impossible to bring them under the classification used here. There are mentioned, therefore, out of a large number of possible forms, only the few for which the description was detailed enough to include statements concerning the gas production and the milk reaction. Of the type that coagulates milk, nearly all the forms falling under this head appear to have been isolated from milk. The majority of these milk varieties appear to be non-motile, *e.g. B. punctatus* Adametz[1], *B. No.* 52 Conn[2], and several varieties of *B. acidi lactici* isolated by Conn[3], which according to him produce no gas and were common in milk; *B. limbatum* Marpmann[4], *B. lacticum* Kruse[5], are also from milk, while *B. ubiquitus* Jordan[6] was isolated from sewage. The motile forms described as coagulating milk are *B. No.* 107 and *No.* 137 Conn[7], *B. equi intestinalis* Dyar and Keith[8], and *B. arborescens* Ravenel[9]; the forms described as acidifying milk without coagulation are all non-motile, *e.g.*, Conn's *B. No.* 41, *B. No.* 54, and *B. No.* 56[10].

Group XI. Bacilli which do not liquefy gelatin, and which cause alkalinity of milk.

Twenty-nine cultures imparting a marked alkaline reaction to litmus milk were studied. Thirteen of these were motile. None of them formed indol, several reduced nitrates, and nearly all grew well on potato and at 37°. Morphologically the majority of them were alike, small or medium-sized bacilli; one non-motile organism, however, formed long slender filaments, and another was a large bacillus containing dark granules in the protoplasm of each cell.

Among the motile forms described by other investigators which are of this type are *B. No.* 98 and *B. No.* 95 Conn[11], *B. pinatus* Ravenel[12], which forms indol, *B. alcaligenes* Petruschky[13], while the non-motile forms are *B. solitarius* and *B. geminis* Ravenel[14] and *B. primus* Dyar[15].

[1] *Landwirthsch. Jahrbücher*, 1899; Chester, *loc. cit.* p. 147.
[2] *Report of Storrs Agric. Exp. Sta.*, 1894, p. 81.
[3] *loc. cit.*, 1899, p. 52.
[4] Flügge, *loc. cit.* p. 366.
[5] Flügge, *loc. cit.* p. 356.
[6] *loc. cit.* p. 830.
[7] *loc. cit.* 1899, pp. 50, 58.
[8] *Mass. Inst. of Tech. Quart.*, VI. p. 3.
[9] *loc. cit.* p. 39.
[10] *loc. cit.* 1894, pp. 57, 82, 83.
[11] *loc. cit.*, 1899 p. 56.
[12] *loc. cit.* p. 32.
[13] *Centralbl. f. Bakteriol.*, 1896, Vol. XIX. p. 187.
[14] *loc. cit.* p. 29.
[15] *Report of the New York Acad. of Science*, 1895, Vol. VIII. p. 360.

Group XII. Bacilli which do not liquefy gelatin and which produce very little or no change in litmus milk. Typhosus Group.

This group comprises a large number of organisms, the majority of which were isolated from gelatin plate colonies. Twelve of these were carefully studied, and it was found that although they were typhosus-like in many of their reactions, they exhibited some slight differences from that type, and differed also among themselves. Eight of them were non-motile, and gave an alkaline end-reaction in dextrose broth, instead of the acid of *B. typhosus*. The majority of them also showed more development on potato than is the case with the typical *B. typhosus*. One variety which was motile, and was morphologically exactly like *B. typhosus*, developed only slowly on agar at 37°, and very feebly if at all in bouillon at that temperature; the agglutination test was negative. Another, which in all its biological reactions was extremely like a typhoid culture, was motile, but was smaller than the typical *B. typhosus*. Unfortunately, this culture was lost before the agglutination test could be applied.

Eighteen other cultures isolated by the gelatin plate method were found unable to produce gas, to liquefy gelatin, or to produce any material change in litmus milk. When these were examined they proved to be without exception non-motile. Fourteen of them were exceedingly fine, slender, transparent bacilli, which did not stain easily, and grew feebly; four were short, thick, almost coccus-like organisms.

Several so-called species isolated from water by different investigators are unquestionably of this type, viz., the *Typhusähnliche* bacilli of Maschek[1] and of Lustig[2], *B. paradoxus* Kruse[3], which, however, forms indol; the several forms of *B. aquatilis sulcatus* I.—V. Weichselbaum[4], which vary morphologically and in growth on potato and at 37°; the non-motile *B. refractans* Wright[5], *B. rodonatum* Ravenel[6], and *B. No.* 55 Conn[7], isolated from milk.

Group XIII. Chromogenic bacilli not included in the above groups.

Bacilli characterized by a marked production of pigment, and not classed on other grounds with the Proteus or other groups were separated into the following subgroups: *A*, Red Chromogenic Bacilli, *B*, Orange Chromogenic Bacilli, *C*, Yellow Chromogenic Bacilli. As the norms for

[1] *Untersuch. des Leitmeritzer Trinkwassers*, Leitmeritz, 1887. Migula, *loc. cit.* p. 730.
[2] *loc. cit.* p. 18. [3] Flügge, *loc. cit.* p. 373.
[4] *Das Oesterreichische Sanitätswesen*, 1889, Nos. 14—23. Migula, *loc. cit.* p. 731.
[5] *loc. cit.* p. 442.
[6] *loc. cit.* p. 40. [7] *loc. cit.* 1894, p. 83.

these three colours there have been taken for red, *B. prodigiosus*, for orange, *Sarcina aurantiaca*, for yellow, *Sarcina lutea*.

A. Red Chromogenic Bacilli. Two varieties were studied. One of these strongly resembled *B. prodigiosus*, except that its pigment at room temperature was more violet, like that produced by *B. ruber balticus*[1] at 37°. It also differed from *B. prodigiosus* and from *B. ruber balticus*, *B. ruber indicus*[2], *B. plymouthensis*[3], and *B. miniaceus*[4] in producing no gas in dextrose bouillon. It grew rapidly, with luxuriant pigment, which extended throughout the closed arm of the fermentation tube, although no pigment was produced in the entire absence of oxygen.

The other variety was studied from two separate isolations, the cultures proving identical except for slight differences in rapidity of growth and colour of pigment. It differs from the red forms above named in the production of alkalinity in milk, in its non-motility and its very slow liquefaction of gelatin. It did not develop in the closed arm of the fermentation tube, and produced no gas. The pigment was bright rose-red.

An extremely short bacillus that occurred twice was in cultural features almost indistinguishable from the pink chromogenic coccus described under Group X.

B. Orange Chromogenic Bacilli. Of the 7 varieties examined, all liquefied gelatin, and grew at body temperature. Two of these varieties were thin, non-motile rods, which in old cultures often showed long bent spirilla forms. They produced a dark-orange pigment, liquefied gelatin only slowly, and turned litmus milk alkaline. These are probably of the type *B. fulvus* Zimmermann[5]. Another non-motile organism curdled milk without acidity, produced indol, and liquefied gelatin rapidly. The other four varieties were motile, and acidified milk with coagulation; two of them produced indol. These forms might possibly be classed with such forms as *B. arborescens* Frankland[6], and *B. aurescens* Ravenel[7].

C. Yellow Chromogenic Bacilli. These cannot be definitely distinguished from the last-named subgroup, owing to the variability of both orange and yellow pigments and the occurrence of many

[1] Laurent, *Ann. de l'Institut Pasteur*, 1890, Vol. IV. p. 465.
[2] Flügge, *loc. cit.* p. 302.
[3] Fischer, *Zeitschr. f. Hygiene*, 1887, Vol. II. p. 74.
[4] Zimmermann, *loc. cit.* p. 46.
[5] *Ibid.* p. 44.
[6] *loc. cit.* p. 482.
[7] *loc. cit.* p. 8.

nondescript intermediate forms. However, 7 varieties were studied and recorded as yellow. Three of these liquefied gelatin, one proving to be a typical *B. lactis erythrogenes* Hueppe[1], with yellow pigment and red fluorescence. The other two were slow liquefiers, and rendered milk slightly alkaline, but differed from each other in motility and in the power of growth on potato. Of the yellow liquefying bacilli already described, *B. lutescens* Lustig[2], *B. aquatilis* Frankland[3], and *B. helvolus* Zimmermann[4] are non-motile, and have a limited growth or none at all on potato.

Four cultures which did not liquefy gelatin were studied. Two of these curdled milk with rapid and complete liquefaction of the precipitated casein; they were motile, medium-sized bacilli, possibly related to *B. radiatus* and *B. ochraceus* Zimmermann[5]. Fuller and Johnson seem to be alone in describing the latter organism as large and spore-forming; such characters would relate it to the varieties which have here been classed as of the yellow Subtilis type. Other writers describe *B. ochraceus* as less than 1μ in diameter and sporeless. Ward, and Boyce and Hill connect certain yellow non-liquefying forms, which they identify as *B. radiatus* and *B. ochraceus* Zimmermann and *B. arborescens* Frankland, with the Proteus group. The cultures here treated have been separated from that group chiefly because of their non-production of gas. In many respects they are closely related to it.

Group XIV. Chromogenic Micrococci.

Of these, 9 yellow and 5 red forms were worked out in detail. The most striking of the yellow varieties was one which produced luxuriant bright pigment like that of *Sarcina lutea*, which it resembled in cultural features. It was, however, a larger coccus and showed no packet grouping. It is probably identical with the variety isolated once from the Thames by Ward, and described in his Group XX. Like three others of the six liquefying varieties observed it coagulated and acidified milk. Of the other two varieties one grew in milk with production of alkali, the other with no effect upon the medium; the latter may have been a weaker variety of the one first mentioned, to which it was otherwise similar except that it showed no growth on potato.

Three non-liquefying cultures were alike in producing alkali in milk. All of the yellow forms grew at body temperature.

[1] Grotenfelt, *Fortschritte d. Medizin*, 1889, Vol. VII. p. 41.
[2] *loc. cit.* p. 781.
[3] *Zeitschr. f. Hygiene*, Vol. VI. p. 381.
[4] *loc. cit.* p. 52.
[5] *loc. cit.* pp. 58, 60.

It seemed impossible to relate these to the yellow cocci isolated by other investigators because of incomplete descriptions. Migula (*System der Bakterien*) gives 14 liquefying and 8 non-liquefying forms.

A coccus which produced a pink pigment appeared very frequently in the water of the Illinois River at Grafton, at times forming from 50 to 90 per cent. of the colonies on gelatin plates. This organism was isolated and exhaustively studied on five separate occasions, showing always the same characteristics. It grew slowly without liquefaction on gelatin plate, luxuriantly on agar, was alkaline in milk, and failed to develop on potato or anaerobically. For a complete description of this organism, as first isolated in 1899 from the Mississippi River, see a paper by Miss Mary Hefferan[1].

Group XV. Non-chromogenic Micrococci.

A. The great majority—27 out of 35—of the colourless coccus forms isolated from time to time were non-liquefying varieties. Of these, 7 different cultures proved to be alike, *i.e.* alkaline in litmus milk with peptonization, growth at 37°, and luxuriant growth on potato.

Only a few cocci have been described as rendering litmus milk alkaline, viz., Conn's *No.* 85[2] and *No.* 47[3] (*M. nivalis* Chester). Cohn's *M. candidus*[4], *M. aquatilis* Vaughan[5], and *M. aquatilis* Bolton[6] may be of the same type.

Thirteen cultures were found to render litmus milk slightly acid in 10 or 15 days, but not enough so for coagulation. With 5 others the milk was amphoteric during 15 days, although microscopic examination showed that development took place. All but 3 of these 16 grew at body temperature, 2 of them reduced nitrates, and they usually grew luxuriantly on potato. The whole series, so far as the tabular reactions show, belonged to the type *M. candicans* Flügge[7], like those mentioned by Ward in his Group XIX. They have not been under observation long enough to determine whether any liquefying power was acquired after long-continued cultivation, as Ward observed with his series.

B. The 8 liquefying forms showed slight differences, which may have been due to variation in vigour. All of them acidified and 4

[1] *Botan. Gazette*, 1900, Vol. xxx. p. 261.
[2] *Report of Storrs Agric. Exp. Sta.* 1894, p. 28.
[3] *loc. cit.* p. 80.
[4] *loc. cit.*, Vol. I. p. 160, 1875.
[5] *Am. Jour. Med. Science*, 1892.
[6] *Zeitschr. f. Hygiene*, 1886, Vol. I. p. 94.
[7] *loc. cit.* p. 117.

coagulated milk; 5 of them also peptonized casein and blood-serum. The others seemed less luxuriant varieties, those which acidified milk without coagulation also refusing to develop at body temperature. Type: *M. coronatus* Flügge[1], *M. acidi lactis* Kruger[2], *M. simplex* Wright[3].

Group XVI. Sarcinae.

Three cultures were isolated, one being in most respects a typical *Sarcina lutea*, forming a bright yellow pigment, and producing slow liquefaction in gelatin, but with no effect upon milk. The other two were white forms which did not peptonize gelatin, and which made litmus milk alkaline.

Group XVII. Streptococci.

In examining the forms described in Group XI., short chains of 3 or 4 cocci were often seen, and 4 varieties showed such well-marked and constant chains in fresh agar or bouillon cultures that they have been definitely separated as streptococci. The possible significance of the occurrence of *Staphylococci* and *Streptococci* in water was first pointed out by Houston in the report of the Medical Officer to the Local Government Board of London, 1898—1900[4]. From experiments extending over several years Houston concludes that micrococci are readily demonstrable in polluted water and sewage, and that the presence of *Streptococci*, a class of germs unlikely to persist long outside the animal body, seems always to coincide with "animal pollution of extremely recent and therefore specially dangerous kind." Several investigators, among them Horrocks[5] in England, and recently Winslow[6] in America, consider with Houston that *Streptococci* are typical sewage forms, although it is probable that some of these may be non-pathogenic varieties that are able to exist for some time outside the animal body, and hence may not always indicate recent contamination.

No special method of search for *Streptococci* was employed in this investigation; it is therefore interesting to note that two varieties, the non-liquefying type of Houston and Horrocks and a second type, found also by Winslow[6] (differing from the other only in liquefaction of gelatin), appeared in the Illinois River at Pekin, where the great majority of *Staphylococcus* forms also occurred. These *Streptococci*

[1] *loc. cit.* p. 178. [2] *Centralbl. f. Bakteriol.*, 1890, Vol. VII. p. 495.
[3] *loc. cit.*, 1895, Vol. v. p. 32.
[4] Houston, *Parliamentary Blue Books*, 1898, 1899, 1900, Supplem. XXVIII.
[5] Horrocks, *Bacteriological Examination of Water*; London, 1901.
[6] Winslow and Hunnewell, *Science*, May 23, 1902.

were isolated from culture plates after use of the carbol broth method; two other cultures, both non-liquefying, were isolated from the Mississippi River, Missouri shore, St Louis, by the fermentation tube. All of these organisms grew at 37°, and developed rather better in the depths than on the surface of agar stab cultures; in agar slant cultures they showed a typical transparent growth of small round colonies. They all curdled milk with acid reaction; the variety which liquefied gelatin also rapidly peptonizing the precipitated casein. None of these forms produced indol or reduced nitrates.

The material in this paper, which is itself a condensed summary, does not readily lend itself to a final summing-up, but a few of the points that it illustrates may be noted.

Summary.

1. The kinds of bacteria that are isolated by the gelatin plate method from certain river waters freshly polluted with sewage are different from those found in the same water collected a long distance below the point of pollution.

2. In the freshly polluted river water non-chromogenic *Staphylococci* were found much more abundantly than in the purer waters.

3. In the freshly polluted water the fluorescent bacteria and a group of non-gas-producing, non-liquefying bacteria (Group XI.) were less abundant than in the purer waters.

4. A larger proportion of organisms belonging to the *Proteus* group were isolated from gelatin plates than from fermentation tubes. The reverse is true of the *B. coli* and *B. lactis aerogenes* types. A certain selective influence even upon gas-producing organisms would seem from this to be exerted by the conditions within the fermentation tube.

5. The study of a rather large number of separately isolated cultures belonging to the class of fluorescent microorganisms shows that the differences between the 'liquefying' and 'non-liquefying' varieties are more constant than is sometimes assumed. The action of these forms upon milk is just as diagnostic as their action upon gelatin. All the strains of fluorescent bacteria that were encountered (58) proved to be motile.

6. Considering as a whole the various physiological tests applied to the several groups of microorganisms, it is found that within almost

every group as constituted in the accompanying tables divergence is shown by closely allied organisms in respect to indol formation and reduction of nitrates. The formation of a surface pellicle on broth is also a phenomenon that presents no apparent correlation with more salient physiological characteristics.

TABLE I.

Group	Gelatine Plate	Fermentation Tube	Carbol Broth	Neutral Red Broth	Total
B. coli communis	7	23	11	5	46
B. lactis aerogenes	1	11	11	4	27
B. coli communis and lactis aerogenes	2	7	8	12	29
Proteus	23	9	3	5	40
B. cloacae	3	10	2	6	21
B. enteritidis		4	1	1	6
B. fluor. liq.	26	4	2	1	33
B. fluor. non-liq.	15	5		5	25
B. subtilis	18	4	4	20	46
B. gelat. liquef.					
milk acid	50	7	4	13	74
milk alk.	22	4	2	2	30
B. non-gelat.-liquef.					
milk acid	15	2	3	12	32
milk alk.	26	1		2	29
milk amphot.	29			1	30
Chromogenic Bac. [1]	17				19
Chrom. Staphylococci	11	1	2		14
Non-chrom. Staphylococci	28	1	3	3	35
Sarcinae	2		1		3
Streptococci			2	2	4
	295	93	59	94	543

[1] Two cultures of the red chromogenic group are without data as to place or mode of isolation.

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TABLE II.

Type	Illinois River			Mississippi River					Missouri River	Total
					Chain of Rocks					
	Averyville	Pekin	Grafton	Grafton	Missouri Shore	St Louis Intake	Channel	Illinois Shore	Fort Bellefontaine	
B. coli communis	3	22	1	7	4	2	1	3	3	46
B. lactis aerogenes	7	8	1	5		3	1	1	1	27
B. coli communis and B. lactis aerogenes	6	12	1		4	1		3	2	29
Proteus	13	4	5	7	2	3	2	2	2	40
B. cloacae	5	1	5	1	2	4	1	2		21
B. enteritidis	3	2			1					6
B. fluor. liq.	8	3	7	9	1		2	3		33
B. fluor. non-liq.	7	1	2	2	4	1	5	1	2	25
B. subtilis	6	2	12	4	4	6	4	2	6	46
B. gelat.-liquef.										
milk acid	19	11	10	15	4	6		1	8	74
milk alk.	7	10	7	2	2	1		1		30
B. non-gelat.-liquef.										
milk acid	6	8	2		3	2	6	4	1	32
milk alk.	17	1	1		2	1	4	2	1	29
milk amphot.	17	6	2	1		1		1	2	30
Chromogenic Bac.[1]	7	3	1	1	2	1	1	1		19
Chrom. Staphylococci	2	2	5	1	1	1	1	1		14
Non-chrom. Staphylococci	4	17	3	2	2		4	1	2	35
Sarcinae	2			1						3
Streptococci		2			2					4
	139	115	65	58	40	33	32	29	30	543

[1] Two cultures of the red chromogenic group are without data as to place or mode of isolation.

Table III.

GROUPS	SOURCE	MORPHOLOGY				BIOLOGY										
						Cultural Features										
						Nutrient broth tube		Nutrient agar tube		Gelatin plate	Gelatin stab.			Potato tube		Fermentation tube
	No. of cultures	Bacillus	Diameter greater than 1μ	Motile	Spores	Scum	Turbidity	Dull	Wrinkled	Characteristic appearance	Deep funnel	Surface growth	Needle growth	Visible	Luxuriant	Growth in closed arm
Group I. B. coli, var. (*a*)	25	+	−	+	−	±	+	−	−	−	−	+	+	+	+	+
var. (*b*)	21	+	−	+	−	±	+	−	−	−	−	+	+	+	+	+
Group II. B. lactis aerogenes, var. (*a*)	16	+	−	−	−	±	+	−	−	−	−	+	+	+	+	+
var. (*b*)	11	+	−	−	−	±	+	−	−	−	−	+	+	+	+	+
Groups I. and II. undifferentiated further	29	+	−	±	−	±	+	−	−	−	−	+	+	+	+	+
Group III. (1) Proteus vulgaris (type)	23	+	−	+	−	±	+	−	−	±	+	+	+	+	±	+
(2) Proteus (varieties)	17	+	−	+	−	±	+	−	−	±	±	+	+	+	±	+
(3) B. cloacae	21	+	−	+	−	±	+	−	−	±	±	+	+	+	±	+
Group IV. B. enteritidis	6	+	−	+	−	±	+	−	−	−	−	+	+	+	−	+
Group V. Fluorescens liquefaciens	33	+	−	+	−	+	+	−	−	−	+	+	+	+	+	+
Group VI. Fluorescens non-liquefaciens	25	+	−	+	−	+	+	−	−	−	+	+	+	+	+	−
Group VII. Subtilis	26	+	−	+	+	+	+	−	+	+	+	−	−	+	+	+
Mesentericus vulgatus	8	+	−	+	+	+	+	−	+	+	+	−	−	+	+	+
,, fuscus	3	+	−	+	+	+	+	−	+	+	+	−	−	+	+	−
,, ruber	3	+	−	+	+	+	+	−	+	+	+	−	−	+	+	+
Yellow Subtilis	4	+	+	+	+	+	+	−	−	−	+	−	−	+	+	+
Megatherium	1	+	−	+	+	+	+	−	−	−	+	−	−	+	+	+
Non-liquefying	1	+	−	+	+	+	+	−	−		−	+	±	+	+	+
Group VIII. Gel. liquef.-Milk acid	74	+	−	±	−	+	+	−	−	−	+	+	+	+	±	+
Group IX. Gel. liquef.-Milk alkaline	20	+	−	±	−	+	+	−	−	−	+	+	+	+	+	+
		+	−	±	−	+	+	−	−	−	+	+	+	±	±	+
Group X. Gel. not liquef.-Milk acid	32	+	−	±	−	+	+	−	−	−	−	+	+	+	+	+
Group XI. Gel. not liquef.-Milk alkaline	29	+	−	±	−	+	+	−	−	−	−	+	+	+	+	+
Group XII. Gel. not liq.-Milk amphoteric	30	+	−	±	−	+	+	−	−	−	−	+	+	+	±	+
Group XIII. Chromogenic Bacilli																
Red	1	+	−	+	−	+	+	−	−	+	+	+	+	+	+	+
	2	+	−	−	−	+	+	−	−	+	−	+	+	+	+	−
	2	+	−	−	−	+	+	−	−	+	−	+	+	−	−	−
Orange	4	+	−	+	−	+	+	−	−	−	+	+	−	+	+	+
	3	+	−	−	−	+	+	−	−	−	−	+	+	+	−	−
Yellow liquefying	6	+	−	±	−	+	+	−	−	−	+	+	±	±	±	±
Lact. erythrogenes	1	+	−	−	−	+	+	−	−	−	+	+	+	+	+	+
Group XIV. Chromogenic Cocci																
Yellow liquefying	1	−	−	−	−	−	+	−	−	−	+	+	+	+	+	−
	5	−	−	−	−	−	+	−	−	−	+	+	+	+	+	±
Yellow non-liquefying	3	−	−	−	−	+	+	−	−	−	−	+	+			−
Pink	5	−	−	−	−	+	+	−	−	−	−	+	+	−	−	−
Group XV. Non-chromogenic Cocci																
Liquefying	8	−	−	−	−	±	+	−	−	−	+	±	±	+	±	±
Non-liquefying	27	−	−	−	−	±	+	−	−	−	−	+	+	+	+	±
Group XVI. Sarcinae																
Yellow	1	−	−	−	−	+	+	−	−	−	−	+	+	+	−	−
White	2	−	−	−	−	−	+	−	−	−	−	+	+	+	+	+
Group XVII. Streptococci																
Liquefying	1	−	−	−	−	−	−	−	−	−	−	+	+			
Non-liquefying	3	−	−	−	−	−	+	−	−	−	−	+	+			+

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Table III.

BIOLOGY

Biochemical Features

Grows at body temperature	Facultative anaerobe	Affected by range of reaction	Liquefaction			Gas production			Nitrate reduced	Indol produced	Milk			Fecal odour	Nutrient agar tubes	
			Gelatin	Casein	Blood serum	Dextrose broth	Lactose broth	Saccharose broth			Curdled	Acid	Alkaline		Chromogenesis	Fluorescence
+	+	+	−	−	−	+	+	+	±	±	+	+	−	±	−	−
+	+	+	−	−	−	+	+	−	±	±	+	+	−	±	−	−
+	+	+	−	−	−	+	+	+	±	±	+	+	−	±	−	−
+	+	+	−	−	−	+	+	−	±	±	+	+	−	±	−	−
+	+	+	−	−	−	+	+	±	±	±	+	+	−	±	−	−
+	+	+	+	+	+	+	−	+	±	±	+	+	−	±	±	−
+	+	+	±	±	±	+	−	+	±	±	±	+	−	±	±	−
+	+	+	±	±	±	+	±	+	±	±	+	+	−	±	−	−
+	+	+	−	+	+	+	−	−	±	±	−	−	+	±	−	−
+	±	+	+	+	+	−	−	−	±	±	+	+	−	±	−	+
±	−	+	−	+	+	−	−	−	±	±	−	−	+	±	−	+
+	−	+	+	+	±	−	−	−	+	−	+	+	−	−	−	−
+	−	+	+	+	+	−	−	−	+	−	±	+	−	−	−	−
+	−	+	+	+	+	−	−	−	+	−	+	+	−	−	brown	−
+	−	+	+	+	+	−	−	−	+	−	+	+	−	−	rose	−
+	−	+	+	+	+	−	−	−	−	−	+	−	+	−	yellow	−
+		+	+	+		−	−	−	+	−	−	−	+	−	−	−
+			−	−		−	−	−		−	−	−	+	−	−	−
+	+	−	+	+	+	−	−	−	±	±	±	+	−	−	−	−
+	+	−	+	+	+	−	−	−	±	±	+	−	+	−	−	−
±	+	−	+	+	+	−	−	−	±	±	−	−	+	−	−	−
+	+	−	−	−	−	−	−	−	±	±	±	+	−	−	−	−
+	+	−	−	±	−	−	−	−	±	−	−	−	+	−	−	−
±	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	+	+	+	+	−	−	−	−	+	−	+	+	−	−	red	−
+	−	+	+ s	−	−	−	−	−	−	−	−	−	+	−	red	−
+	−	−	+	−	−	−	−	−	+	−	−	−	+	−	red	−
+	−	−	+	+	−	−	−	−	−	−	+	+	−	−	orange	−
+	−		+	+	−	−	−	−	−	+	+	+	−	−	orange	−
+	+	+	± s	+	−	−	−	−	±	−	±	−	±	−	yellow	−
+	+	+	+	+	−	−	−	−	−	−	−	−	+	−	yellow	+ red
+		+	+ r	−	−	−	−	−	−	−	−	−	+	−	bright yel.	−
+	−	+	+	+	−	−	−	−	+	±	+	+	−	−	yellow	−
+			−	+	−	−	−	−			−	−	+	−	yellow	−
+	−	−	−	+	−	−	−	−	+	−	−	−	+	−	pink	−
+	±	±	+ r	±	−	−	−	−	−	−	+	+	−	−	−	−
+	±	±	−	±	−	−	−	−	−	−	−	−	+	−	−	−
+		−	+ s	+	+	−	−	−	−	−	−	−	−	−	yellow	−
+	−	−	−	−	−	−	−	−	+	+	−	−	+	−	−	−
+			+	+	−	−	−	−	−	−	+	+	−	−	−	−
+			−	−	−	−	−	−	−	−	±	+	−	−	−	−

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