THE ENTEROCOCCUS AND THE FÆCAL STREPTOCOCCI: THEIR PROPERTIES AND RELATIONS.

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(PLATE I.)

No organism has been invoked as the pathogenic agent in such a variety of disease conditions as the streptococcus; and in few groups of organisms is the problem of classification and differentiation so difficult and so involved.

Occurring as important saprophytic inhabitants of the human body, and having their chief seat of pullulation in the alimentary tract, they constitute a source of potential infection from which, it is held, disease may be set up whenever conditions arise favourable to the departure of these organisms from their saprophytic mode of life and permitting the invasion of the body tissues. To be able to recognise the pathogenic streptococci when such organisms are recovered from diseased tissues, and to appreciate what their rôle in such conditions may be, the first essential for the bacteriologist is to have a clear conception of the normal saprophytic forms occurring in the human body, in order that he may compare their characters with those of the organisms found in disease and, if possible, differentiate between the two. The very fact of the difficulties in the way of streptococcus classification being so great renders the subject all the more important, and it is only when the differentiation of these organisms is better and more widely understood that a fuller comprehension of the pathology and treatment of streptococcus infections can be expected.

Pioneer work in the direction of classification was carried out by Mervyn Gordon (1903-11¹), who introduced the series of biological tests associated with his name, and, applying them to a long range of salivary streptococci, was able to classify these into a number of types. Prior to this the most important work on differentiation had been that of Schottmüller (1903²) who showed that the property of hæmolyzing, or of producing methæmoglobin from blood, might be made a basis for classification. A. C. Houston (1903-4³), working on the lines proposed by Gordon, similarly examined and determined the main features of streptococci of the fæces, and Andrewes and Horder (1906⁴), utilising the results of Gordon and Houston, and applying a slightly extended series of tests to a

large number of streptococci isolated by them from disease conditions, undertook a wide statistical study of the genus, as a result of which they were able to arrange the whole series of organisms, so studied, into seven large groups (including the pneumococci), each group centering in a definite type, demarcated by its biological activities, and connected with other types by a graded series of intermediate forms, which these authors look upon as variants of the central type, either by the suppression or by the addition of certain properties. Thus a short-chained organism, growing moderately well on gelatin at 20°C., clotting milk and reducing neutral red, producing acid in saccharose, lactose, and raffinose, and of little virulence for rodents, is considered as the central type of the *salivarius* group; a similar organism, failing to ferment raffinose, is looked upon as a variant of the *salivarius* type by defect, in respect of this property; whilst one giving the reactions of the central type but, in addition, producing acid in glucosides is considered as a variant, by addition, of this property.

In the course of the recent war considerable attention has been directed to the possible importance of the fæcal group of streptococci, both as a source of infection of suppurating wounds and as ætiological agents in the whole gamut of minor pyrexial conditions collectively grouped under the vague designation of "Pyrexia of Unknown Origin"; often, be it said, with the production of no very definite reasons for the assignation of the organisms found to this section of the streptococcus group.

It should be insisted upon that where Andrewes and Horder's classification is made use of, the tests employed by these authors in arriving at this classification should invariably be carried out. The practice of designating organisms *salivarius*, *fæcalis*, etc., upon such uncertain grounds as morphology and naked-eye appearances on culture media, cannot be too strongly deprecated as only leading to confusion.

The enterococcus, and the fæcal cocci, have long been suspected of causal relationship to certain varieties of enteritis; but it is mainly as wound-infecting organisms that they have been implicated in the late campaign.

This was done by Fleming (1915⁵) in one of the earliest papers on the bacteriology of septic war wounds: he, however, did not employ any differentiating tests and confined himself to drawing attention to the resemblance of encapsulated diplococci, seen in the films of pus, to certain of the intestinal streptococci. Shortly afterwards Wright (1915⁶), writing upon wound infections, described a streptococcus which he found to be the most universally present organism in wounds and, differentiating it from the *Streptococcus pyogenes*, considered it to be identical with the *Enterococcus* of French authors,—basing his identification upon the morphology of the organism and certain of its cultural characters. He states that this organism is the ordinary streptococcus of the fæces. Similarly, Weinberg and Seguin (1916⁷), Cottet (1918⁸), Rosenthal (1917⁹), Weissenbach (1918¹⁰), Hautefeuille and Soulié (1918¹¹), Sacquépée (1918¹²) and others all note the presence of the enterococcus in the wounds studied by them.

On the medical side, T. Houston and M'Cloy (1916¹³) have described the finding of the enterococcus, very constantly, in the blood, sputum, and urine of

patients suffering from Trench Fever, and those myalgic and pyrexial conditions often included under this heading, which have been so common amongst the troops in the late campaign. They found the same organism in 41 per cent. of 110 wounds examined. In their paper a comprehensive description of the organism is given, with their reasons for classifying it as such.

The streptococcus-like organisms found in the fæces have been the subject of very numerous descriptions, and a whole host of loosely described types encumber bacteriological literature. It is evident that many of these should be entirely disregarded in the light of more modern work, since the distinctions made use of in defining them are now recognised as insufficient. There appear, however, to be certain constantly recurring and outstanding types, all considered more or less distinct by those who have worked with them. A survey of a mass of confused literature suggests the following three groups as worthy of critical consideration.

1. Described by Continental workers:—

- (a) The *Enterococcus* of Thiercelin (*Micrococcus ovalis* of Escherich).
- (b) The *Streptococcus enteritis* (Hirsh and Libman 1897¹⁷).

2. Described by English workers:—

- (a) The *Streptococcus fæcalis* of Andrewes and Horder.

The *Enterococcus*, which was described by Thiercelin in (1899¹⁴), before the introduction of more recent methods, and accounted by him the causal agent in certain diarrhoeas, biliary infections, and cases of appendicitis, has received little attention in English bacteriological literature; whereas the *Streptococcus fæcalis* is well represented. It seems to have been tacitly accepted, by English writers, that the two organisms are identical, a conclusion which appears open to doubt when the literature assembled about these two names is studied. The enterococcus, as such, is not mentioned in the majority of English text-books, and the descriptions of this organism in French and American works differ in some important details from those of the *Streptococcus fæcalis* of A. C. Houston and Andrewes and Horder, as well as from such descriptions of the enterococcus as have appeared in recent war-literature.

Winslow (1908¹⁵) summarises the characters of the *Streptococcus fæcalis* as follows:—"It is short-chained and renders broth uniformly turbid. It grows readily at 20° C. and forms sulphuretted hydrogen in broth cultures. It has no hæmolytic power and little virulence, but produces a positive reaction to all Gordon's tests except raffinose and inulin. That is, it clots milk, reduces neutral red, and ferments saccharose, lactose, salicin, coniferin, and mannite. The mannite reaction is specially characteristic of this intestinal type."

This description applies equally well to the characters of the commonest type of streptococcus found by A. C. Houston in the examination of 300 organisms isolated from 19 different stools. Houston differentiated these cultures into 40 groups. Thirteen per cent. of the whole series had the characters just described. A considerable proportion of the organisms studied by this worker fermented raffinose (32 per cent.), whilst only a very small number (4·6 per cent.) fermented inulin.

Turning to the descriptions of the *enterococcus*, the following summary of its characteristics is given by Besson (1914¹⁰):—

- (a) The organism shows considerable morphological pleomorphism. It resembles the pneumococcus in the body fluids and the streptococcus in cultures of some days old.
- (b) It grows abundantly in gelatin at 20° C.
- (c) Its growth in liquid serum is minimal.
- (d) The vitality of the organism is very great.
- (e) Sometimes it shows the characteristic peculiarity of inability to grow aerobically unless previously cultured under anaerobic conditions.

The *Streptococcus enteritis* of Hirsh and Libman (1897¹⁷) comprises long and short-chained groups, considered as distinct by these writers, and which, since then, have been variously considered as identical with, or distinct from, the enterococcus.

Tissier (1900¹⁸) pays considerable attention to these organisms, which he found of frequent occurrence in the stools of infants. He lays stress upon the diplococcal character of the enterococcus and its tendency to be surrounded by an areola. He differentiates it from the *Streptococcus enteritis* (q.v.) by these two points and by its failure to form chains of more than a few individuals; whereas the *S. enteritis* forms chains of from ten to fifteen elements. The enterococcus is the more luxuriantly growing of the two forms, produces more sharply contoured colonies upon agar, and has a more rapid action in clotting milk. In his hands neither of these organisms showed much pathogenicity.

Dopter and Sacquépée (1914¹⁹) largely concur with the description of Besson, and state, in addition, that the enterococcus inconstantly coagulates milk, produces a toxin capable of killing rabbits by progressive cachexia, and is sometimes pathogenic for mice. They, also, remark upon its pleomorphism. Macé (1912²⁰), considers the enterococcus as identical with the *Streptococcus enteritis* of Escherich (1899²¹), which appears to be the chain-forming type described under the same name by Hirsh and Libman. He lays stress upon the pleomorphic morphology of the organism and further states that it is able to grow at 46° C.; it never liquefies gelatin, and inconstantly causes coagulation in milk. It is killed by an exposure of fifteen to twenty minutes to a temperature of 60° C., and is without action upon any of the sugars. It is pathogenic for mice and slightly so for rabbits. He places it in an intermediate position between the pneumococcus and the streptococcus proper.

Park and Williams (1914²²), in America, agree in general with the description given by Macé. They, also, state that the organism does not ferment sugars and is pathogenic to mice. They add that it resembles the staphylococcus on agar and gelatin but fails to liquefy the latter. It is sensitive to exposure to heat or sunlight.

Amongst recent writers, T. Houston and M'Cloy (*loc. cit.*) give a full account of the organism they have isolated from Trench Fever patients and from suppurating wounds. They describe it as a luxuriantly growing organism of great vitality, which, in preparations from ordinary media, appears as a diplococcus or short streptococcus. It coagulates milk and ferments glucose, lactose, saccharose, and salicin, but is without action upon mannite. They lay much stress upon the ability of the organism to withstand heat, and they found it capable of surviving an exposure of one and a half hours to a temperature of 55° C. This may be comparable with an earlier observation of Logan (1914²³), who found a coccus in the fæces of infants capable of surviving Pasteurisation for ten minutes at 80° C.

The organism found by Wright (*loc. cit.*), in wounds, generally appeared in pus films as a lanceolate diplococcus and its microscopical appearance, in agar

and broth cultures, was similar. It grew more luxuriantly than did the *Streptococcus pyogenes* and was able to grow at room temperature. No hæmolysis was produced on blood-smeared plates. Wright identifies the organism with the enterococcus and also with the *Streptococcus faecalis*, although he does not appear to have employed Gordon's tests. He states that it grows luxuriantly in pure serum, sometimes forming convoluted chains of great length and, further, when cultured upon agar from a growth in blood, produces large colonies, resembling the staphylococcus, as much as a half to one centimetre (!) in diameter. Georges Rosenthal (*loc. cit.*) distinguishes two types of cocci in infected wounds: short-chained, non-hæmolytic enterococci, of great vitality; and hæmolytic, true streptococci, of low vitality. Similarly, Weissenbach (*loc. cit.*) notes the occurrence of both streptococci and enterococci in his cases, and remarks upon the necessity for differentiation between these two organisms as a point of much surgical importance. Levaditi (1918²⁴) classifies the wound streptococci as: (1) Typical Pyogenic Streptococci, which are hæmolytic and virulent, but show little tendency to affect mannite; and (2) Atypical Streptococci, which are non-hæmolytic and avirulent, and which show diverse sugar reactions.

Donaldson (1917²⁵) gives a brief summary of the characters of the enterococcus, apparently based upon the study of a single case of puerperal septicæmia, from which he isolated the organism. He notes that it grew generally as a pneumococcus-like diplococcus which showed some pleomorphism, especially in older cultures: it did not give rise to hæmolysis on blood agar, and grew well on gelatin at 18° C., without producing liquefaction. Acid was produced from glucose, lactose, maltose, saccharose, raffinose, glycerol, mannite, and inosite. The organism was moderately pathogenic to mice and rabbits. Weissenbach (1918^{26a}) studied 104 strains of cocci, including 36 enterococci, derived from stools and pathological processes. He devised a differential test to distinguish these from the *Streptococcus pyogenes* consisting in the employment of a fluid medium containing 10 per cent. of bile which gave good growths of enterococci but in which the streptococci failed to grow. In a further paper (1918^{26b}) he has shown that non-hæmolytic true streptococci, obtained from suppurative conditions, comport themselves similarly to the *Streptococcus pyogenes*, *vis à vis* this medium.

From the foregoing short summary of several descriptions it is evident that, although the subject is approached from rather different aspects, there are considerable divergencies between the characters of the *S. faecalis* of English writers and those of the enterococcus of others. Whilst descriptions of the *Streptococcus faecalis* are all comparable these differ widely from those of the enterococcus and, in addition, those of the latter differ considerably amongst themselves. Although most workers are agreed that the enterococcus is an organism growing well on ordinary media, tending to pleomorphism, of great vitality and devoid of hæmolytic power, there are notable divergencies between the descriptions of its other properties. For example, whilst both Macé and Park and Williams state that the organism is without action upon the sugars, T. Houston and M'Cloy, and others, ascribe considerable ability to split sugars to their strains, which as regards morphology and cultural characters generally correspond to Besson's description. The *Streptococcus faecalis* of Andrewes and Horder is one of the most actively saccharolytic members of the streptococcus group and is

characteristically a mannite fermenter; a property which is stated not to have been possessed by Houston and M'Cloy's strains. In general, the differences alluded to are such as to suggest that in many cases different organisms are under description.

In view of the increasing attention paid to this section of the streptococci, and the above-mentioned conflicting descriptions that are found in the literature of the subject, it seemed desirable to undertake a systematic examination of a series of these organisms, with the object of portraying the relationship between the enterococcus and the *Streptococcus faecalis* and, should the two organisms not prove identical, of endeavouring to define the characters of the first-named and aid its identification. Experience has shown that such classification as can be arrived at within the streptococcus genus is one of groups, and that this necessitates the study of as wide a series of strains as is possible. The restricted results derived from the examination of a few strains are, for this purpose, worthless.

The present paper is based upon the study of 152 strains, and deals solely with the bacteriology of the organisms as found in the saprophytic state in the human body: it makes no attempt to deal with them as agents of pathological processes. The work was carried out in a Field Laboratory, and certain discontinuities in the observations are due to the conditions of working.

METHODS.

The matter is approached both from the morphological standpoint, and that of the biological activities of the organisms. Morphological differences within the streptococcus group are, generally, considered unsafe grounds for differentiation and some, following Marmorek, would completely disregard them. It was necessary, however, to observe morphology in this work as so many of the existing descriptions of the enterococcus, particularly the French ones, are largely based upon microscopical and cultural appearances.

Source of the Organisms.

These were, without exception, isolated from faeces. In most instances a few colonies from each stool were examined, so as to obtain an idea of the types most commonly occurring in a wide selection of individuals; some of these colonies were taken from the MacConkey plates in use in the ordinary routine work of the laboratory. In a few other cases a more exhaustive examination of an individual was made, to obtain information as to the whole streptococcal flora of the individual stool: in these cases a variety of methods was made use of, so as to include all the types present, in case certain methods should favour the isolation of any particular type.

Method of Isolation.

The strains to be described were obtained as follow:—

- (1) By the direct plating of fresh faeces upon plain agar.
- (2) By the direct plating of fresh faeces upon MacConkey's bile-salt-lactose

agar. (A proportion of the stools, in these instances, had been previously passed through brilliant-green, in the routine search for typhoidal organisms.)

(3) By the methods advocated by Thiercelin²⁷ as being especially favourable for the isolation of the enterococcus, *i.e.*,

(a) Filtration of the fæcal emulsion prior to plating.

(b) Anaerobic cultivation of the fæces in broth, followed by plating upon agar.

(4) To obtain Houston and M'Cloy's heat-resisting forms the stool was cultivated in broth for eighteen to twenty-four hours, then heated for thirty minutes to 60° C.; reincubated, with the addition of some fresh broth, for a further eighteen to twenty-four hours, and finally plated.

Isolated colonies were picked off and replated. It was found that this was not always sufficient to ensure purity, so, early in the work, the practice of replating all colonies at least twice was instituted. When finally isolated in pure culture the organism was subcultured into serum or ascitic broth for detailed examination. All chromogenic organisms were excluded.

Methods of Examination.

The following properties were studied :—

(a) *Morphology*.—Macroscopic and microscopic features of growth on agar, in neutral broth, serum broth and, occasionally, in other media.

(b) *Biological Properties*.

(α) The characters of the growth in gelatin at 20 to 22° C.

(β) *Fermentative ability*. The action on milk, and ability to produce acid from lactose, saccharose, raffinose, inulin, salicin, and mannite were routine tests, and were carried out in His' serum-water medium, with a reaction distinctly alkaline to litmus which was used as an indicator. The reactions were read at the end of three or four days and were usually sharp. No sugar reaction was accepted as negative unless the organisms could be shown to be alive in the medium at the end of this period. Dulcitol, sorbitol, and glucose were occasionally used; the former was never acted upon and the latter was invariably fermented in the cases in which it was employed.

Gordon's neutral-red reduction test was not applied as, in the author's hands, the reaction was rarely obtained and was then often equivocal.

[A. C. Houston (*loc. cit.*) also found this reaction unreliable and notes that the personal factor, in reading the results, is undesirably great. Floyd and Wolbach (1914²⁸) in a study of 247 strains of streptococci only obtained a positive reaction once. Hopkins and Lang, quoted by Chalmers and Atiyah (1919²⁹) and concurred with by those authors, do not consider this to be a reliable test. Possibly samples of the dye differ in the readiness with which they undergo reduction, as, in the cases in which the writer applied the test, it was carried out exactly as kindly directed by Gordon in a personal communication.]

The decomposition of urea, and the production of sulphuretted hydrogen, were studied in a number of cultures.

The production of hæmolysis, and the formation of methæmoglobin, were tested for by allowing a series of dilutions of an 18-hour serum-broth culture (horse-serum 1 part, broth 4 parts) to act for two and a half hours at 37° C., on an equal volume of a 10 per cent. suspension of washed rabbit's corpuscles; the tubes were placed in the cool overnight and read the following morning. This method is available for the quantitative estimation of methæmoglobin formation, as well as of hæmolysis, as has been shown by Blake (1916³⁰).

Neufeld's test (1912³¹), of solubility in 1 per cent. sodium taurocholate, was applied in most cases either to a washed emulsion from an agar culture or, less usually, to a 24-hour culture in serum-broth, as advocated by Mair (1916³²).

Pathogenicity was tested as soon after isolation as possible. Quantities of 1.0 c.c. and 0.5 c.c. of a 24-hour culture in serum-broth were injected subcutaneously into white mice. Rabbits were also used in some cases. The vitality of the organism was roughly estimated by testing its ability to survive for fifteen days on an agar slope at 37° C. The thermo-resistance was tested by immersing Pasteur pipettes, each containing a small quantity of a broth culture, in a bath kept at a temperature of 60° C. At the end of 5, 10, 20, and 30-minute periods, a pipette was withdrawn and the contents blown out into a tube of glucose-broth. The tubes were incubated for four days and examined daily for growth.

RESULTS.

It may be stated at the outset that, with three exceptions, all the strains examined proved to have some saccharolytic powers. It is thought well to give a complete list of these (Table I.), along with certain other properties considered vital for purposes of classification, before proceeding to analyse the results obtained. To each organism an identification number, for purposes of future reference, is attached. It will be noted that very frequently several strains giving identical reactions were isolated from the same stool.

It will further somewhat clear the ground to mention and dismiss, for the present, certain negative properties. Hæmolysin-formation was only met with in two strains which occurred together in a single stool, and methæmoglobin-formation was only found in one strain. It may be therefore stated that the overwhelming majority of the organisms examined were without the actions on red corpuscles which are so commonly associated with the pathogenic streptococci.

The absence of pathogenicity for mice and rabbits was striking. Of the 69 strains tested against mice, 56 were found to be entirely devoid of pathogenic action, either immediate or delayed. The animals showed no ill-effects from the comparatively enormous doses injected and remained well until observation was discontinued at the end of three to four months. Thirteen organisms showed some degree of pathogenicity, viz.: Nos. 50, 51, 52, 53, 79, 91, 112, 209, 255, 260, 264, 266, and 269; the significance of these results will be discussed at a later stage. Organisms Nos. 100, 110, 187, 206, 207, 264, 266 were examined for pathogenicity towards rabbits. In all cases the results were entirely negative, even when large doses, *e.g.*, 5 c.c. of a 24-hour serum-broth culture, were injected intravenously. Efforts were made to exalt the virulence, and to produce pathological effects, with strains 100 and 110. These were injected intravenously along with killed typhoid cultures and the organisms recovered from the blood when death ensued. The blood was citrated, incubated for some eighteen hours to increase the number of organisms present, and reinoculated, along with a killed typhoid emulsion, into a second animal. After the death of this animal it was recovered and injected into a third. In each case the organisms were found incapable of maintaining themselves in the animal's tissues, whatever the route might be

that was adopted for inoculation and even when massive doses were employed.

The bile-solubility test was found to be invariably negative. Some strains, as will be seen, closely resembled the pneumococcus in many particulars, but none of these underwent lysis in the taurocholate solution. Control tests with known pneumococci were positive. No action was found upon dulcitol or urea.

Sugar Types.

Considering the organisms upon the accepted grounds of classification of Andrewes and Horder, we find that the whole series can be artificially divided, with seeming reason, into four saccharolytic groups, viz.:—

- I. A mannite-fermenting group.
- II. A raffinose- or inulin-fermenting group.
- III. A "Combined" group: comprising types which split mannite as well as raffinose, or the polysaccharide.
- IV. An "Undifferentiated" group; which lacks action on any of these three substances.

Table II. shows such a grouping, drawn up upon saccharolytic characters alone.

Following the classification of Andrewes and Horder, the types in Group I. comprise the frequently-occurring and well-recognised Central Type (A) of the *fæcalis* group; along with a number of other types varying in different degrees from this central type. Type B varies by the addition of the property of liquefying gelatin; and the remainder by defect of different saccharolytic properties.

Group II. consists of strains fermenting raffinose or inulin, or both of these substances, but being without action on mannite. Raffinose and inulin fermentation are considered characteristics (the former especially so) of the salivary streptococci, but have been found not infrequently (raffinose 32 per cent., inulin 4·6 per cent.—A. C. Houston) in organisms from the fæces. From the fact that organisms of this type were recovered from a number of stools, they would appear to be frequently present, though in lesser numbers than certain other forms. It may be noted that all these raffinose-inulin sugar types appear in Gordon's analysis of the salivary organisms.

Considering Group III., we come face to face with the necessary but extremely inconvenient weakness of Andrewes and Horder's classification—the variants. Indeed, it is the number of variants and the multiplicity of forms intergrading between the Central Types, that has tended to lessen the practical value of their most important work, and to make the clinical pathologist despair of differentiating streptococci and pursue the easier road of denying the subject its merited importance. This group combines the salient features of the salivary

TABLE I.

Stool Number.	Organism Number.	Method of Isolation.	Character of Colonies on Agar.	Morphology of Organism.	Involution.	Capsule Formation.			Growth at 22° C.	
									Growth	Liquefaction.
1 (F)	2	Thiercelin "A"	Coarse	Diplococci	+	-	-	+	+	-
2	12	"	Medium	Diplococci and short chains	-	-	-	+	+	-
3	17	Thiercelin "B"	Coarse	Diplococci	-	-	-	+	+	-
4	18	"	Fine	Streptococcus medius	-	-	-	+	+	-
5	19	MacConkey plate	Medium	"	+	-	-	+	+	-
6	20	"	Coarse	"	+	-	-	+	+	-
7	27	MacConkey plate, after brilliant green	"	Diplococcus	-	-	-	+	+	-
8	28	"	"	Diplococci and short chains	+	-	-	+	+	-
9	29	"	"	Diplococci	-	-	-	+	+	-
10	30	"	"	"	-	-	-	+	+	-
11	34	MacConkey plate	"	Growth in irregular clumps	+	-	-	+	+	-
12	36	"	"	"	+	-	-	+	+	-
13	37	MacConkey plate, after brilliant green	"	Diplococci	+	-	-	+	+	-
14	38	MacConkey plate	"	"	-	-	-	+	+	-
15	39	"	"	"	-	-	-	+	+	-
16	40	"	"	"	-	-	-	+	+	-
17	41	"	"	Diplococci and short chains	+	-	-	+	+	-
18	46	MacConkey plate, after brilliant green	"	Diplococci	+	-	-	+	+	-
19	47	"	"	"	-	-	-	+	+	-
20	48	"	"	"	-	-	-	+	+	-
21	49	"	"	"	+	-	-	+	+	-
22	50	"	"	Diplococci and short chains	-	-	-	+	+	-
23	51	"	"	Diplococci	-	-	-	+	+	-
24	52	"	"	"	-	-	-	+	+	-
25	53	"	"	"	-	-	-	+	+	-
26	60	Thiercelin "B"	Very fine	Irregular clumps and tetrads	+	-	-	+	+	-
27	61	"	"	Irregular clumps, tetrads and chains	+	-	-	+	+	-
28	62	"	"	"	+	-	-	+	+	-
29	66	Plating on plain agar	Medium	Short chains and diplococci	-	-	-	+	+	-
30	67	"	Fine	Streptococcus longus	-	-	-	+	+	-
31	68	Thiercelin "A"	Coarse	Short chains and diplococci	-	-	-	+	+	-
32	69	"	Medium	Kidney-shaped diplococci and irregular clumps	+	-	-	+	+	-
33	70	"	"	"	+	-	-	+	+	-
34	71	Plating on plain agar	Moderately coarse	Short chains and diplococci	-	-	-	+	+	-
35	72	Thiercelin "A"	Moderately fine	Kidney-shaped diplococci	+	-	-	+	+	-
36	73	Plating on plain agar	Moderately coarse	Diplococci	-	-	-	+	+	-
37	77	MacConkey plate	Coarse	"	+	-	-	+	+	-
38	78	Thiercelin "B"	"	"	-	-	-	+	+	-
39	79	"	Medium	"	-	-	-	+	+	-
40	80	"	Coarse	Diplococci and short chains	-	-	-	+	+	-
41	83	MacConkey plate, after brilliant green	Fine, conglomerate growth	Streptococcus longus	+	-	-	+	+	-
42	84	"	Fine	"	+	-	-	+	+	-
43	85	"	Coarse	Diplococci	+	-	-	+	+	-
44	86	"	"	"	+	-	-	+	+	-
45	88	"	"	"	-	-	-	+	+	-
46	89	"	"	"	-	-	-	+	+	-
47	90 (a)	MacConkey plate	"	Streptococcus longus	+	-	-	+	+	-
48	90 (b)	"	"	Diplococci	-	-	-	+	+	-
49	91	"	Fine	Streptococcus longus	-	-	-	+	+	-
50	92	"	Coarse	Diplococci	+	-	-	+	+	-
51	93	MacConkey plate, after brilliant green	Medium	Streptococcus longus	-	-	-	+	+	-
52	99	"	"	Diplococci	-	-	-	+	+	-
53	100	Thiercelin "A"	Coarse	"	-	-	-	+	+	-
54	101	"	"	"	-	-	-	+	+	-
55	102	"	"	"	-	-	-	+	+	-

TABLE I.

[illegible]

TABLE I.—Continued.

Stool Number.	Organism Number.	Method of Isolation.	Character of Colonies on Agar.	Morphology of Organism.	Involution.	Capsule Formation.	Gelatin.	
							Growth at 22° C.	Liquefaction.
19	103	Thiercelin "A"	Coarse	Diplococci	-	-	+	-
19	104	"	"	Diplococci and short chains	-	-	+	-
19	105	Plating on plain agar	Moderately fine	Short chains	-	-	+	-
19	106	"	"	Diplococci and short chains	-	-	+	-
19	107	"	"	Short chains	-	-	+	-
19	109	Thiercelin "A"	Coarse	Diplococci	-	±	+	-
19	110	"	"	"	-	±	+	-
19	111	"	Moderately fine	Short chains and diplococci	±	-	+	-
19	112	Plating on plain agar	Very fine	Streptococcus longus	-	-	-	-
20 (F)	113	"	Fine	"	-	-	-	-
20 (F)	115	"	Coarse	Short chains and diplococci	-	±	+	-
20 (F)	116	"	"	Streptococcus medius	-	-	+	-
20 (F)	117	"	"	Diplococci	-	-	+	-
20 (F)	125	"	"	"	-	-	+	-
21 (A)	127	MacConkey plate, after brilliant green	"	"	-	+	+	-
21 (A)	128	"	"	"	-	±	+	-
21 (A)	129	"	"	"	-	+	+	-
21 (A)	130	"	"	"	-	+	+	-
22	132	Plating on plain agar	Coarse conglomerate growth	Streptococcus longus	-	-	+	-
22	133	"	Coarse	Short chains	±	-	+	-
22	134	"	"	"	-	-	+	-
22	135	"	Coarse conglomerate growth	Streptococcus longus	-	-	+	-
22	136	"	Coarse	Diplococci	-	-	+	-
22	137	"	Coarse conglomerate growth	Streptococcus longus	-	-	+	-
22	138	"	"	"	-	-	+	-
22	139	"	"	"	-	-	+	-
22	140	"	"	"	-	-	+	-
22	141	"	"	"	-	-	+	-
23	143	"	Coarse	Irregular clumps	+	-	+	-
23	146	"	"	"	+	-	+	-
24	148	"	"	Short chains	+	-	+	-
24	149	"	"	"	+	-	+	-
24	150	"	"	"	+	-	+	-
24	155	"	"	"	+	-	+	-
25 (1)	163	"	"	Diplococci and short chains	±	-	+	-
25 (1)	164	"	"	Diplococci	±	-	+	-
25 (1)	165	"	"	Diplococci and short chains	±	-	+	-
25 (1)	168	"	Medium	Streptococcus medius	-	-	±	-
25 (1)	169	"	Coarse	"	±	-	+	-
25 (1)	170	"	"	Diplococci	-	-	+	-
25 (1)	171	"	Coarse conglomerate growth	Streptococcus medius et conglomeratus	-	-	+	-
25 (1)	174	Thiercelin "B"	Coarse	Diplococci	±	-	+	-
25 (1)	175	"	"	"	-	-	+	-
25 (1)	179	"	"	"	-	-	+	-
25 (1)	184	Preliminary heating of growth to 60° C. for 30 minutes.	"	"	-	-	+	+
25 (1)	185	"	"	"	-	-	+	+
25 (1)	187	"	"	"	±	-	+	-
25 (1)	188	"	"	"	-	-	+	+
25 (1)	189	"	"	Diplococci and short chains	±	-	+	-
26	190	MacConkey plate, after brilliant green	"	Diplococci	-	-	+	-
27	192	"	"	"	-	-	+	-
28	195	"	"	Short chains	-	-	+	-
29 (F)	196	MacConkey plate	"	Diplococci	-	-	+	-
25 (2)	198	"	"	"	-	-	+	-
25 (2)	199	"	Coarse, doubly contoured colonies	Short chains	-	-	+	-
25 (2)	203	"	Coarse	"	-	-	+	-

TABLE I.—*Continued.*

Stool Number.	Organism Number.	Method of Isolation.	Character of Colonies on Agar.	Morphology of Organism.	Involution.	Gelatin.		
						Capable Formation.	Growth at 32° C.	Liquefaction.
25 (2)	204	MacConkey plate	Coarse, doubly contoured	Short chains	±	-	+	-
25 (2)	206	"	"	"	-	-	+	-
25 (8)	207	Plating on plain agar	Coarse "	Diplococci	-	-	+	+
25 (8)	208	"	"	Diplococci and short chains	-	-	+	-
25 (8)	209	"	"	Diplococci	-	-	+	+
30	222	MacConkey plate	"	Diplococci and short chains	-	-	+	-
30	223	"	"	"	-	-	+	-
30	224	"	"	Diplococci	-	-	+	-
30	225	"	"	"	-	-	+	-
25 (4)	228	Thiercelin "B"	"	Streptococcus medius	-	-	+	-
32	230	Plating on plain agar	"	Diplococci	-	-	+	-
32	231	"	Fine	Streptococcus medius	-	-	+	-
32	232	"	Coarse	Diplococci	-	-	-	-
32	233	"	Fine	Streptococcus medius	-	-	-	-
32	234	"	"	"	±	-	-	-
32	235	"	Coarse	Diplococci	-	-	+	-
32	236	"	"	"	-	-	-	-
33	239	MacConkey plate	"	Diplococci and short chains	-	-	+	+
33	240	"	"	"	-	-	+	+
34	241 (F)	"	"	Diplococci	-	-	+	-
34	242	"	"	Short chains and diplococci	-	-	+	-
34	243	"	"	Streptococcus medius	-	-	-	-
35	248	Plating on plain agar	Fine	"	-	-	±	-
35	250	"	"	"	-	-	±	-
35	253	"	"	"	-	-	-	-
25 (5)	254	"	"	Streptococcus longus	-	-	±	-
25 (5)	255	"	Medium	Streptococcus medius	-	-	±	-
25 (5)	256	"	Coarse	Diplococci	-	-	±	-
25 (5)	257	"	"	"	-	-	±	-
25 (5)	258	"	"	"	-	-	±	-
25 (5)	259	"	"	"	±	-	+	-
25 (5)	260	Preliminary heating of growth to 60° C. for 30 minutes.	"	Diplococci and short chains	-	±	+	-
25 (5)	261	"	"	"	±	±	+	-
25 (5)	262	"	"	Diplococci	-	-	+	-
36	263	Plating on plain agar	"	"	+	-	+	-
36	264	"	Fine	Streptococcus longus	-	-	-	-
36	265	"	Coarse	Diplococci	-	-	+	-
36	266	"	Medium	Streptococcus medius	-	-	-	-
36	268	"	Fine	Short chains	-	-	+	-
36	269	"	Medium	"	-	-	+	-
36	270	"	"	Conglomeratus (chains)	-	-	+	-

EXAMPLES OF CERTAIN OTHER COCCI, ISOLATED FROM

12	56	Thiercelin "A"	Staphylococcus albus	Staphylococci	-	-	+	+
12	59	"	Lemon yellow colonies	Gram negative staphylococci	-	-	+	+
13	65	Plating on plain agar	Large staphylococcus albus-like colonies	Staphylococci	±	-	+	-
13	76	MacConkey plate	Coli-like sticky colonies	"	-	-	+	+
14	81	"	Dirty yellow colonies	"	-	-	+	-
25	180	Thiercelin "B"	Staphylococcus albus	"	-	-	+	±
23	144	Plating on plain agar	Fine opaque colonies	Tetrads	-	±	+	+

TABLE I.—Continued.

Sugar Reactions.							Heat Resistance. 5, 10, 20, 30 Minutes at 60° C.	Pathogenicity for Mice.		Hemolysis.	Methemoglobin.	Solubility in Bile.	Vitality.	Sulphuretted Hydrogen.	Remarks.
Clothing of Milk.	Saccharose.	Lactose.	Raffinose.	Inulin.	Saltin.	Mannite.		0.5 c.c. Dose.	1.0 c.c. Dose.						
+	+	+	+	+	+	-	- - - -			-	-	-			Avirulent to rabbit. " "
+	+	+	+	+	+	-	- - - -			-	-	-			
+	+	+	+	+	+	-	+++++		+	-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			* Mouse died 8 days after inoculation.
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			Avirulent to rabbit. " "
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			* Mouse died 14 days after inoculation.
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			
+	+	+	+	+	+	+	+++++			-	-	-			

STOOLS, BUT NOT INCLUDED IN ABOVE SERIES.

-	+	+	-	-	+	- - - -				-	-	-	+		Staphylococci or allied organisms. Gelatin softened. M. Tetragenus.
-	+	+	-	-	-	- - - -				-	-	-	+		
+	+	+	-	-	-	- - - -				-	-	-	+		
-	+	+	-	-	+	- - - -				-	-	-	+		
-	+	+	-	-	+	- - - -				-	-	-	+		
-	+	+	-	-	+	- - - -				-	-	-	+		
-	+	+	-	-	+	- - - -				-	-	-	+		
-	+	+	-	-	+	- - - -				-	-	-	+		
-	+	+	-	-	+	- - - -				-	-	-	+		
-	+	+	-	-	+	- - - -				-	-	-	+		

TABLE I.—*Continued.*

Stool Number.	Organism Number.	Method of Isolation.	Character of Colonies on Agar.	Morphology of Organism.	Involution.	Capsule Formation.	Gelatin.	
							Growth at 22° C.	Liquefaction.
COMPARISON WITH								
		Houston and M'Cloy's enterococcus (from blood culture)	Coarse	Short chains and diplococci	-	-	+	-
		Pneumococcus, recently isolated from pneumonic sputum	Transparent, fine, ringed colonies	Diplococci	-	±	-	-
		Typical wound streptococci	Fine colonies	Streptococcus medius or longus	-	-	-	-

STOOLS—

F = Fluid stool. T = From case of Typhoid.
 J = From case of Catarrhal Jaundice (no bile in stool).
 D = From case of Flexner Dysentery.
 A = From case of Paratyphoid "A."
 All others were normal stools.

INVOLUTION AND CAPSULE FORMATION—

+ = Definite. ± = Slight. - = Not observable.

GELATIN—

Growth at 22° C. + = Well marked. ± = Minimal. - = No growth.
 Liquefaction. + = Gelatin liquefied. - = No liquefaction.

SUGAR REACTIONS—

+ = Acid formation. ± = Slight acid formation.
 - = No change.

TABLE I.—*Continued.*

Clotting of Milk.	Sugar Reactions.						Heat Resistance. 5, 10, 20, 80 Minutes at 60° C.	Pathogenicity for Mice.		Hæmolysis.	Methæmoglobin.	Solubility in Bile.	Vitality.	Sulphuretted Hydrogen.	Remarks.
	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.		0·5 c.c. Dose.	1·0 c.c. Dose.						
OTHER ORGANISMS.															
{	+	+	+	-	-	+	+	++++			-	-	-		Sorbito +
	+	+	+	+	+	+	-	----	+	+	-	+	+	-	Sorbito :—Neg.
	+	+	+	+	+	+	-	----	+	+	-	+	+	-	
	+	+	+	+	+	+	-	----	+	+	-	+	+	-	

HEAT RESISTANCE—

++++	=	Organism survived 30 minutes' exposure to 60° C.
+++	=	„ 20 „ „ 60° C.
++	=	„ 10 „ „ 60° C.
+	=	„ 5 „ „ 60° C.
-	=	„ killed by 5 „ „ 60° C.
±	=	Different results in two experiments.

PATHOGENICITY FOR MICE—

+	=	Mouse died within a few days of the inoculation.
-	=	Mouse survived.
+ -	=	Different results in two experiments.

SULPHURETTED HYDROGEN FORMATION—

+	=	Definite reaction.
±	=	Slight reaction.
-	=	No change.

MORPHOLOGY—

Under this head is given the form which predominated in the various media used.

TABLE II.

Stool in which this type was found.	Reference Letter	Clotting of Milk.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Liquefaction of Gelatin.	Heat Resistance.		Remarks.
										Untested.	Thermo-Resistant.	
											Thermo-Sensitive.	

GROUP I.—MANNITE FERMENTERS.													
3, 5, 10, 11, 17, 19, 20, 25, 29, 36	A	+	+	+	-	-	+	+	-	2, 17	28, 41, 46, 48, 49, 50, 51, 52, 53, 68, 85, 93, 100, 101, 102, 103, 104, 105, 106, 107, 109, 110, 111, 115, 174, 179, 187, 189, 196, 203, 208, 225, 258, 263	125	The central type of the S. faecalis group.
25	B	+	+	+	-	-	+	+	+	Nil	184, 185, 188, 207, 209	Nil	Variant of Type "A" by addition of the property of liquefying gelatin.
1, 16, 17, 25 5, 13, 22, 28	C	+	+	+	-	-	+	+	-	2, 90 (b)	88, 256	73 (partially resistant)	Variants of Type "A" by defect of various properties. Not placed: a very unusual organism with atypical morphology.
	D	+	+	+	-	-	+	+	-	Nil	27, 66, 71, 77, 78, 79, 80, 136, 195	Nil	
33	E	+	+	+	-	-	+	+	-	"	239, 240	"	
3, 4, 25 24, 26 22	F	+	+	+	-	-	+	+	-	"	18, * 19, * 20, * 262	"	
	G	+	+	+	-	-	+	+	-	"	148, 149, 150, 155, 260, 261	133, 134	
	H	+	+	+	-	-	+	+	-	"	Nil		

GROUP II.—RAFFINOSE-INULIN GROUP.												
13, 21, 25, 32	I	+	+	+	+	+	+	-	-	98, * 127, 128, 129, 130	Nil	199, 204, 229, * 231, * 232, * 233, * 234, * 236
17, 18, 19, 20 32 7	J	+	+	+	+	+	+	-	-	Nil	"	91, * 99, * 112, * 113 *
14, 17 30	K	+	+	+	+	+	+	-	-	"	"	34, 36
	L	+	+	+	+	+	+	-	-	"	"	83
	M	+	+	+	+	+	+	-	-	90* (a)	"	225
	N	+	+	+	+	+	+	-	-	Nil	"	

* Organisms showing chain-formation as a characteristic of their morphology are marked with an asterisk.

TABLE II.—Continued.

Stool in which this type was found.	Reference Letter to Sugar type.	Clotting of Milk.	Saccharose.	Lactose.	Raffinose.	Inulin.	Sallein.	Mannite.	Liquefaction of Gelatin.	Heat Resistance.			Remarks.
										Untested.	Thermo-Resistant.	Thermo-Sensitive.	
GROUP III.—COMBINED GROUP (RAFFINOSE-INULIN-MANNITE).													
16, 30	Q	++	++	++	++	—	++	++	—	89	22, 23, 24 37 Nil	Nil	Consists of two groups, sharply differentiated by their morphology, which separation is confirmed by the heat test.
8	P	++	++	++	++	—	++	++	—	„	Nil	69, 70, 72	
13	Q	++	++	++	++	—	++	++	—	„	Nil	„	
GROUP IV.—UNDIFFERENTIATED GROUP.													
6, 9, 10, 22, 25, 26, 27, 34, 35	R	+	+	+	—	—	+	—	—	Nil	29, 30, 33, 39, 190, 192, 257	132, * 135, * 137, * 138, * 139, * 140, * 168, * 169, * 141, * 242, 258 * 84 *	Consists of two groups, sharply differentiated by their morphology, which separation is confirmed by the heat test.
9, 10, 15, 16, 20, 25, 32	S	+	—	+	—	—	+	—	—	40	47, 86, 117, 163, 164, 165, 170, 230	67, * 171, * 241, * 248, * 248, * 250, * 254, 256 *	
13, 25, 34, 35	T	—	+	—	—	—	+	—	—	Nil	Nil	148, 146, 265 116 * 268 270 266, * 269, 264 *	
23, 25, 36	U	—	+	+	—	—	+	—	—	60, 61, 62	259	148, 146, 265	Consists of two groups, sharply differentiated by their morphology, which separation is confirmed by the heat test.
12, 20	V	—	—	+	—	—	+	—	—	Nil	Nil	116 *	
36	W	—	—	+	—	—	+	—	—	„	„	268	
36	X	—	—	—	—	—	—	—	—	„	„	270	Consists of two groups, sharply differentiated by their morphology, which separation is confirmed by the heat test.
36	Y	—	—	—	—	—	—	—	—	„	„	266, * 269, 264 *	

* Organisms showing chain-formation as a characteristic of their morphology are marked with an asterisk.

and faecal streptococci, comprising, as it does, organisms which ferment both raffinose and mannite. The question therefore rises as to whether these organisms are to be looked upon as variants of the salivary group by the addition of mannite-fermentation, or of the faecal group by the addition of raffinose. Moreover, is it possible to decide upon this point?

The same difficulty confronts us in the study of Group IV. Here the first type upon the list (R.) has every appearance of being a variant of the Central Faecalis Type by defect of the single property of mannite fermentation. It corresponds to a type occurring second in point of frequency amongst A. C. Houston's faecal cocci, as it does in my own series. In Andrewes and Horder's grouping this type is classified amongst the variants of *salivarius*,—these authors not recognising non-mannite-fermenting variants of the *Streptococcus faecalis*, although they note that the mannite test is probably not an absolute one. The rest of the strains in this group are harder to place: they may be either extreme variants from the salivary or faecal centres or belong to the *mitis* type, which may or may not be looked upon as a centre of variation *per se*, and was regarded by its creators as a probable offshoot of the main salivary type.

Early in the present work the bearing of the statement by T. Houston and M'Cloy, as to the remarkable ability of their enterococcus to survive exposure to heat, upon the attempt at classification now undertaken, was tested. It soon became apparent that this property did not apply to all the types of streptococci found in the faeces, and, furthermore, that the organisms under examination fell into two very sharply defined groups: the one surviving an exposure of thirty minutes to a temperature 60°C., the other being killed in about five minutes at the same temperature. This phenomenon was thereafter systematically studied, and it has shed a good deal of light upon the true nature of some of these puzzling intergrading forms. At the same time the study of morphology has been profitable, especially so when taken in conjunction with the ability of the organism to withstand heating, and it tends to strengthen and confirm opinions arrived at by the former method.

It is strongly suggested by further reference to Table II., that chain-formation is definitely connected with inability of the organism to survive the heat test. To study further the association of morphology and heat-resistance with other biological properties, an examination of the degree of association in different cases has been made. It is found that the fermentation of saccharose, lactose, and salicin, and the clotting of milk, are properties largely common to the whole series and therefore not available as criteria for differentiation. On the other hand, this does not hold good for many of the remaining properties, and the association of these is more particularly studied in the succeeding table (Table III.). In drawing up this, the analysis has had to be restricted to 134

organisms in which observations were made on each and every of the properties under consideration. The Table shows the actual association of properties in this universe (N) and also the corresponding theoretical figures calculated on the assumption of complete independence, which serve as a ready comparison.

TABLE III.—*Association of Properties within the Universe N.*

	H.	h.	R.	r.	M.	m.	C.	c.
Number of individuals within the series possessing each attribute	85	49	23	111	75	59	34	100
H (Heat resistance)	..	..	4	81	69	16	3	82
h (Heat not resisted)	..	..	19	30	6	43	31	18
R (Raffinose fermented)	(14·6)	(8·4)	..	..	7	16	9	14
r (Raffinose not fermented)	(70·4)	(40·6)	..	..	68	43	25	86
M (Mannite fermented)	(47·6)	(27·4)	(12·9)	(10·1)	..	..	3	72
m (Mannite not fermented)	(37·4)	(21·5)	(10·1)	(48·9)	..	..	31	28
C (Chain formation)	(21·6)	(12·4)	(5·8)	(28·1)	(19·0)	(15·0)	..	..
c (Chains not formed; diplococci)	(68·4)	(86·6)	(17·1)	(82·8)	(56·0)	(44·0)	..	..

N. Total number in the series = 184.

The figures in clarendon are the actual observed figures. The bracketed figures in ordinary type are those calculated for the case of independence of the properties studied in this table, and are given for purposes of reference.

The degree of the definite association of properties, evident in this series, may be shown by the use of one or other of Yule's co-efficients of association (1919³³) as calculated from these results, for the following properties:—

PROPERTIES.	Co-efficient "Q"	Co-efficient "ω"
Heat-resistance and Mannite fermentation	+0·93	+0·69
Heat-resistance and Raffinose fermentation	−0·85	−0·56
Heat-resistance and chain-formation	−0·96	−0·74
Raffinose-inulin fermentation and Mannite fermentation	−0·56	−0·31
Raffinose-inulin fermentation and chain formation	+0·36	+0·19
Mannite fermentation and chain-formation	−0·93	−0·67

These two co-efficients each have values between 0, in the case of independence of the two attributes under consideration, and ± 1 in the case of complete dependence, the sign denoting association or dissociation as the case may be. Their significance is slightly different, Q in general giving higher values than ω . It is therefore seen that there is a very well-marked degree of association between certain of these properties.

Table IV. gives, in percentages, the incidence of a large number of characters over series of organisms, each such universe being constituted upon the basis of the presence of one of the above-mentioned properties.

TABLE IV.—*Showing, in percentages, the distribution of characters in populations possessing the attribute denoted in the column on the left. (Compiled from the whole series detailed in Table I., with the exception of those few organisms which were only incompletely studied.)*

	Clotting of Milk.	Saccharose.	Lactose.	Raffinose and/or Inulin.	Salicin.	Mannite.	Growth in Gelatin at 22° C.	Liquefaction of Gelatin.	Resistant to Heat.	Sensitive to Heat.	Long-Chain Production.	Diplococcal.
Whole series (137 organisms)	72	72	78	17	98	58	90	6	62	88	24	78
Heat resistant series . . (85)	82	66	98	5	100	81	100	9	..	..	4	97
Heat sensitive series . . (52)	52	83	73	38	79	11	65	0	..	..	58	85
Chain formation charac- teristic (88)	57	79	70	27	82	9	58	0	9	95	..	..
Diplococcal shape char- acteristic (100)	80	69	89	12	96	73	100	8	82	18	..	..
Mannite fermenters . . (75)	80	67	89	9	98	..	100	10	98	6	4	96
Raffinose and/or inulin fermenters (24)	71	100	96	..	83	29	80	5	17	83	33	58

It will be seen from these results that the association of heat resistance with mannite fermentation, and its negative association with chain production and raffinose fermentation are very well-marked, as is also the dissociation between chain formation and mannite fermentation, which latter is associated with a diplococcal form. Raffinose fermentation is dissociated from the fermentation of mannite and tends to be somewhat associated with the formation of chains, but this last is the least marked of these associations and is susceptible of an interpretation which enhances its value in certain cases as will be shown later on.

Classifications in bacteriology are in many cases arbitrary conveniences for dividing organisms into groups convenient for practical purposes. The more detail with which the various characters are examined and the more of these that are taken into consideration, the greater will the number of the sub-classes become, and, for practical purposes, it is necessary to seek for lines of division between groups which will collect a maximum of common characters in each of the different sub-groups so constituted. The coincident existence of various characters, which is brought out in the above tables, points to lines of

divergence which may be legitimately employed for classification, and it is further seen that the more widely distributed characters also tend to group themselves along the same lines.

We find an intimate connection between, on the one hand, heat resistance, mannite fermentation, a diplococcal morphology, ability to grow in gelatin at 22° C., and a generally high saccharolytic activity, with but little tendency to act on raffinose or inulin.

On the other hand, we find another series of organisms exists, in which sensitiveness to heat is associated with failure to ferment mannite, a tendency to the formation of long chains, a certain ability to split the polysaccharides, and a decreased activity towards salicin with a relatively high degree of activity towards saccharose. The association of morphology and other properties is not so strong here, and it will be found, from further study, that this group consists of two main classes; the one of diplococci, the other of long-chained streptococci, which differ in their other properties (Plate I., Figs. 5 and 6).

The practically invariable occurrence of heat-resistance as a character of those organisms which give the sugar reactions of the Central Type of the *fæcalis* group, as defined by Andrewes and Horder (*vide* Table I.), and its absence from those organisms whose fermentative reactions are most widely separated from this type (Group II., Table II.), suggest that this reaction is closely contingent upon the other properties that define the fæcal group, and also suggest that it may be utilised as a further criterion for the identification of variants of this group, which cannot be otherwise identified. It has been shown that all the properties should be taken together in placing any given type. The position would be strengthened if it could be shown that the property of heat-resistance is as characteristic of the *pyogenes* and *salivarius* types by its absence, as it is of the *fæcalis* by its presence. This has been found to be the case. Out of a large number of hæmolytic streptococci, isolated from wounds and other suppurative processes, none were found to be possessed of this property. Fourteen salivary cocci, of which nine were chain-formers and five conglomerate, were all killed by five minutes' exposure to 60° C. Four specimens of saliva, examined by the method of cultivating in broth, heating the growth at 60° C., and recultivating, were found to be devoid of heat-resistant types, whilst two others were found to contain such organisms, but these were definitely not the *Streptococcus salivarius*, as will be shown later on.

To sum up:—There is a type of organism occurring characteristically in the fæces which has the classical fermentative properties of the central type of the *Streptococcus fæcalis* group of Andrewes and Horder, and, in addition, the various other properties which have been discussed above. Principal amongst these is the property of withstanding heating, which property may also appear in variants lacking the most

characteristic saccharolytic property of this group, the fermentation of mannite. Applying this finding to the further differentiation of the variants shown in Table II., it appears that the identity of many undifferentiated organisms, hitherto slumped into the *mitis* group, may be decided upon. Thus the group "R" Table II., although comprising organisms of the same sugar reaction, is found to consist of a number of variants of the *faecalis* type, and a further number, differentiated from these by their morphology and their failure to withstand heating. The heat-resistant forms are diplococcal, and in other respects correspond to the *faecalis* type; the heat-sensitive forms are long-chained, or conglomerate, and must be referred to the *salivarius* group. This same differentiation is found to occur very constantly throughout the whole series; in most cases the heat-sensitive forms either having sugar reactions which differ so widely from those of the *faecalis* type as to make it improbable that they are variants of this type, or else being differentiated from it by their morphology.

These results lead to the final grouping of the organisms studied into two large sections (see Table V.).

(a) The *faecalis* group proper: comprising a central type, and those variants which show the properties of fermenting mannite or resisting exposure to heat. These are characteristic of the faeces and are deserving of the specific names of *Enterococcus* or *Streptococcus faecalis*.

(b) Organisms which do not ferment mannite and are rapidly killed at 60° C. This series includes a number of sub-types which may be arbitrarily differentiated into *Streptococcus salivarius* and *Streptococcus mitis*, the criterion being the fermentation of inulin or raffinose. Detailed examination shows, within this group, the presence of forms tending, on the one hand, towards the *pneumococci* (Nos. 89, 99, 127, 128, 129, 130, see Plate I., Fig. 4), and, on the other, delicate forms which are closely related to the *Streptococcus pyogenes*. The majority of these forms are described by Gordon (*loc. cit.*) in his paper upon the salivary types.

Description of the Enterococcus.

As already stated all but three of the organisms met with in the stools, in the course of this work, showed, in some degree, the power of splitting sugars. Many of the existing descriptions of the *enterococcus* deny this organism such power. How this statement has arisen is not easy to see, unless gas-production has been taken as a criterion of fermentation, for that type of coccus which alone is characteristic of the faeces actively produces acid from sugars. It is evident to the writer that the *enterococcus* of different workers is one or other of the characteristic faecal streptococci, but that in the existing descriptions it is generally considered as a unique organism instead of as one of a group. Similarly, the *Streptococcus faecalis* of Andrewes and Horder, though recognised by these authors as a group, has not included any

TABLE V.

Reference to Table II.	Clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Saltin.	Mannite.	Heat Resistance.	Liquefaction of Gelatin.	Number of this type met.	Remarks.
A. SUGAR REACTIONS OF THE GROUP OF THE ENTEROCOCCUS.											
A	+	+	+	-	-	+	+	+	-	37	Central type.
B	+	+	+	-	-	+	+	+	+	5	Variant by excess.
C	+	+	+	-	-	+	+	+	+	4	defect.
D	+	+	+	-	-	+	+	+	+	10	"
E	+	+	+	-	-	+	+	+	+	2	" excess and defect.
F	+	+	+	-	-	+	+	+	+	4	defect.
G	-	-	-	-	-	+	+	+	-	6	"
H	+	+	+	+	-	+	+	+	-	3	" excess.
P	+	+	+	+	-	+	+	+	+	1	"
R	+	+	+	+	-	+	+	+	-	8	defect.
S	+	+	+	-	-	+	-	+	-	8	"
U	+	+	+	-	-	+	-	+	-	1	"
B. SUGAR REACTIONS OF FORMS HAVING THEIR AFFINITIES WITH OTHER OF THE DESCRIBED GROUPS OF STREPTOCOCCI.											
I	+	+	+	+	+	+	-	-	-	8	Gordon's salivary type, 8a.
J	+	+	+	+	+	+	-	-	-	4	" 5a.
K	+	+	+	+	+	+	-	-	-	1	" 5b, 6a, 6b, 7a.
(1) L	+	+	+	+	+	+	-	-	-	2	" 5c.
M	+	+	+	+	+	+	-	-	-	2	" 5d, 6d, 6e.
N	+	+	+	+	+	+	-	-	-	1	" 3f, 4c, 5f.
(2) Q	-	+	+	+	-	+	+	-	-	3	Vide footnote.
R	+	+	+	-	-	+	-	-	-	11	Gordon's salivary types, 4b, 5c, 5i, 6c.
S	+	-	+	-	-	+	-	-	-	1	"
T	-	-	+	-	-	+	-	-	-	8	" 2c, 3d, 3e.
(3) U	-	+	+	-	-	+	-	-	-	3	" 3g, 4e, 4h, 6b.
V	-	+	+	-	-	+	-	-	-	1	" 2a and 3b.
W	-	-	+	-	-	+	-	-	-	1	"
X	-	-	-	-	-	+	-	-	-	1	"
Y	-	-	-	-	-	-	-	-	-	3	Includes two hæmolytic strains. S. pyogenes.
Types A to P, Q, S, W, X, and Y were not encountered in Gordon's analysis of 300 strains from human saliva.											
C. UNCLASSIFIED ORGANISMS.											
(4) H	-	-	-	-	-	-	+	-	-	2	
(5) A	+	+	-	-	-	+	+	-	-	1	

(1) Strains 34 and 36. (2) Strains 69, 70, and 72. (3) Strains 143 and 146. All of these, as well as strains 60, 61, and 62, which were not subjected to the heat test, show the same type of aberrant morphology. They grow badly in fluid media, though well enough on solid. In films they appear as mulberry-like masses dividing in three planes. They show a good deal of variation in size and shape of the individual cocci and some variation in their retentiveness of Gram's stain. Their relationship to the other forms is uncertain. They are referred to in the text as showing an "aberrant morphology."

(4) Two strains, Nos. 133 and 134, from the same stool, give this reaction. It is a very unusual type and cannot be placed.

(5) This strain, No. 125, is the solitary example of a strain having the sugar reactions and morphological characters of the typical enterococcus but being sensitive to heating.

non-mannite fermenters, some of which, as shown in the present work, cannot be justly excluded. The name of *Streptococcus faecalis* is perhaps a little unfortunate, as it may give rise to the assumption that all of the streptococci found in the faeces belong to this group; whereas, actually, though this species is the predominating one, there are present a number of salivary organisms and organisms of the *mitis* group. It is perhaps the incomplete recognition of this fact that has led to a certain amount of confusion, since these two types differ considerably from the *Streptococcus faecalis* and its closely connected variants. When the *salivarius* and *mitis* group are excluded, the properties of the faecal cocci are found to be considerably narrowed down and the group to present fairly characteristic features. It is to this group that the name of *enterococcus* should apply. This term is becoming more and more widely adopted, and it is well to retain it, since it is the prior title, remembering always that at present it must refer to a group and not to a single type.

Morphology.—The *enterococcus* is characteristically a lanceolate diplococcus, the elongation of the units being usually less marked than is the case with the *pneumococci*. Growing in fluid media the organism produces a uniform fine turbidity and, in films, appears as a diplococcus resembling the *pneumococcus*, and sometimes showing a slight halo. No capsule has been demonstrated. Short chains are often seen but the diplococcal forms predominate, and the chains, which rarely exceed six or eight elements, are usually found to consist of associated diplococci rather than of a string of round cocci, as is more usual in true chain-forming streptococci. This diplococcal form is a matter of considerable importance in differentiating the organism from other streptococci,—as important, here, as in the *pneumococcus* group. Wherever long-chained organisms, conglomerate cocci, or others showing an aberrant morphology are recovered from the faeces, it may confidently be expected that further tests will show that they do not belong to this group. There is, however, some evidence that in pathological conditions the diplococcal character may be less marked, but this point has not been cleared up. The composition and reaction of the medium has little influence on the morphology of the organisms: the presence of large amounts of serum often causes the growth to appear as a deposit in the tube consisting of clumped masses of diplococci; in pure serum the growth is only moderate. The organism will grow in media alkaline to phenolphthalein, but the growth is not luxuriant. In media acid to Congo Red (*i.e.*, H^+ -ion concentration = $N \times 10^{-3}$) no growth can be seen.

Upon agar it grows well, producing colonies which are definitely larger than those of the *Streptococcus pyogenes*. The average diameter of discrete colonies, after twenty-four hours' cultivation, was found to be 0.75 mm.—this being approximately twice the size of pyogenic streptococcus colonies under the same conditions. Where the colonies are well-spaced they are often 1.0 mm. in diameter. The colonies are sharply contoured, round, and lenticular. When viewed under a low magnification they are uniformly and finely granular. The growth upon agar, when the inoculation is made from a broth culture, is frequently as a uniform film, resembling a growth of *B. typhosus*; this follows from the physical characters of the growth in broth,—its uniform distribution. The chain-forming streptococci, on the other hand, give discrete colonies.

In films made from agar cultures the organisms appear in staphylococcal formation with no very definite arrangement of individual units. Usually a

number of scattered diplococci are seen in the more sparse portions of the field, and, not infrequently, the individual cocci are definitely lanceolate (Plate I., Fig. 1): this character is not invariably present but when seen is very typical.

Upon gelatin plates the characters are similar to those upon agar. In gelatin stab-cultures, at 22° C., and at room temperature, the organism grows well. The gelatin is not as a rule liquefied; exceptionally certain strains do this.

Much has been written about the pleomorphism of this organism; a certain amount of involution has been met with in some strains, showing itself as a tailing off of a short-chain in yeast-like fashion, or the association of two individuals of different sizes in a diplococcus (Plate I., Fig. 3). This variation in form has not been frequently noted and has usually been found to disappear after a short period of growth upon artificial media. It cannot be said to be a character of the organisms in young cultures under ordinary conditions.

Biological Properties.—The vitality of the organism is very great. Cultures in gelatin and serum-broth have been found alive after the lapse of two years; frequently these had dried up but the organisms were recovered when the inspissated material was dissolved in broth. The organisms may be confidently expected to survive for a year in serum-broth or gelatin cultures.

Fermentative Reactions.—The central type of the group—the Typical Enterococcus—clots milk and produces acid in glucose, lactose, cane sugar, salicin, and mannite. No effect is produced upon dulcitol, raffinose, or inulin. The three principal variants are shown below:—

	Milk Clot.	Sacch- arose.	Lac- tose.	Raff- inose.	Inulin.	Sal- icu.	Man- nite.	Dul- cite.
Typical Enterococcus	+	+	+	—	—	+	+	—
VARIANT I.	+	—	+	—	—	+	+	—
VARIANT II.	+	+	+	—	—	+	—	—
VARIANT III.	+	—	+	—	—	+	—	—

Occasionally variants, by addition, are met with which are capable of splitting raffinose as well as mannite. Some of the scarcer varieties do not clot milk.

The value of sugar reactions in the streptococcus group has been greatly opposed (Ainley Walker 1911-12³⁴, Beattie and Yates 1911³⁵, Rosenow 1914³⁶) on the grounds of their inconstancy. Forty-eight of the present series of enterococci were re-examined at varying periods, from a few days up to two years from the time of their isolation, being kept meanwhile upon ordinary media. Of these, forty-two showed no change in their reactions, some of these having retained their original reactions for two years. Six showed changes, usually involving the loss of ability to ferment a single sugar; in none of these was change of reaction observed earlier than eight months after isolation. The details of these cases cannot be discussed here, but it may be said that the reactions are of considerable constancy.

Heat Resistance.—This is a characteristic property of the enterococcus. Most strains can withstand an exposure of thirty minutes to a temperature of 60° C. This property appears to be due to the presence of some specially resistant cells, since much of the culture seems to be killed by this treatment. It is probable that if larger quantities of culture had been used, as in the classical method of determining the thermal death-point of an organism, the ability to withstand heating might be found to be even greater. Using small quantities of culture, as in the technique employed in the present work, it is suggested that a useful way of applying the test, as a routine practice, would be to examine the ability

of a broth culture to resist exposure for fifteen minutes to 60° C. All the enterococci studied would survive this treatment, none of the other streptococci would do so, most of these being killed in five minutes, and none surviving over ten minutes.

To this property there was only a single exception—Strain 125. It is, of course, probable that like most other tests for the differentiation of streptococci this test is not an absolute one; but as far as the experience of the writer goes it is more constant than any other test yet introduced.

Other Properties.—The organism is insoluble in bile and the bile salts. It is devoid of action on red blood corpuscles, causing neither hæmolysis nor methæmoglobin formation. In lead-acetate broth it inconstantly produces a small quantity of sulphuretted hydrogen. It is without action on urea. According to Weissenbach (*loc. cit.*) it grows well in glucose-peptone-broth, containing 10 per cent. of bile; a character which differentiates it from the pathogenic streptococci.

Pathogenicity.—Of the thirty-three strains tested against mice only six showed some degree of pathogenicity. It is perhaps worthy of note that four of these were isolated from a typhoid stool, in which condition it is stated that the virulence of the coliform flora is much increased. None of the five strains examined were found to be pathogenic for rabbits. It may therefore be said that the organisms are of little or of no virulence for these animals. The true streptococci from the fæces which were examined, though not generally virulent, showed a greater tendency towards virulence than did the enterococci.

Occurrence.—The organism is very constantly present in the stools. It was found present in twenty-six out of the thirty-five stools examined; in many of these cases only one or two organisms were selected from each stool. Where a large number of organisms from a single stool were examined it was always found.

Influence of Diarrhœa.—In plating a series of 359 stools upon MacConkey's medium the connection between the consistence of the stool and the proportion of streptococci to coliform colonies was noted. The following results were obtained :—

	No. of Stools Examined.	Percentage showing excess of Streptococci.	Percentage showing absence of Streptococci.
Whole series . . .	359	7·7	3·6
Fluid stools . . .	39	7·7	Nil
Soft stools . . .	111	3·6	3·3
Solid stools . . .	209	10·0	2·6

These figures do not show any very great differences, there being a slightly increased streptococcus incidence in the solid stools. Mutch (1918³⁷), in a paper read before the Royal Society of Medicine, lays stress upon the delaying of the intestinal contents as one of the chief factors favouring streptococcus growth in the colon; especially is this the case, when it is associated with the presence of carbohydrate foodstuffs.

Diarrhœa has, however, a definite influence on the type of cocci present. The hurrying on of the intestinal contents seems to favour the appearance of a greater proportion of the mouth cocci and to give less time for the pullulation of the characteristic intestinal forms, which probably takes place in the lower intestine. This is illustrated by the following observation.

Stool No. 25 was under examination, and in twenty-five strains the following proportions had been found: enterococci 68 per cent., salivarius type 16 per cent., mitis type 16 per cent. An attack of acute diarrhœa occurred and from a culture

made on the second day of this eight strains were isolated, all of which were of the *mitis* type. No enterococci were found.

The organism is rarely found in the mouth. Gordon, in his 300 strains isolated from the saliva, failed to find any mannite-fermenters. In the present work a number of types isolated from the mouth have been found to belong to the *salivarius* or *mitis* groups.

An attempt was made, on five healthy individuals, to isolate the organism from the saliva by the method of utilising its heat-resisting powers, which enables large amounts of material to be examined (*loc. cit.*). Three of the individuals showed absence of heat-resisting forms, the other two gave growths. In both of these cases enterococci were recovered,—in the one the central type and in the other a mannite-fermenting variant which failed to attack saccharose or clot milk (Type F, Table IV.). The result emphasises the rarity of these organisms in the saliva.

French writers describe the organisms as occurring in the respiratory and genital passages. These have not been systematically examined in the present work, but the organism has been found in the male urethra, and in the lung at autopsy. Houston and M'Cloy appear to have definitely isolated it from the sputum in their cases of Trench Fever. The organism appears to be frequently present in infected wounds, having been found by Sacquépée (*loc. cit.*) in 40 per cent. of wounds examined within twenty-four hours of infliction.

Method of Isolation.—The organism is readily isolated on the ordinary media. It grows readily on bile-salt media, which inhibit the more delicate streptococci. The special methods advocated by Thiercelin have not appeared to the writer to possess any advantages.

The organism can best be obtained by the procedure of inoculating a tube of broth with faeces and, after incubating for eighteen to twenty-four hours, heating the tube for thirty minutes to 60° C. Some fresh broth is then added and the tube reincubated and afterwards plated upon agar. An abundant growth of enterococci results. Most other non-sporing organisms are killed.

Summary—Relationship to other Forms.—The relationship of the *enterococcus* to the *streptococcus faecalis* has already been dealt with; as has also the question of nomenclature.

The power of withstanding exposure to heat is not a property of all intestinal streptococci. By its use these are divisible into two classes, one of which largely consists of organisms having fermentative reactions corresponding to those of the *streptococcus faecalis*. The other group consists of types which frequently occur in the saliva, and includes those raffinose-fermenters of the faeces which are not thermo-resistant and may be regarded as survivors of the salivary organisms. Such raffinose-fermenters as are heat-resistant are variants of the enterococcus. They have its morphology and ferment mannite; whereas the heat-sensitive types have either an aberrant morphology (*vide* note to Table IV.), or resemble in this respect the true streptococci or the pneumococci.

The non-mannite, non-raffinose fermenters (*str. mitis*), are divisible into the same two classes, according to whether they are heat-resistant or not. The thermo-resistant types appear to be variants of the mannite-fermenting type (enterococcus), and their other properties support this view. The non-resistant organisms have, possibly, the

same relationship to the raffinose-fermenting, heat-sensitive types as the resistant ones have to the enterococcus. In this case, the group of the *streptococcus mitis* would disappear and the alimentary tract streptococci be classified into *enterococci* and *streptococci salivarii*.

The *enterococci* of Houston and M'Cloy appear to be identical with the organisms here described. These writers did not meet with any strains which fermented mannite. One of their strains, which was kindly placed at the writer's disposal, had this property and, in every respect, corresponded to what is here described as the Central Type of the Enterococcus.

With regard to many of the older descriptions these are frequently so fragmentary as to be of little use. The organisms described above, as comprising the *enterococcus* group, would appear to include the *enterococcus* of Thiercelin and the *micrococcus ovalis* of Escherich. The *streptococcus enteritis* of Hirsh, Libman, and Escherich, is evidently one or other of the true streptococci met with in the fæces, many of which appear to be identical with the salivary organisms. They are certainly distinct from the *enterococcus*.

The relationship of the *enterococcus* to the *streptococcus pyogenes* does not need discussion. The morphology of the latter, its restricted sugar-splitting powers and more limited conditions of growth, serve to distinguish it; as do also its commonly-present action on blood cells and its virulence. It is, in addition, heat-sensitive.

It is noteworthy that several strains of the streptococci encountered in the present work closely simulate the *str. pyogenes* (e.g., Strains 67, 250, 254, 255, 264, 266, 268, 269), and that strains 264 and 266 seem identical with it in all respects. This appears to be the first description of a hæmolytic, virulent, streptococcus in the fæces.

Certain other such strains present a resemblance to the *pneumococci*, both in their action on sugars and their appearance in films (e.g., Nos. 89, 99, and 127-130, Plate I., Fig. 4). None of these are bile-soluble or give rise to methæmoglobin formation. They all grow much more luxuriantly in artificial media than do the true pneumococci, and also lack their characteristic virulence for mice.

CONCLUSIONS.

The *Streptococcus enteritis* comprises the large group of true chain-forming streptococci of the fæces, many of which are undoubtedly surviving salivary types.

The *Streptococcus fæcalis* of Andrewes and Horder comprises the group of the *Enterococcus* and possibly some of the above-mentioned types. It omits many true *Enterococci*, since non-mannite-fermenting variants are not recognised.

The characters of the *Enterococci* have been described, as well as those of certain other fæcal streptococci. It would appear that the former comprise a well-defined group, worthy of specific rank within the streptococcus genus.

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FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

FIG. 6.

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DESCRIPTION OF PLATE I.

- FIG. 1.—Enterococcus No. 109. Growth on agar showing elongated character of the individual cocci. ($\times 1300$.)
- FIG. 2.—The same culture in neutral broth. ($\times 900$.)
- FIG. 3.—Enterococcus No. 111, showing some pleomorphism. Growth in ascitic broth.
- FIG. 4.—A Raffinose-fermenting diplococcus, No. 99, not an enterococcus, having pneumococcus-like characters and showing a definite halo. Growth in serum-broth. ($\times 1300$.)
- FIG. 5.—Organism No. 113. A true streptococcus from the fæces fermenting Raffinose. Growth in ascitic broth. ($\times 600$.)
- FIG. 6.—Organism No. 98. A true streptococcus from the fæces fermenting Inulin. Growth in neutral broth. ($\times 600$.)

Fig. 3 is a drawing by Mr A. K. Maxwell. The other illustrations are photographs kindly taken by Dr C. Powell White, to whom I wish to express my sincere thanks.