SOME OBSERVATIONS ON THE CLASSIFICATION OF ENTEROCOCCI

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Introduction

The term "Enterococcus" was first used by Thiercelin (1899) to describe a diplococcus or short-chained streptococcus of bowel origin which differed in morphological characters from other intestinal streptococci. The subsequent division of the genus by Andrewes and Horder (1906), using the tests for differentiation devised by Gordon (1905), resulted in the recognition of seven large groups of streptococci, one of which they regarded as characteristic of the intestinal tract and designated *Streptococcus faecalis*. The essential feature of this classification of streptococci was the recognition of a relatively small number of predominant groups, to which specific names were applied, though they were not well defined species. Between these groups were intermediate forms which were regarded as varieties, differing slightly either by excess or defect of some property.

It is difficult at present to define the term "species" as applied to members of the genus *Streptococcus*, but most observers use the term loosely as implying "a predominant group" in the sense used by Andrewes & Horder. There appears to be little doubt that the names Enterococcus and *Str. faecalis* employed respectively by Thiercelin and by Andrewes & Horder were used to indicate the same species of streptococcus, and the term "enterococcus" has subsequently been used by many workers (Houston & McCloy, 1916; Dible, 1921; Bagger,

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1926) for describing this "predominant group" which is characteristic of the faeces. On account of its prior use and the difficulty in defining a streptococcus species this "common name" is probably preferable to *Str. faecalis*.

Some confusion, however, exists in the literature with reference to the relationship of these groups. Some consider enterococcus as synonymous with *Str. faecalis* (Bergey, 1926); others have used the term "enterococcus" to designate faecal and other streptococci which have some of the outstanding characters of the *Str. faecalis*, whilst Sherman (1937) in a recent review uses the term "enterococcus" for a "division" of streptococci, which contains groups and species, the biological characters of which are admittedly ill defined. From a review of the literature it is evident that in its present use the term "enterococcus" cannot be regarded as synonymous with *Str. faecalis* of Andrewes & Horder, as it appears unlikely that all the varieties included in the latter species were enterococci. There is also evidence to show that the *Str. faecalis* group does not include many varieties of true enterococci which fail to ferment mannitol, the fermentation of which was regarded by Andrewes & Horder as specially characteristic of the *Str. faecalis*.

In addition to the latter species a number of other streptococci, which show properties in common with enterococci, have been given specific rank; these include *Str. lactis* (Lister, 1873), *Str. zymogenes* (MacCallum & Hastings, 1899), *Str. liquefaciens* (Orla-Jensen, 1919), and group D haemolytic streptococci (Lancefield, 1933). A further reference to these will be made later in this paper.

In order to avoid ambiguity with reference to the above-mentioned groups, species or varieties it is proposed in this communication to use the term "enterococcus" as a "common name" for faecal streptococci, which have the morphological and other properties of the *Str. faecalis* of Andrewes & Horder and of varieties of the latter which either ferment mannitol or resist exposure to heat.

Enterococci are regarded as saprophytes and facultative parasites of the intestinal tract of man and animals. They are also frequently found in infections, either in pure culture or in association with other bacteria. In many of these infections, however, their exact etiological role is difficult to assess. As the majority of faecal strains have little or no pathogenicity for laboratory animals (Dible, 1921), such invasive powers as they possess may depend on a lowered resistance of the host. Differentiation of the enterococcus group is of practical importance on account of its wide distribution on the skin in the genital tract, etc., where the haemolytic varieties may be confused with more pathogenic streptococci.

OBJECT OF THE PRESENT INVESTIGATION

Although good descriptions are to be found in the earlier literature, the definition of the enterococcus may be regarded as commencing with the work of Dible (1921). Since then many observations have shown that the biochemical

and other tests proposed for defining the group cannot be rigidly applied on account of the occurrence of varieties, which may fail to show some property regarded as characteristic, such as mannitol fermentation or heat resistance. In view of the limitations imposed by biochemical and other tests in defining bacterial species from closely related varieties, an attempt is here described to define the enterococcus group by the method of antigenic analysis, employed by Lancefield (1933) for demonstrating the group relationships of haemolytic streptococci. It has been shown that haemolytic streptococci possess a complex carbohydrate which serves to separate them into well defined sub-groups. One of the latter (group D, Lancefield, 1933) shows properties in common with enterococci, and we thought that the method might also be applied to define the relationship of the enterococcus and group D haemolytic streptococci to each other and to the other groups of haemolytic streptococci already established by Lancefield.

Preliminary experiments demonstrated that extracts made from varieties considered as typical enterococci were readily precipitated by antisera prepared by inoculating formalinized suspensions of the same varieties. These results suggested that the method might also be applied to define varieties within the group which failed to show some of the characteristic properties of enterococci, such as mannitol fermentation, resistance to heat or reduction of methylene blue. It also appeared desirable, using the same method, to investigate the relationship of enterococci to *Str. zymogenes*, *Str. liquefaciens*, group D haemolytic streptococci, and *Str. lactis*, all of which show properties in common with this group but have been regarded as distinctive species of streptococci.

History

It is not proposed to review the earlier observations on the general characters of enterococci. This has already been done by Dible (1921), whose excellent contribution has led to a clearer and broader understanding of the group, and by Bagger (1926). Bagger records significant observations on growth and survival at various temperatures, the *p*H limits for growth and survival, and growth on media containing bile, all of which are now recognized to be of special importance in differentiating enterococci from closely related groups and species, such as the *Str. lactis* and *Str. viridans*. Of the characters described by earlier workers, special attention must be directed to fermentation of mannitol which was considered by most workers to be a specially characteristic property and to the remarkable ability of enterococci to withstand exposure to heat, described by Houston & McCloy (1916). Dible (1921) has shown the close association of these properties and has applied the heat-resistance test as a means of showing the existence of varieties, such as non-mannitol fermenters, which cannot be differentiated in any other way. The heat-resistant forms were diplococcal and in other respects corresponded with the *Str. faecalis*. Although the property of survival after heating at a temperature of 60° C. for 30 min. is shared with the *Str. thermophilus* and the capacity

to ferment mannitol is not limited to this group, these properties have been used most frequently as criteria for defining enterococci.

The fact that enterococci grow on media containing bile, while other groups of streptococci do not, was first observed by Weissenbach (1918). The use, however, of pure bile containing peptone as a differential culture medium for enterococci was first investigated by Bagger (1926). In a series of experiments he showed that strains of enterococci not only survived but could be subcultured indefinitely in this medium. The observation of Houston (1934) that enterococci invariably grow in chains in pure bile and that no other streptococci can do so still awaits confirmation, but, if confirmed, the systematic application of this test might provide another means of differentiating enterococci from *Str. lactis* and "*viridans*" streptococci. The ability to split aesculin in a medium containing bile, which was first used for differentiating enterococci from other streptococci by Rochaix (1924) and extensively applied by Meyer & Schonfeld (1926), was later criticized by Weatherall & Dible (1929) on the grounds that it is a less severe criterion for enterococci than the property of heat resistance. The latter and other workers have pointed out that the test, as usually applied, is in reality a test for bile tolerance; when carried out in an aesculin-containing medium from which bile is omitted the glucoside may be hydrolysed by other streptococci as well as enterococci.

In recent work on differentiation various workers have applied a wider variety of tests. Tests such as the ability to grow at 10 and 45° C., or in presence of 6·5% NaCl, or in alkaline media of *p*H 9·6, or to reduce methylene blue have been used by Sherman & Stark (1934), and Sherman, Stark & Mauer (1937) in the differentiation of the enterococcus-lactis group from other groups of streptococci. Other tests, such as the final *p*H attained in 1% glucose broth (Ayers, 1916; Avery & Cullen, 1919; Brown, Frost & Shaw, 1926; Hare & Colebrook, 1934; Lancefield & Hare, 1935) and the ability of certain streptococci to hydrolyse sodium hippurate (Ayers & Rupp, 1922), are of more limited value when applied to this group. It will be more convenient to consider these tests in relation to the strains studied in this series.

The earlier investigation of Andrewes & Horder (1906) showed that *Str. faecalis* produced no change during growth on blood agar, and until recently it was assumed that all enterococci behaved in a similar manner, but the results obtained by Wordley (1921, 1922), Meyer (1926), and Weatherall & Dible (1929) have clearly shown the existence of haemolytic enterococci, i.e. enterococci which exhibit clear zones of beta haemolysis on blood agar but do not produce a filterable haemolysin when grown on a suitable fluid medium and tested by the usual method. Pseudo-haemolytic streptococci, isolated from the genital tract by Hare & Colebrook (1934) and considered by them to be related to haemolytic enterococci, were shown by Lancefield & Hare (1935) to belong to group D haemolytic streptococci of Lancefield. Enterococci showing greenish zones around colonies on fresh blood agar are not infrequently referred to (Orcutt, 1926; Meyer & Schonfeld, 1926; Ehrismann *et al.* 1935).

Houston (1934) considered that, when grown on fresh blood agar, true enterococci may be mistaken for "*viridans*" or even "*haemolytic*" streptococci.

From the information available it is clear that the enterococcus group may be regarded as containing haemolytic and non-haemolytic varieties, but further information with reference to the action of some strains during growth on fresh and heated blood agar will be necessary before the group as a whole can be adequately described.

As stable suspensions of enterococci can readily be prepared, it is not surprising that many workers have attempted to define antigenic types by agglutination and agglutinin absorption (Houston, 1934; Takeda, 1935; Shigeno, 1938). The findings recorded by these workers show the existence of a variable number of serological types which exhibit a group relationship to one another, but it is impossible at present to determine the relationship of these types to the other groups defined by the precipitin test of Lancefield. Other workers (Bagger, 1926; Ehrismann *et al.* 1935) have considered agglutination methods of no value in defining antigenic types.

The use of sensitivity to the action of bacteriophages as a method of distinguishing closely related bacteria, (Bail, 1921), has been applied by Lancefield (1933) to haemolytic streptococci. Observations on the application of this method to strains of enterococci will be found later in this communication.

Origin of the strains of enterococci examined

The strains of enterococci of human origin used in this investigation were selected at random from a large collection placed at our disposal by Sir Thomas Houston. They had been frequently cultured and were regarded as typical enterococci. The bovine strains were freshly isolated by one of us (N. C. G.) from bovine faeces. The haemolytic streptococci (group D of Lancefield) were supplied through the courtesy of Dr Leonard Colebrook.

Methods

The following tests were applied:

(*a*) *Cultural and biochemical tests*

(1) Ability to grow in broth at 10 and 45° C., in broth containing 6·5% NaCl at 37° C., and in alkaline broth of *p*H of 9·6 (Sherman & Stark, 1931, 1934; Sherman *et al.* 1937).

(2) Fermentation of trehalose, sorbitol, mannitol, salicin, raffinose and inulin.

(3) Heat resistance (Houston & McCloy, 1916; Dible, 1921).

(4) Reduction of methylene blue (Sherman & Albus, 1918; Avery, 1929).

(5) Production of chains during growth in pure ox bile (Houston, 1934) and ability to grow on 40% bile blood agar (Belenky & Popowa, 1929; Lancefield, 1933; Hare & Colebrook, 1934).

(6) Splitting of aesculin in a medium containing bile (Rochaix, 1924).

(7) Final *p*H in 1% glucose broth (Avery & Cullen, 1919).

(8) Liquefaction of gelatine (Andrewes & Horder, 1906; Dible, 1921).

(9) Type of haemolysis shown by deep colonies in blood agar, using 6% horse blood (Brown, 1919), and production of soluble haemolysin (Hare & Colebrook, 1934).

(10) Fibrinolysis of human serum (Tillet & Garner, 1933; Hare & Colebrook, 1934).

(11) Hydrolysis of sodium hippurate (Ayers & Rupp, 1922).

(12) Reduction of nitrates to nitrites.

(13) Production of sulphuretted hydrogen (Andrewes & Horder, 1906).

(14) Sensitivity to bacteriophage (Hadley & Dabney, 1926; Lancefield, 1933).

In this investigation Wright's broth of *p*H 7·5 was used except where otherwise stated and all test cultures were made from 12 to 18 hr. old broth cultures incubated aerobically at 37° C.

The *media* used for fermentation of sugars, production of soluble haemolysin, hydrolysis of sodium hippurate, final *p*H on 1% glucose broth and growth on bile blood agar and the biochemical tests applied were the same as those used by Hare & Colebrook (1934).

Heat resistance. Sealed capillary glass tubes, each containing approximately 0·1 c.c. of 18 hr. broth culture were completely immersed in a water-bath for 30 min. at a temperature of 60° C. The contents of each capillary tube were then discharged into a fresh tube of broth which was examined for growth after 24 and 48 hr. incubation and then plated.

Splitting of aesculin in a medium containing bile. Cultures were grown on the medium used by Harrison & Van der Leck (1909) (as recorded by Meyer, 1926), at 37° C. for a week and observed for "blackening".

Growth on pure ox bile. Cultures were heavily seeded into sterile ox bile and incubated for 3 days. These were examined by the hanging drop method for evidence of growth and occurrence of chain formation after 24 hr. and at the end of 3 days.

Reduction of methylene blue. Two methods were used:

(1) Cultures were made in sterile milk containing a methylene blue at a concentration of 1/5000, and observed for 7 days (Avery, 1929).

(2) One drop of 1% aqueous solution of methylene blue was added to a 10 c.c. 24 hr. old broth culture, which was then incubated at 37° C. for an hour and the results noted.

Reduction of nitrates. Cultures were grown for 5 days on broth containing 0·1% KNO_3 and then tested for the presence of nitrites, using Greiss-Ilosva's reagent.

Production of sulphuretted hydrogen. Liver extract broth was used and cultures were incubated for 4 days. The presence of H_2S was detected by the use of strips of filter paper soaked in 10% lead acetate solution, dried, sterilized,

and inserted between the plug and the glass so that the free end projected into the culture tube well above the level of the fluid medium.

Liquefaction of gelatine. Shake cultures were made in 10% gelatine and incubated at room temperature. Duplicate cultures incubated at 37° C. for the same period and subsequently refrigerated gave precisely the same results.

(*b*) *Serological tests*

Precipitating antisera were prepared by injecting rabbits intravenously with formalinized suspensions of strains of enterococci and group D haemolytic streptococci by the method of Lancefield (1933). Twelve sera were prepared in this way of which eleven were regarded as suitable group-precipitating antisera. One antiserum prepared from a group D haemolytic streptococcus was unsuitable as it exhibited marked cross-precipitation with extracts prepared from strains representative of Lancefield's groups A, B, C, G and F.

All the strains used for preparing group precipitating antisera were apparently enterococci, both morphologically and culturally. They grew in broth at 10 and 45° C., curdled milk, showed strong reduction of methylene blue when added to a 24 hr. broth culture and when cultures were grown in skimmed milk containing a 1/5000 concentration of the dye, split aesculin when growing in a medium containing bile and produced a final acidity of 4·0–4·2 in 1% glucose broth.

A summary of other properties of eight of the strains is given in Table I since the antisera prepared from these were most used in the precipitin tests tabulated in Table II.

Table I. *Showing varieties of enterococci and group D haemolytic streptococci used for preparing group precipitating antisera*

No. of strains	Source	Group	Haemolysis	Liquefaction of gelatine	Heat resistance at 60° C.	Fermentation of mannitol	Variety
1	Human	–	–	–	+	+	*Faecalis*
1	Bovine	–	+	–	+	–	*Haemolyticus*
3	—	D	+	–	+	+	*Haemolyticus*
2	Human	–	–	+	+	+	*Liquefaciens*
1	—	D	+	+	–	+	*Zymogenes*

On the basis of haemolytic and proteolytic properties the strains shown in Table I can be classified into the more commonly recognized varieties of the enterococcus.

At first the extracts used as precipitinogens were prepared from the deposits obtained by growing strains in 80 c.c. of broth at 37° C. for 12–18 hr. by Lancefield's method (1933); later it was found that the method of preparation devised by Fuller (1938) gave equally satisfactory results. A large number of extracts were prepared by both methods but finally Fuller's method was adopted on account of the facility with which an extract could be made from a culture grown in a smaller amount of medium.

The precipitin tests were carried out by the capillary tube method of

Lancefield & Hare (1935). Control tests carried out with larger volumes of the reagents gave comparable results, but were more difficult to interpret. The results were read immediately after the completion of the test, and also after incubation at 37° C. for 2 hr. A final reading of the tests after 24 hr. at room temperature was also made.

RESULTS OF THE TESTS

The results of precipitin, biochemical and other tests are recorded in Table II. All the antisera prepared by inoculating enterococci reacted with extracts prepared from the latter and group D haemolytic streptococci by showing a flocculent precipitate. Antisera prepared from strains of group D haemolytic streptococci reacted with extracts prepared from the latter and enterococci. With extracts prepared from the majority of strains the precipitate was observed immediately after the two reagents were mixed; with extracts from a few strains the precipitate was not observed until the tubes were incubated at 37° C. for 2 hr. With the antisera used no differences were noted between extracts prepared from enterococci isolated from human and bovine sources and the group D haemolytic streptococci shown in the table. Of the forty-one strains tested forty gave positive results, the remaining strain was negative with all the antisera tried. It will be observed, however, that this strain could not be regarded as an enterococcus on biochemical reactions or on other characters. Control precipitation tests using extracts prepared from strains representative of haemolytic streptococci (Lancefield), other than group D, were carried out with the same antisera and in each case the result was negative. In these tests two strains each of groups A, B, and C and one strain each of groups F and G were employed. Other control tests carried out with extracts prepared from four strains of *Str. viridans*, two strains of *Str. lactis* and two strains of *Str. agalactiae*, types I*b* and I*c* (Stableforth, 1937) were negative, except that minimal precipitation was observed after 24 hr. when extracts of *Str. viridans* were tested with three of the antisera. The control tests are not shown in the table.

The group relationships of the strains summarized in Table II were also investigated by testing extracts of them with group specific antisera prepared from strains representative of groups A, B, C, D, and G (Lancefield). The results are summarized in Table III and show that all the strains of enterococci and haemolytic streptococci (group D) reacted strongly with group D antiserum only. No reactions were observed with any of the other antisera in 2 hr., but minimal cross-reactions were sometimes observed with the specimen of the group G antiserum used, when readings were taken after 24 hr.

Table II also shows in summary form some of the biochemical and other characters correlated with the results obtained by the precipitin test. Of the strains classified, all showed luxuriant growth in 24 hr. in broth of *p*H 9·6 and in broth incubated at 45° C. When grown at 10° C. a few strains showed little

Table II. *Results of combined precipitation, biochemical and other reactions of all strains of enterococci and group D haemolytic streptococci investigated*

Strains	Precipitation with antisera prepared from: Enterococci: Human	Enterococci: Bovine	Group D	Heat resistance	Fermentation of mannitol	Growth at: 10° C.	Growth at: 45° C.	Growth in broth: With 6·5% NaCl	Growth in broth: Of pH 9·6	Reduction of methylene blue	Final pH	Liquefaction of gelatine	Haemolysis in deep blood agar	Soluble haemolysin
Enterococci (human)														
3	+	+	+	−	+	+	+	+	+	+	4·0	+	None	−
1	+	+	+	−	+	+	+	+	+	+	4·0	−	None	−
1	+	+	+	+	+	+	+	+	+	+	4·0	+	None	−
1	+	+	+	+	+	+	+	+	+	+	4·0	−	None	−
1	+	+	+	+	+	+	+	+	+	+	4·2	−	None	−
1	+	+	+	+	+	+	+	+	+	+	4·0	+	Alpha	−
1	+	+	+	−	+	+	+	−	+	+	4·0	−	None	−
1	+	+	+	+	+	+	+	+	+	+	4·0	−	Beta	−
1	+	+	+	+	+	+	+	+	+	+	4·2	−	Beta	−
Enterococci (bovine)														
8	+	+	+	+	+	+	+	+	+	+	4·0	+	None	−
2	+	+	+	+	+	+	+	+	+	+	4·0	−	None	−
4	+	+	+	−	+	+	+	+	+	+	4·0	+	None	−
1	+	+	+	+	−	+	+	+	+	+	4·2	−	Beta	−
1	+	+	+	+	−	±	+	+	+	−	4·0	−	Alpha	−
1	+	+	+	+	+	+	+	+	+	+	3·8	+	None	−
Enterococci type strains														
Houston I	+	+	+	+	+	+	+	+	+	+	4·0	+	None	−
Houston II	+	+	+	+	+	+	+	±	+	+	4·0	+	None	−
Houston III	+	+	+	+	+	+	+	+	+	+	3·8	−	None	−
*Type I (K.M.)	+	+	+	−	+	+	+	+	+	+	4·4	+	Alpha	−
*Type II (K.M.)	+	+	+	+	+	+	+	±	+	+	4·2	−	None	−
*Type III (K.M.)	+	+	+	+	−	+	+	+	+	−	4·0	−	None	−
*Type IV (K.M.)	−	−	−	−	−	.	.	.	.	−	4·2	−	Beta	−
**Str. faecalis* (Hucker)	+	+	+	+	+	+	+	+	+	−	4·0	−	None	−
Lancefield group D														
3	+	+	+	+	+	+	+	+	+	+	4·0	−	Beta	−
1	+	+	+	+	+	+	+	+	+	+	4·0	+	Beta	−
1	+	+	+	+	+	+	+	+	+	+	4·2	−	Beta	−

* These strains were obtained from the National Collection of type cultures.

Table III. *Summary of cross-precipitin reactions using extracts of enterococci and group D haemolytic streptococci with antisera prepared against groups A, B, C, D, and G (Lancefield)*

	Antisera				
	A	B	C	D*	G
Enterococci, 35 strains	–	–	–	+	–
Group D haemolytic streptococci, 5 strains	–	–	–	+	–

* We are indebted to Dr Leonard Colebrook for a supply of this group precipitating antiserum.

or no growth in 24 hr., but all showed a good growth in 48 hr. Only one strain (enterococcus) failed to grow in broth containing 6·5% NaCl. The majority of strains of enterococci showed no haemolysis in deep blood agar plates. All strains of group D haemolytic streptococci and a few strains of enterococci showed well marked beta haemolysis. In three strains the exact type of colony on deep blood agar was difficult to determine. These showed a weak alpha haemolysis and are classified as such in the table. None formed a soluble haemolysin when tested by the method used by Hare & Colebrook (1934). Of the forty strains tested thirty-seven reduced methylene blue, thirty were heat resistant at 60° C. for half-an-hour and thirty-seven fermented mannitol. In thirty-seven strains the final *p*H in 1% glucose broth after incubation for four days was 4·0–4·2.

In a further study of the biochemical and other properties usually regarded as characteristic of this group of streptococci a close agreement was also found between enterococci and group D haemolytic streptococci. All strains split aesculin in a medium containing bile and failed to ferment inulin and raffinose, except one strain which fermented the latter. All strains reduced nitrates (Conn, 1936), except one bovine enterococcus. When grown on liver extract broth all produced sulphuretted hydrogen except two bovine strains, one of which also failed to reduce nitrates. In microscopic examination of hanging drop preparations of cultures grown in ox bile (Houston, 1934) all strains showed active growth with chain formation. Confirmatory evidence of their high tolerance for bile was also obtained by the growth of all strains on 40% bile blood agar.

Whilst the majority of strains show uniform biochemical and other properties it is apparent that varieties lacking one or more properties may be encountered and it is evident that these cannot be excluded from the group on antigenic grounds.

SUMMARY OF THE VARIETIES OF ENTEROCOCCI DEFINED BY ANTIGENIC, BIOCHEMICAL AND OTHER PROPERTIES AND THE RELATIONSHIP OF THESE VARIETIES TO CLOSELY RELATED GROUPS OR SPECIES OF STREPTOCOCCI

The enterococci and group D haemolytic streptococci investigated serologically showed the characters of enterococci as defined earlier in this communication.

The enterococci included strains derived from human, bovine and other sources. Twenty of these fermented mannitol and were heat resistant, the remainder possessed one or other of these properties; the group D haemolytic streptococci showed both. These characters clearly differentiated both groups from other species of streptococci. The enterococci and group D haemolytic streptococci showed other properties in common, including ability to grow at 45° C., in broth containing 6·5% sodium chloride and in broth of high alkalinity (*p*H 9·6); the latter characters differentiate the enterococcus from the *Str. lactis*, which has a lower maximum growth temperature and is less tolerant to sodium chloride and alkali (Sherman & Stark, 1931, 1934; Sherman *et al.* 1937).

The literature dealing with descriptions of enterococci contains frequent references to their proteolytic and haemolytic properties; gelatine liquefying enterococci have been described by many workers and there is now no doubt of the occurrence of enterococci, which exhibit beta haemolysis on blood agar (Meyer, 1926; Weatherall & Dible, 1929).

A classification based on these properties is shown in Table IV, from which it will be observed that, in addition to the usual (*faecalis*) variety, which shows non-proteolytic and non-haemolytic properties, three other varieties can be recognized. One of these (var. *liquefaciens*) is similar to *Str. liquefaciens* (Orla-Jensen, 1919) and is probably that species. The proteolytic, haemolytic variety, which we have designated *zymogenes*, possesses similar properties to the *Str. zymogenes* of MacCallum & Hastings (1899) and to the group D haemolytic streptococci of Lancefield (1933).

From the results shown in Table II, it is clear that the group D haemolytic streptococci investigated comprise both the *zymogenes* and its closely related non-proteolytic variety of enterococcus. It will also be observed that of the strains of group D haemolytic streptococci shown in this table the majority are non-proteolytic, haemolytic types similar to a few other strains, shown as enterococci.

These results are in accordance with the conclusions of Sherman *et al.* (1937) that group D haemolytic streptococci (Lancefield, 1933) were similar to *Str. zymogenes* (MacCallum & Hastings, 1899) and that both the proteolytic and non-proteolytic types of the latter species are related serologically to the group D haemolytic streptococci of Lancefield.

It is also probable that the haemolytic enterococci described by Meyer (1926) and by Weatherall & Dible (1929), as well as the group D haemolytic streptococci described by Hare & Maxted (1935), were either the *zymogenes* or *haemolyticus* varieties of the enterococcal group referred to in this communication.

Table IV. *Varieties of enterococci and group D haemolytic streptococci classified by the precipitin test*

No. of strains	Haemolysis	Proteolysis	
1	+	+	Var. *zymogenes*
7	+	+	Var. *haemolyticus*
21	−	+	Var. *liquefaciens*
11	−	−	—

SUSCEPTIBILITY TO LYSIS BY BACTERIOPHAGE

Several authors have shown that streptococci of the enterococcal type are readily lysed by bacteriophages (Beckerich & Hauduroy, 1922; Hadley & Dabney, 1926). Houston (unpublished observations) has shown that bacteriophages active against the majority of typical strains of enterococci can be readily isolated from sewage. Bail (1921) suggested that sensitivity to bacteriophage might serve to differentiate closely related bacteria, and there are reasons for believing that serological characters and phage sensitivity are closely related (Gough & Burnet, 1934). Lancefield (1933) used susceptibility to lysis by phage as confirmatory evidence for the validity of grouping haemolytic streptococci by precipitin tests, and Evans (1936) used sensitivity to phages in conjunction with fermentation reactions as differential tests for groups and species of haemolytic streptococci.

With the object of confirming the group relationships of some of the types studied in this series, the sensitivity of thirty-six strains was investigated to three bacteriophages, two of which (A and B) were "stock" and one (M) freshly isolated. Phages A and B had each been previously propagated on a special strain of enterococcus. The tests for sensitivity to lysis were carried out by making heavy inoculations of young broth cultures of the strains on plates containing 1·5% agar. When the plates had dried, a drop of phage was placed in the centre of each inoculation. From Table V it will be observed that of the thirty-six strains tested thirty-four were sensitive to the three phages used and two were resistant. The latter were non-mannitol fermenters. Control observations made with phages B and M using 1·5% agar containing 5% blood showed that no lysis was produced when representative strains of other groups and species of streptococci were used for inoculating the medium. Of the strains used, thirteen were haemolytic streptococci representative of groups A, B, C, G and F of Lancefield, two were *Str. lactis* and one was *Str. equi*.

Table V. *Lysis of varieties of enterococci by bacteriophages A, B and M*

	Reaction to bacteriophages		
No. of strains	Sensitive	Resistant	
1	1	0	Var. *zymogenes*
4	3	1	Var. *haemolyticus*
19	19	0	Var. *liquefaciens*
12	11	1	—

DISCUSSION

From the serological results shown in Table II a brief summary of which has already been reported by Houston (1936) it is clear that enterococci and group D haemolytic streptococci have a common antigen, the chemical nature of which was not investigated.

This antigen is precipitated by antisera prepared against formalinized whole cultures of either enterococci or group D haemolytic streptococci, but not by extracts prepared from other streptococci, including Lancefield's groups A, B, C, G and F, *viridans* streptococci, and *Str. lactis*.

It is therefore justifiable on antigenic grounds to infer that the enterococci and group D haemolytic streptococci investigated are the same group, which, on the grounds of priority, may be called by the "common name" enterococcus. It is also reasonable, on antigenic grounds, to include within this group non-mannitol fermenting, heat resistant as well as mannitol fermenting, non-heat resistant types, since the precipitating antisera prepared from the latter types, reacted equally well with extracts prepared from all enterococci and group D haemolytic streptococci. This conclusion confirms the work of Dible (1921) in which the property of heat resistance was used as a criterion for defining non-mannitol fermenting types as enterococci. These results are also in conformity with the results obtained in the present investigation using susceptibility to lysis by bacteriophages as a criterion for this group.

From the occurrence of varieties, which can be distinguished by their proteolytic and haemolytic properties, but containing a common antigen, it can be concluded that neither the possession of proteolytic nor of haemolytic properties, or the absence of these is related to antigenic structure as shown by the method used. It is therefore reasonable to assume that the enterococcus group may contain either non-haemolytic or haemolytic varieties analogous to *Str. agalactiae*, the haemolytic and non-haemolytic varieties of which may be of the same serological type (Stableforth, 1937).

As there is at present no justification on antigenic or other grounds for the use of the specific names, *Str. zymogenes* or *Str. liquefaciens*, it is probably better to adopt the terms "*zymogenes*", "*liquefaciens*", and "*haemolyticus*" for distinguishing varieties of enterococci, which may show proteolytic and haemolytic properties. Such a procedure, if adopted, would indicate that these varieties are not species of *Streptococcus*, independent of the enterococcal group.

It is also suggested that the term group D haemolytic streptococcus to designate either a variety of the enterococcus or as a name for a group or species of streptococcus is unnecessary. Its use might with advantage be discontinued and the term Enterococcus var. *zymogenes* substituted. The conclusion already reached by Sherman *et al.* (1937) on biochemical grounds that *Str. zymogenes* and group D haemolytic streptococci are identical, is amply confirmed by the results obtained in this investigation. The negative results obtained with extracts prepared from *Str. lactis*, the identity, or non-identity of which with the enterococcus has been questioned, support the view that these groups are unrelated on antigenic grounds and in other properties to which reference has already been made.

Summary

An investigation of enterococci from human and bovine sources by the method of Lancefield (1933) shows that they possess a specific antigen by which the enterococcus group can be defined.

The validity of this method as a means of differentiating the enterococcus

from other groups of streptococci is confirmed by the biochemical and other properties, such as the association of mannitol fermentation with heat resistance of the strains used, which already differentiate the group in some degree. The tests for susceptibility to lysis by bacteriophages, carried out during the present investigation, are also confirmatory of the results obtained by the precipitin method.

As the specific antigen characteristic of enterococci cannot be demonstrated in strains representative of groups A, B, C, (Lancefield, 1933) and *Str. lactis* (Lister, 1873) it is concluded that these are unrelated to the enterococcus.

A number of other streptococci, however, including *Str. zymogenes* (MacCallum & Hastings, 1899), *Str. liquefaciens* (Orla-Jensen, 1919), and group D haemolytic streptococcus (Lancefield, 1933) have been shown to possess an antigen in common with the enterococcus, as well as the other properties of enterococci, including susceptibility to lysis by bacteriophages; these are usually considered as distinct species, but reasons are advanced in the present communication for regarding them as varieties of enterococci.

The name group D haemolytic streptococcus of Lancefield, is unnecessary and may give rise to confusion. As an enterococcus, it possesses no characteristic antigenic, or haemolytic or other property which differentiates it from the *zymogenes* or *haemolyticus* varieties of enterococci referred to in this communication.

The more important growth characters and fermentation and other properties of the enterococci, classified by the precipitin method in this investigation, may be summarized as follows; ability to grow at 10 and at 45° C., in broth containing 6·5% NaCl, in broth of *p*H 9·6, to produce chains in pure bile, to produce a final *p*H in 1% glucose broth of 4–4·2, to reduce methylene blue, and to ferment trehalose, sorbitol and salicin; but deficiency in one or more of these properties was occasionally noted.

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