THE SEROLOGICAL GROUPING OF *STREPTOCOCCUS LACTIS* (GROUP N) AND ITS RELATIONSHIP TO *STREPTOCOCCUS FAECALIS*

By P. M. F. SHATTOCK and A. T. R. MATTICK, *National Institute for Research in Dairying, University of Reading*

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Introduction

The work of Lancefield in defining the serological groups of haemolytic streptococci has greatly facilitated the task of differentiating and characterizing these organisms. There has been much excellent progress in respect of the frankly pathogenic types, but little advance has been recorded in clearing up the position of the non-pathogenic types such as the milk or 'lactic' streptococci. Prior to the work of Hucker (1932) no systematic attempt appears to have been made to apply serological methods to these organisms, and it seems that, with agglutination, he had little success. Our own work, using the Lancefield technique, began some years ago, and in 1936 (*Report*, 1937) we prepared group-specific sera for *Streptococcus lactis*. Since then Seelemann & Nottbohm (1940) and Seelemann & Flint (1941) have confirmed its group specificity, and Sherman, Smiley & Niven (1940), in a note, have also recorded its serological identity. It is obviously important to be able to distinguish unequivocally those organisms which occur in milk and dairy products and which are used in processes such as cheesemaking and buttermaking, from the enterococci, some strains of which may perhaps give rise to enterotoxins. Moreover, from the manufacturing standpoint *Str. faecalis* may have other objectionable properties.

In recent years it has been established that many of the Lancefield groups, including the classical group A, embrace non-haemolytic as well as haemolytic strains. If, therefore, it can be shown that the lactic streptococci, which are non-haemolytic, fall into a clear-cut group, they are as much entitled to a group letter as any other streptococci. We have developed this point later in the paper.

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History

Streptococcus faecalis

The name *Str. faecalis* was first given by Andrewes & Horder (1906) to a group of streptococci characteristic of the human intestine. They described it as a non-haemolytic, short-chained streptococcus giving uniform turbidity in broth, growing well on gelatin at 20° C., forming H_2S and chemically very active. They found that sucrose, lactose, salicin, coniferin and mannitol were fermented, but not raffinose and inulin. They showed further that milk was clotted and neutral red reduced, and observed that the strains were very resistant to desiccation. They regarded the fermentation of mannitol as particularly characteristic, although they admitted exceptions and noted that variations in other respects were numerous.

It was soon recognized that *Str. faecalis* of Andrewes & Horder was very similar to the *Enterococcus* described morphologically by Thiercelin (1899). It was, however, left to Dible (1921) to resolve the confusion then existing regarding the faecal streptococci, and his classical paper contains a full review of the literature to that date. Dible regarded *Str. faecalis* as synonymous with the *Enterococcus*, the biochemical reactions of which are given, together with those of the three main variants:

	Milk clot	Sucrose	Lactose	Raffinose	Inulin	Salicin	Mannitol	Dulcitol
Typical *Enterococcus*	+	+	+	−	−	+	+	−
Variant I	+	−	+	−	−	+	+	−
Variant II	+	+	+	−	−	+	−	−
Variant III	+	−	+	−	−	+	−	−

Dible, while recognizing the importance of mannitol fermentation, drew attention to the heat-resistant properties of the *Enterococcus* (noted earlier by Houston & McCloy, 1916) and found a high correlation between heat resistance and the formation of short chains or diplococci. He regarded the ability 'to withstand exposure to 60° C. for 15 min.' as one of the most constant characters, as those forms which do not ferment mannitol are heat resistant. Dible defined the '*faecalis* group proper' as '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*.'

Some workers, e.g. Sherman (1937), use the term *Enterococcus* to designate a group or division of streptococci which includes haemolytic, non-haemolytic and gelatin-liquefying types (*Str. zymogenes*, *Str. durans* and *Str. liquefaciens*). Lancefield (1933) on serological grounds divided haemolytic streptococci into groups, now well recognized, according to their origin, and it became evident that her group D, comprising haemolytic streptococci isolated from cheese, showed characteristics similar to the enterococci. This was particularly apparent as haemolytic strains of the *Enterococcus* had already been described (Wordley, 1921; Meyer, 1926; Weatherall & Dible, 1929). Graham & Bartley (1939) have shown that the *Enterococcus* possesses an antigen in common with Lancefield's group D and suggest that the name group D haemolytic *Streptococcus* is unnecessary and tends to give rise to confusion as this organism cannot be differentiated either serologically or by other characteristics from haemolytic varieties of the *Enterococcus* already recognized. Graham & Bartley have done much to clarify the classification of the enterococci, and as their paper contains a full review of the literature since Dible's (1921) paper, it is not proposed to recapitulate it here.

The literature contains references to heat-resistant streptococci resembling the *Enterococcus* which display other characteristic features. Thus *Str. glycerinaceus* was given specific rank by Orla Jensen (1919) who isolated strains from cheese. It is particularly characterized by a strong fermentation of glycerol. He drew attention to the similarity of this organism to a proteolytic streptococcus associated with milk and milk products which he called *Str. liquefaciens*, the only real difference between the two organisms being liquefaction of gelatin by the latter. Orla Jensen (1919) identified *Str. liquefaciens* with *Micrococcus casei amari* of Freudenreich. *Str. liquefaciens* has been widely recognized as a distinct species (e.g. Long & Hammer, 1936), although on the original description its similarity to *Str. faecalis* is evident. Andrewes & Horder (1906) in their description of *Str. faecalis* noted that some strains might liquefy gelatin, and Dible (1921) also encountered liquefying strains. Later it was claimed by Sherman, Mauer & Stark (1937) that *Str. glycerinaceus* is only a variety of *Str. faecalis*, and that the use of a specific name for this variant is not warranted. Sherman, Stark & Mauer (1937) drew attention to the

similarity of *Str. liquefaciens* and *Str. faecalis*, while Sherman (1938) reported that Lancefield, in a private communication, found that strains of *Str. faecalis* and *Str. liquefaciens* which gave typical cultural and biochemical reactions, belonged to her group D. In the same paper, Sherman reported the confirmation of these findings, and Graham & Bartley (1939), going thoroughly into these relationships, agreed with Lancefield. The right of *Str. liquefaciens* to specific rank is discussed later.

Streptococcus lactis

The common organism associated with the souring of milk was first described by Lister (1878) as '*Bacterium lactis*'. In the next thirty years a variety of names and descriptions, including *Str. lacticus* of Kruse (1903), were added to the literature, and it was left to Löhnis (1909) to clarify the situation. He agreed that it should be placed in the genus *Streptococcus* and suggested that it should be named '*Streptococcus lactis*', thus keeping Lister's original species name. The various synonyms encountered are very numerous and were summarized by Breed (1928). Bergey (1939) has adopted this list in its entirety.

Orla Jensen's (1919) description of *Str. lactis* is as follows. The optimum temperature for growth is 30° C., and when freshly isolated it will coagulate sterile milk at this temperature in less than 24 hr. At the optimum temperature it occurs as a diplococcus or as short chains. Growth below 10° C. and above 40° C. is in general poor. *Str. lactis* is characterized by failure to ferment sucrose. The main sugar reactions (according to Orla Jensen) are:

Arabinose	Mannitol	Dextrose	Sucrose	Lactose	Maltose	Raffinose	Dextrin	Inulin	Salicin	Starch
±	±	+	−	+	+	−	+	−	+	−

The origin of *Str. lactis* has not yet been determined. It is generally recognized that it does not occur in aseptically taken milk samples, and yet it is a common contaminant of dairy utensils. Stark & Sherman (1935) were able to isolate *Str. lactis* from some, but not all, of the plants examined by them. They failed to isolate it from the mouths and throats of cows, bovine and human faeces and soil, and suggested that the organism may be of plant origin. Orla Jensen (1919) found that strains cultivated on artificial substrates tended to lose their ability to thrive in milk and suggested that this, together with the fact that many strains prefer maltose to lactose, shows that milk is not the most natural substrate for *Str. lactis*, which may originate in cow dung. The similarity of the sugar reactions and morphology of *Str. lactis* to *Str. faecalis* has led many workers to conclude that *Str. lactis* is only a variety of *Str. faecalis*. Hammer & Baker (1926) endeavoured to differentiate varieties of *Str. lactis* according to certain growth characteristics. They defined what they termed the '*Str. lactis* group' as 'any organism coagulating litmus milk rapidly or fairly rapidly with reduction of the litmus but without digestion or the formation of gas and which appears in strains from milk as a Gram positive coccus arranged in chains or pairs, the pairs rarely being grouped in clumps'. Such descriptions have added to the tendency to regard *Str. lactis* and *Str. faecalis* as very similar if not identical organisms. Ayres & Johnson (1924) compared *Str. lactis* with faecal streptococci noting morphology, survival at 60° C. for 30 min., reactions in litmus milk at 30 and at 10° C., reduction of Janus green, the final *p*H in a medium of reduced surface tension, the production of CO_2 and of NH_3 from peptone, and the hydrolysis of sodium hippurate as well as sugar fermentations. They concluded that the two organisms were similar if not identical. Meyer & Schönfeld (1926), observing aesculin splitting in the presence of bile, the fermentation of raffinose and mannitol, growth in 10 and 20% bile-peptone water, heat resistance and morphology, differentiated the *Enterococcus* from *Str. viridans*. Using the same tests they endeavoured to differentiate the lactic acid streptococci from these two groups. They concluded that the lactic acid streptococci could be divided into two types, one (presumably *Str. lactis*) being similar to the *Enterococcus* and the other (presumably *Str. cremoris*) being similar to *Str. viridans*. Demeter (1929) could find no reason for believing that *Str. lactis* was other than a variant of *Str. faecalis* which had become adapted to a milk medium. Kleckner (1935) isolated lactic acid streptococci from unpasteurized milk, buttermilk and cottage cheese, and, using biochemical and serological (agglutination) tests, compared them with faecal streptococci. He concluded that there was no real difference between the two types. It is evident, however, that some of Kleckner's so-called lactic acid streptococci were in fact *Str. faecalis*. Chapman (1936) studied the resistance of the *Enterococcus* to a number of bactericidal substances and considered *Str. lactis* to be a possible synonym of *Str. faecalis* and the *Enterococcus*. Topley & Wilson (1936) in discussing the literature were unable to find any real difference between these two organisms and suggested that they

should be regarded as one group. Sherman & Stark (1934), however, maintained that *Str. lactis* and *Str. faecalis* were separate entities and could readily be differentiated in that *Str. lactis* had a lower maximum temperature for growth, a low resistance to heat, and less tolerance to alkali and to NaCl.

The identity of *Str. lactis* is of particular interest and importance to dairy bacteriologists, and at an earlier date we carried out some preliminary work on its serological differentiation from *Str. faecalis* using Lancefield's (1933) technique. We stated (*Report*, 1937): 'From the serological results obtained with *Str. lactis* and *Str. faecalis* it appears that they can be distinguished and are probably worthy of specific rank.' Sherman (1938), in discussing the relationship of *Str. faecalis* to group D of Lancefield, found that hydrochloric acid extracts of *Str. lactis*, unlike those of *Str. faecalis*, did not react with group D sera. Later, Sherman, Smiley & Niven (1940), in a note on 'The serological integrity of *Str. lactis*', state, without details, that group sera against *Str. lactis* gave no reactions with *Str. faecalis* or other enterococci, with representatives of other serological groups (A–H inclusive), nor with *viridans* and other non-haemolytic streptococci outside the so-called 'lactic group'. Graham & Bartley (1939), dealing with the classification of the enterococci, found that no reactions were obtained with group D sera and extracts of two strains of *Str. lactis* used as controls. Seelemann & Nottbohm (1940) showed that a group serum for *Str. lactis*, which precipitated with fifteen other strains of *Str. lactis*, gave no cross reactions with sixteen group D strains or with any other groups tested. Two group D sera gave good precipitin reactions with fifteen other strains of group D, but no cross-reactions were obtained with sixteen *Str. lactis* strains.

Experiments

At the outset of this work it was necessary to secure an adequate number of strains of heat-resistant, mannitol-fermenting streptococci possessing those other properties now associated with the name *Str. faecalis*. These organisms were all non-haemolytic and were used for comparison with *Str. lactis*. It soon became clear, however, that those haemolytic organisms, with other properties similar to those of *Str. faecalis*, assembled in Lancefield's group D, would also have to be considered in relation to *Str. lactis*. Moreover, the question of the identity of those heat-resistant mannitol-fermenting streptococci, atypical in other respects, e.g. *Str. glycerinaceus* (Orla Jensen) and *Str. liquefaciens* (Orla Jensen), both commonly found in milk products, would also have to be taken up. Therefore for comparison with *Str. lactis* a number of authentic haemolytic group D strains were secured together with strains of *Str. glycerinaceus* (Orla Jensen) and *Str. liquefaciens* (Orla Jensen) for inclusion with the original *Str. faecalis* strains. The opportunity thus occurred of determining the interrelationship of the group D strains with the non-haemolytic *Str. faecalis* strains and of exploring the identity of *Str. liquefaciens* (Orla Jensen) and of *Str. glycerinaceus* (Orla Jensen).

Methods

(*a*) *Biochemical and cultural*

'Sugar' fermentations were carried out in Lemco broth containing 0·5% of the test sugars with litmus as indicator. Tubes were incubated at 37° C. for *Str. faecalis* and group D strains and at 28–30° C. for *Str. lactis*. Sugar reactions were recorded after 24 hr., 48 hr. and 10 days. The splitting of aesculin was confirmed by the production of a black precipitate with ferric chloride.

Gelatin liquefaction. Stab cultures were incubated at 22° C. and examined as for the sugar reactions except that where no liquefaction was recorded after 10 days the tubes were reincubated and finally read after 1 month.

Haemolysis. Poured plates seeded to give 100–200 colonies per plate on incubation were prepared in nutrient agar containing 5% horse blood. The *Str. faecalis* and group D were incubated at 37° C. and the *Str. lactis* at 30° C. Plates were examined after 24 and 48 hr. incubation and again after storage in the ice chest at about 33–35° F. for a further 24 hr.

Heat resistance. Two drops of an 18 hr. culture in yeast-dextrose broth* were inoculated into 10 ml. of the same medium previously heated to 60° C. The tubes were held in a water-bath at 60° C. for 30 min., cooled and incubated at the optimum temperature. The presence or absence of growth, compared with unheated control tubes, was recorded after 24 and 48 hr.

(*b*) *Serological*

Serum preparation

It was originally intended to use living organisms for the production of sera, but some deaths of rabbits, which could only be attributed to the organisms inoculated, were sustained. As trouble ceased when killed cultures were used it was likely that some strains of *Str. lactis* and *Str. faecalis* had some pathogenicity for the rabbit when introduced intravenously.

Early in the work reported here formalin-killed suspensions were prepared from organisms grown for 24 hr. at the optimum temperature in yeast-dextrose broth, at *p*H 7·0 for *Str. lactis* and *p*H 7·4 for *Str. faecalis* and group D strains. These broth cultures were centrifuged, the deposit suspended in 0·85% NaCl containing 0·2% formalin and stored in the ice chest until sterile (Lancefield, 1933). One and the same suspension, standardized to give an opacity equivalent to Brown's tube no. 7, was used throughout for inoculations, which were carried out on 4 successive days followed by 3 days' rest. The first two doses were 0·25 and 0·5 ml., and 1 ml. was used for each subsequent injection. The production of potent group sera by this method proved to be very slow, and in many cases it was found necessary to continue inoculations for 3 months or longer before usable sera could be obtained. Unfortunately, we found that when long periods of inoculation were required for the production of sera, non-specific antibodies often appeared. Moreover, in many cases certain organisms gave rise to strain- or type-specific sera only, which would react with the homologous organism but not with other members of the same group. The difficulty in producing group-specific sera, particularly for *Str. lactis*, has been encountered by other workers, e.g. Sherman (1938), Seelemann & Nottbohm (1940) and Seelemann & Flint (1941). In an attempt to overcome these difficulties, treatment of the organism before inoculation was considered. From experience in the absorption of sera with certain streptococci it was thought that little if any of the species-specific or group antigens were present at the surface. Attempts were therefore made to expose these antigens without significant alteration. With this in view suspensions were treated with sodium lauryl sulphate (Hunwicke, 1935). This method was devised for the production of endotoxins, the antigenic properties of which were said to be unimpaired by the treatment. Bacteria were dissolved in an alkaline aqueous solution of the sodium salt of lauryl alcohol sulphuric ester, and the neutralized, sterilized product used as a vaccine. Satisfactory sera were not obtained with this product, and as its injection caused considerable local injury to the ears it had to be abandoned.

Further experiments were carried out in which various crude extracts of bacteria were prepared and used for inoculation:

(1) Organisms which had been extracted with acetone, dried in vacuo over P_2O_5 and finally disintegrated by grinding, in the cold (not above 15° C.), in a ball mill.

(2) The ground organisms from (1) extracted with CO_2-free water.

(3) The residue from (2) extracted with 5% NaCl in distilled water.

(4) The residue from (3) extracted with 3% Na_2CO_3.

The most satisfactory method for the preparation of sera was to use the acetone-extracted ground organisms. As it was possible that some essential fraction might have been lost on acetone extraction, unextracted organisms dried over P_2O_5 were also used for inoculation. That no significant differences could be detected in the sera showed these fears to be groundless. It was, however, found that satisfactory sera could be more quickly prepared from the extracted ground organisms. The main benefit of extraction lay in the fact that grinding was greatly facilitated because the organisms did not adhere to the glass. It is not, however, certain that the use of ground organisms will completely overcome strain specificity, and individual rabbit response, as always, has still to be contended with. Latterly, inoculations have been carried out every fourth day instead of every 4 consecutive days with 3 days' rest. Sera were stored in the cold without preservative.

* Peptone 2%, lemco 1%, yeastrel 0·3%, NaCl 0·5%, dextrose 0·5%; *p*H 7·0.

Extracts

Various methods of preparing extracts for precipitation test were compared. These were the sodium lauryl sulphate extraction of Hunwicke already referred to, extraction with sodium hypochlorite according to Kurt Meyer's (1930) method for the extraction of the Shiga bacillus, the formamide method of Fuller (1938) and Lancefield's method of extracting with HCl (1933). It was decided to adopt that of Lancefield, as the others gave extracts which were unreliable because of disconcerting cross-reactions or prozones. The possibility of encountering prozones was obviously dangerous unless a large number of extract dilutions involving the use of large amounts of sera was tested.

Precipitin tests

Precipitin tests were carried out according to Lancefield's (1933) technique. Ring formation was recorded after standing at room temperature for 10–20 min. The tubes were then shaken and incubated in a water-bath at 37° C. for 2 hr. and final readings made after standing in the ice chest overnight. Latterly ring tests only have been carried out, using small tubes of 3 mm. internal diameter and the minimum quantity of serum. The results obtained by this semi-micro method have been checked from time to time by the incubation method using the larger quantities of reagents.

Precipitin-absorption test

Organisms for absorption were grown in yeast-dextrose broth at 37° C. for 24 hr. The resulting cultures were centrifuged, the organisms resuspended in 0·85% NaCl and killed by heating in a water-bath at 60° C. for 1 hr. The concentration of the suspension was adjusted so that it was equivalent to 50 times Brown's opacity tube no. 10. The organisms from 1 ml. were centrifuged, and 1 ml. of the serum to be absorbed was added to the packed bacteria and thoroughly mixed. Absorption was carried out in a water-bath at 37° C. for 1 hr. and the tubes left in the ice chest overnight. The organisms were then removed by centrifuging. A control absorption using the homologous organism for the serum was always included.

Results

(*a*) *Cultural and biochemical reactions of* Str. faecalis *and group D streptococci*

With one exception (J.M.A.1) all the strains of *Str. faecalis* examined fermented mannitol and all survived heating at 60° C. for 30 min. On the basis of the sugar reactions (Table 1), *Str. faecalis* and group D strains are indistinguishable. None of the *Str. faecalis* strains liquefied gelatin, but four of the group D strains did so. Sherman & Stark (1934) stated that *Str. faecalis* would grow at 45° C., survive heating at 65° C. for 30 min., grow at a *p*H of 9·6 and in a medium containing 6·5% NaCl. These tests and methods of carrying them out will be dealt with later, in discussing the differentiation of *Str. lactis* from *Str. faecalis*, but it will be seen from Table 1 that the group D streptococci examined showed reactions similar to those of *Str. faecalis*.

The one constant distinguishing characteristic was the reaction on horse-blood agar plates. On horse blood agar group D strains all showed true haemolysis, whereas with the exception of J.M.A.1 which produced narrow zones containing incompletely haemolysed corpuscles, the *Str. faecalis* strains were either inert or showed slight zones of greening which usually proved not to be true α haemolysis.

(*b*) *Serological reactions of* Str. faecalis *and group D streptococci*

Precipitating sera were prepared from four strains of *Str. faecalis*. Two of these, O.J. and 370, gave type reactions only. The other two, 2703 and J.M.A.4, gave good group sera. Strain 'Carter' (group D) gave a very satisfactory group serum. A second group D serum (kindly supplied by Dr A. W. Stableforth) was also used in these investigations. All sera were tested for group specificity against HCl extracts of Lancefield groups A–C and E–M inclusive, and all failed to give precipitates either in ring tests or after incubation.

Heavy precipitates (Table 1) were obtained with *Str. faecalis* (2703) serum against extracts of all the strains of *Str. faecalis* and group D which were tested. The two group D sera gave strong reactions with all group D extracts and some extracts of *Str. faecalis*. With other *Str. faecalis* strains, however, weak reactions only were obtained, and in two instances (J.M.A.1 and 2703) precipitates were produced only after 2 hr. in the water-bath at 37° C. This delayed reaction has also been noted by Graham & Bartley

(1939). These two strains are recorded in Table 1 as giving negative results with Stableforth's group D serum, ring tests only being carried out. The strain 'C and G', which must be regarded as *Str. faecalis* on cultural and biochemical grounds, is recorded as giving a positive reaction with a serum for *Str. faecalis*. It was, however, negative with two group D sera. Ring tests only were carried out as sufficient serum was not available for the incubation technique. In the light of our experience with strains J.M.A.1 and 2703 it is probable that incubation of optimal proportions of extracts and serum 'C and G' would have given a positive result with group D. It was observed that whereas HCl extracts of other Lancefield groups could be stored in the ice chest for many months without apparent loss of potency, the HCl extracts of some but not all *Str. faecalis* strains, deteriorated after a few weeks' storage. This was not apparent with the haemolytic members of group D.

In order to clarify these results absorption tests were carried out with the group D 'Carter' serum (Table 2). Absorption with *Str. faecalis* J.M.A.4 resulted in the removal of the group substance, but as J.M.A.4 evidently belonged to a different serological type from 'Carter' group D, the type substances were left intact and were precipitated by other group D and *Str. faecalis* strains which possessed type substances in common with the strain 'Carter'. It was then shown that a typical haemolytic group D strain ('Heasman') and a non-haemolytic strain of *Str. faecalis* (J.M.A.7), both of which reacted with the group D 'Carter' serum which had been absorbed by *Str. faecalis* (J.M.A.4), were apparently of the same serological type as 'Carter'. The evidence for this is not complete, as sera for the strains 'Heasman' and J.M.A.7 were not available for reciprocal absorption tests. This does not alter the fact that typical *Str. faecalis* strains dealt with here fall into group D.

A strain of the so-called *Str. glycerinaceus* and four strains of *Str. liquefaciens* were also investigated and all reacted with a group D serum. The biochemical and serological results are summarized in Table 1.

In collecting strains of *Str. faecalis*, three strains of streptococci, 'Haines', 'Goddard' and 'Elene', isolated from human faeces, proved to be of the *Str. bovis* type (Orla Jensen, 1919). They fermented raffinose but not mannitol, resisted heating at 60° C. for 15 min. and grew at 45° C. but not at *p*H 9·6 or in a salt concentration of 6·5% (Sherman, 1937). HCl extracts of both 'Haines' and 'Goddard' gave good ring tests with *Str. faecalis* and group D sera, but only partial reactions were obtained with 'Elene'. This is similar to the experience of Sherman (1938), who found that some strains of *Str. bovis* gave positive reactions with group D sera, some gave only faint reactions and some were completely negative. The difficulty experienced in preparing group sera for *Str. bovis* has made the relationship of this organism to group D difficult to interpret, although on biochemical grounds *Str. bovis* can be readily distinguished from *Str. faecalis* (Shattock, 1937).

(c) *Cultural and biochemical differentiation of* Str. lactis *from* Str. faecalis *and group D streptococci*

Most of the workers in this field hold that *Str. lactis* is nothing more than a variant of *Str. faecalis*, although some are not prepared to be dogmatic (Topley & Wilson, 1936). This attitude of so many workers is not surprising, as the morphology and biochemical reactions of these two types are remarkably similar. With the exceptions of Sh. 3 and 'C and G', all the *Str. faecalis* and group D strains examined here fermented sucrose while the *Str. lactis* strains did not (Table 1). The fermentation of a single sugar is not in our opinion sufficient to warrant the creation of a species, particularly when sucrose-fermenting strains of *Str. lactis* (Yawger & Sherman, 1937) and non-sucrose-fermenting strains of *Str. faecalis* (Dible, 1921) are not unknown. Mannitol fermentation by *Str. lactis* strains is inconsistent, but the fact that this organism is usually held to be able to withstand heating at 60° C. for 30 min. (e.g. Ayres & Johnson, 1924; Sherman, 1937) brings it very close to the accepted definition of *Str. faecalis*. It will be noted that none of our *Str. lactis* strains survived this temperature.

Sherman and his co-workers have consistently maintained that *Str. lactis* can readily be differentiated from *Str. faecalis*, and Sherman & Stark (1934) devised four 'tolerance tests' by means of which they claimed that the two species could be distinguished. As there is a certain lack of experimental detail in their paper other workers have perhaps not used these tests to the extent that the claims appear to warrant. Those who have used them have recorded their scepticism of their value (e.g. Nichols, 1939; Hobbs, 1939).

Table 1

Strain	Litmus milk—24 hr.	Glycerol	Arabinose	Mannitol	Sucrose	Lactose	Maltose	Raffinose	Dextrin	Inulin	Salicin	Aesculin	Gelatin liquefaction	Haemolysis	Growth at 45° C.	Growth at p_H 9·6	60° C. for 30 min.	Group D 'Carter'	Group D (Stableforth)	*Str. faecalis* 2703	*Str. faecalis* J.M.A.4	*Str. glycerinaceus* 2702	*Str. liquefaciens* 2705	*Str. lactis* O.J.	Origin
														Str. faecalis						Sera					
2703	A.	−	+	+	+	+	+	−	+	−	+	+	−	Sl.Gr.	+	+	+	+	−	+	·	·	·	−	Nat. Coll. Type Cults.
J.M.A.1	·A.	−	−	−	+	+	+	−	+	−	+	+	−	P.	+	+	+	+	−	+	·	·	·	−	Faeces (J. M. Alston)
J.M.A.2	r.A.	+	−	+	+	+	+	−	+	−	+	+	−	Sl.Gr.	+	+	+	+	+	+	·	·	·	−	,,
J.M.A.3	A.	+	+	+	+	+	+	−	+	−	+	+	−	Greening	+	+	+	+	+	+	·	·	·	−	Canned peas (J. M. Alston)
J.M.A.4	A.	+	+	+	+	+	+	−	+	−	+	+	−	Sl.Gr.	+	+	+	+	+	+	·	·	·	−	Urine (J. M. Alston)
J.M.A.5	–	+	+	+	+	+	+	−	+	−	+	+	−	Greening	+	+	+	+	+	+	·	·	·	−	Cervix (J. M. Alston)
J.M.A.7	A.	+	+	+	+	+	+	−	+	−	+	+	−	None	+	+	+	+	+	+	·	·	·	−	Urine (J. M. Alston)
J.M.A.8	A.	+	+	+	+	+	+	−	+	−	+	+	−	Sl.Gr.	+	+	+	+	+	+	·	·	·	−	,,
E.B.54		·	·	·	·	·	·	·	·	·	·	·	·	Sl.Gr.	+	+	+	+	+	+	·	·	·	−	Endocarditis (S. D. Elliot)
E.B.56		+	+	+	+	+	+	−	+	−	+	+	−	Sl.Gr.	+	+	+	+	+	+	·	·	·	−	,, ,,
E.B.57		+	+	+	+	+	+	−	+	−	+	+	−	Sl.Gr.	+	+	+	+	+	+	·	·	·	−	,, ,,
E.B.96		+	+	+	+	+	+	−	+	−	+	+	−	Sl.Gr.	+	+	+	+	+	+	·	·	·	−	,, ,,
370	R.A.	+	+	+	+	+	+	−	+	−	+	+	−	Greening	+	+	+	+	+	+	·	·	·	−	Nat. Coll. Type Cults.
775	R.A.C.	+	+	+	+	+	+	−	+	−	+	+	−	Greening	+	+	+	+	+	+	·	·	·	−	,,
2707	R.A.C.	+	+	+	+	+	+	−	+	−	+	+	−	Greening	+	+	+	+	+	+	·	·	·	−	,,
Str. faecium O.J.	r.A.	+	+	+	+	+	+	−	+	−	+	+	−	α	+	+	+	+	+	+	·	·	·	−	(Orla Jensen via J. G. Davis)
Sh. 3	R.A.C.	+	+	+	+	+	+	−	+	−	+	+	−	α	+	+	+	+	+	+	·	·	·	−	Milk powder (J. L. Shimwell via J. G. Davis)
Clarke	R.A.	+	−	+	+	+	+	−	+	−	+	+	−	Greening	+	+	+	+	+	+	·	·	·	−	Faeces (E. H. Gillespie)
C. and G.	R.A.C.	+	−	+	−	+	+	−	−	−	+	+	−	α	+	+	+	+	−	+	·	·	·	−	Milk powder (J. G. Davis)
														'Group D' (*Str. faecalis*) strains											
Carter	r.A.	+	−	+	+	+	+	−	+	−	+	+	−	β	+	+	+	+	+	+	·	·	·	+	(R. Hare via R. M. Fry)
SHC 136	R.A.	+	−	+	+	+	+	−	+	−	+	+	−	β	+	+	+	+	+	+	·	·	·	+	Vagina of bitch (R. M. Fry)
SHC 159	R.A.C.	+	−	+	+	+	+	−	+	−	+	+	+	β	+	+	+	+	+	+	·	·	·	+	Urine—dog (R. M. Fry)
SHC 167	R.A.C.	+	−	+	+	+	+	−	+	−	+	+	−	β	+	+	+	+	+	+	·	·	·	+	Vagina of bitch (R. M. Fry)
Heasman	R.A.C.	+	−	+	+	+	+	−	+	−	+	+	−	β	+	+	+	+	+	+	·	·	·	+	(R. Hare via R. M. Fry)
Mahoney	R.A.	+	−	+	+	+	+	−	+	−	+	+	−	β	+	+	+	+	+	+	·	·	·	+	,,
Henry	A.	+	−	+	+	+	+	−	+	−	+	+	−	β	+	+	+	+	+	+	·	·	·	+	Vagina—human (R. M. Fry)
Spence	R.A.	+	−	+	+	+	+	−	+	−	+	+	−	β	+	+	+	+	+	+	·	·	·	+	(A. Fleming)
Black	R.A.C.	+	−	+	+	+	+	−	+	−	+	+	−	β	+	+	+	+	+	+	·	·	·	+	,,
Rundle	R.A.C.	+	−	+	+	+	+	−	+	−	+	+	−	β	+	+	+	+	+	+	·	·	·	+	,,
Magill	R.A.C.	+	−	+	+	+	+	−	+	−	+	+	+	β	+	+	+	+	+	+	·	·	·	+	,,

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'*Str. glycerinaceus*', *Str. liquefaciens* and *Str. bovis*

Str. glycerinaceus 2702	R.A.	+	−	+	+	+	+	−	+	−	+	.	−	Sl.Gr.	+	+	+	+	.	+	.	+	.	.	Nat. Coll. Type Cults.
Str. liquefaciens 2705	R.A.C.	−	−	+	+	+	+	−	+	−	−	.	+	Sl.Gr.	.	.	+	+	.	+	.	.	+	.	,,
Str. liquefaciens Z 84	R.A.C.	+	−	+	+	+	+	−	+	−	+	+	+	Sl.Gr.	.	.	+	+	.	.	.	.	+	.	Milk (M. Zein-El-Dine)
Str. liquefaciens Z 93	R.A.C.	+	−	+	+	+	+	−	+	−	+	+	+	Sl.Gr.	.	.	+	+	.	.	.	.	+	.	,, ,,
Str. liquefaciens Z 94	R.A.C.	+	−	+	+	+	+	−	+	−	+	+	+	Sl.Gr.	.	.	+	+	.	.	.	.	+	.	,, ,,
Str. bovis Haines	A.	−	−	−	+	+	+	+	+	−	+	+	−	Sl.Gr.	+	−	−	+	+	+	.	.	.	.	Human faeces (E. H. Gillespie)
Str. bovis Goddard	A.	−	−	−	+	+	+	+	+	+	+	+	−	Sl.Gr.	+	−	−	+	±	+	.	.	.	.	,, ,,
Str. bovis Elene	A.	−	−	−	+	+	+	+	+	+	+	+	−	Greening	+	−	−	±	−	±	.	.	.	.	,, ,,
														Str. lactis										
O.J.	R.A.C.	.	−	+	−	+	+	−	+	.	+	+	−	Sl.Gr.	−	−	−	−	.	−	.	.	.	+	(Orla Jensen via J. G. Davis)
H 195	R.A.C.	.	+	+	−	+	+	−	+	.	+	+	−	None	−	−	−	−	.	−	.	.	.	+	Lancashire cheese (J. Harrison)
H 199	R.A.C.	.	−	−	−	+	+	−	+	.	+	+	−	Greening	−	−	−	−	.	−	.	.	.	+	,, ,,
H 201	R.A.C.	.	+	+	−	+	+	−	+	.	+	+	−	None	−	−	−	−	.	−	−	.	.	+	,, ,,
H 203	R.A.C.	.	+	+	−	+	+	−	+	.	+	+	−	None	−	−	−	−	.	−	.	.	.	+	,, ,,
H 207	R.A.C.	.	+	+	−	+	+	−	+	.	+	+	−	None	−	−	−	−	.	−	−	.	.	+	,, ,,
H 222	R.A.C.	.	−	−	−	+	+	−	+	.	+	+	−	None	−	−	−	−	.	−	−	.	.	+	Cheddar cheese (J. Harrison)
H 240	R.A.C.	.	−	−	−	+	+	−	+	.	−	+	−	None	−	−	−	−	.	−	.	.	.	+	,, ,,
H 257	R.A.C.	.	−	+	−	+	+	−	+	.	+	+	−	Greening	−	−	−	−	.	−	.	.	.	+	,, ,,
H 287	R.A.C.	.	−	+	−	+	+	−	+	.	+	+	−	None	.	.	.	.	.	.	.	.	.	+	Cheshire cheese (J. Harrison)
H 288	R.A.C.	.	−	−	−	+	+	−	+	.	+	+	−	Greening	−	−	−	−	.	−	−	.	.	+	,, ,,
H 290	R.A.C.	.	+	+	−	+	+	−	+	.	+	+	−	None	−	−	−	.	.	.	.	.	.	+	,, ,,
H 299	R.A.C.	.	+	+	−	+	+	−	+	.	+	+	−	None	−	−	−	−	.	−	.	.	.	+	,, ,,
Rice	R.A.C.	.	.	+	−	+	+	−	.	.	+	+	−	Greening	−	−	−	−	.	−	.	.	.	+	Starter (E. B. Rice)
Freshly isolated strains																									
M 3	R.A.C.	.	.	+	−	+	+	−	.	.	+	+	−	α	−	−	−	−	.	−	.	.	.	+	Milk (A. A. Nichols)
M 4	R.A.C.	.	.	+	−	+	+	−	+	.	+	+	−	Greening	−	−	−	−	.	−	.	.	.	+	,, ,,
M 6	R.A.C.	.	.	+	−	+	+	−	.	.	+	+	−	None	−	−	−	−	.	−	.	.	.	+	,, ,,
M 11	R.A.C.	.	.	+	−	+	+	−	.	.	+	+	−	Sl.Gr.	−	−	−	−	.	−	.	.	.	+	,, ,,
M 14	R.A.C.	.	.	−	−	+	+	−	.	.	+	+	−	α	−	−	−	−	.	−	.	.	.	+	,, ,,
M 15	R.A.C.	.	.	+	−	+	+	−	+	.	+	+	−	None	−	−	−	−	.	−	.	.	.	+	,, ,,
M 16	R.A.C.	.	.	+	−	+	+	−	.	.	+	+	−	None	−	−	−	−	.	−	.	.	.	+	,, ,,
M 17	R.A.C.	.	.	−	−	+	+	−	+	.	+	+	−	Sl.Gr.	−	−	−	−	.	−	.	.	.	+	,, ,,
M 18	R.A.C.	.	.	−	−	+	+	−	.	.	+	+	−	Sl.Gr.	−	−	−	−	.	−	.	.	.	+	,, ,,

* The negative results were obtained with the ring test. Incubation of optimal proportions was not carried out.

Note. The three *Str. bovis* strains withstood 60° C. for 15 min. (Orla Jensen, 1919; Dible, 1921).

A. = acid. R. = complete reduction. r. = partial reduction. C. = clot. P. = incomplete β haemolysis. Sl.Gr. = slight greening. ± = slight precipitate.

'*Growth at* 45° C.'

Dextrose (0·5%) Lemco broth inoculated with young actively growing (18 hr.) cultures was incubated at 45° C. in a water-bath (thermostatically controlled ±0·1° C.). All *Str. faecalis* and group D strains grew well in 24 hr. with a strong diffuse growth, while all the *Str. lactis* strains tested failed to grow, the tubes remaining clear. In yeast-dextrose-litmus-milk (Davis, 1935) all *Str. faecalis* and group D strains produced obvious acidity whilst with *Str. lactis* strains the medium remained unchanged.

'*Thermal death-rates*'

Sherman found that *Str. lactis* cultures in sterile skimmed milk heated at 65° C. for 30 min. were killed while *Str. faecalis* cultures survived. The details of the method of carrying out this test are not given in the original paper.

Several experiments were done in order to arrive at a satisfactory technique.

(*a*) Quantities of 0·1 ml. of 18 hr. yeast-dextrose broth cultures were inoculated into 10 ml. of yeast-dextrose-litmus-milk in 6 × ⅝ in. test tubes (previously brought to 65° C.) and heated in a water-bath (thermostatically controlled ±0·1° C.) at 65° C. for 30 min. The tubes were removed, cooled, incubated at 32° C. and read after 24 hr. and after 3 days. At 24 hr. there was no change in acidity in any of the tubes and after 3 days the results were neither clear cut nor reproducible.

Table 2

Extract		Serum group D 'Carter' Unabsorbed	Absorbed J.M.A.4	Absorbed Heasman	Absorbed J.M.A.7
Group D:	Carter	+	+	−	−
	Heasman	+	+	−	−
	Magill	+	−	−	−
	Black	+	−	−	−
	Mahoney	+	±	−	−
	SHC 159	+	−	−	−
	SHC 167	+	±	−	−
Str. faecalis:	J.M.A.2	+	−	−	−
	J.M.A.3	+	.	−	−
	J.M.A.4	+	−	−	−
	J.M.A.7	+	+	−	−
	J.M.A.8	+	+	−	−
	E.B. 54	+	+	−	−
	E.B. 56	+	+	−	−

+ = precipitate. − = no precipitate. ± = slight precipitate.

(*b*) Two drops of an 18 hr. culture were inoculated into dextrose (0·5%) Lemco broth and treated as above. Tubes were examined after 24 and 48 hr. and after 3 days for growth, as shown by turbidity. Again clear-cut results were not obtained.

(*c*) 18 hr. cultures were heated in capillary tubes at 65° C. for 30 min. using as a medium, in different experiments, milk and broth. The contents of the tubes were transferred after 30 min. to 10 ml. of broth and incubated. Again anomalous results were obtained.

It appears that the heat-resistance test at 65° C. must be regarded as unsatisfactory, as *Str. faecalis* strains are inconsistent in their behaviour from day to day and from medium to medium, and it cannot therefore be regarded as a reliable differential method.

'*Limiting hydroxyl-ion concentration of growth*'

Sherman & Stark (1934), using lactose nutrient agar plates which had been adjusted to *p*H 9·6 immediately before use, found that *Str. faecalis* grew rapidly, but that *Str. lactis* was completely inhibited. No mention is made of the precautions necessary to maintain the *p*H at 9·6. Using nutrient lactose agar plates adjusted to *p*H 9·6 immediately before inoculating, incubating at 32° C. and examining after 48 hr. and after 4 days it was found that *Str. lactis* strains could not be clearly differentiated from *Str. faecalis* and group D strains. Moreover, it was found that an uninoculated control plate originally registering a *p*H of 9·6 had fallen to 8·4 over the period of incubation. Further experiments were carried out using dextrose (0·5%) Lemco broth adjusted to *p*H 9·6 immediately before use and checked by the glass electrode. The inoculated tubes were placed in an anaerobic jar with soda-lime and incubated at

30° C. By this method the results after 24 hr. were, in the main, clear cut and reproducible. *Str. lactis* strains failed to grow while *Str. faecalis* and group D strains grew well. In uninoculated control tubes the fall in *p*H over the period of incubation was consistently 0·4–0·45.

Inhibition of growth by sodium chloride

Using poured lactose nutrient agar plates to which 6·5% NaCl was added immediately before use, Sherman & Stark found that *Str. faecalis* strains grew vigorously while *Str. lactis* strains failed to grow. In trying to reproduce their results using the recommended medium it was found that some strains of *Str. lactis* succeeded in growing slightly and some *Str. faecalis* and group D strains were partially inhibited. Further tests were carried out using dextrose (0·5%) Lemco broth to which 6·5% NaCl was added. It was established that in the medium used by us, all the *Str. faecalis* and group D strains grew, though some only poorly. Some *Str. lactis* strains gave slight growth.

In all the 'tolerance tests' controls in dextrose Lemco broth (or in yeast-dextrose milk when used as the test medium) incubated at 30–32° C. were always included.

Of these four tests the 'thermal death-rates' test was found to be inconsistent by all methods tried. Growth in a liquid medium at *p*H 9·6 and growth at 45° C. gave clear-cut and reproducible results, and *Str. lactis* could readily be distinguished from *Str. faecalis* and group D streptococci by these two tests. In the presence of 6·5% NaCl all strains of *Str. faecalis* and group D grew, but in some cases growth was poor. As some strains of *Str. lactis* also gave some growth the test is obviously unreliable for routine purposes.

Table 3. *Summary of precipitin tests*

	Serum						
Extract	Group D 'Carter'	Group D (Stable-forth)	*Str. faecalis* 2703	*Str. faecalis* J.M.A.4	*Str. glycerinaceus* 2702	*Str. liquefaciens* 2705	*Str. lactis* O.J.
Group D	+ (11)	.	+ (7)	.	+ (4)	+ (4)	− (11)
Str. faecalis	− (1)*	− (3)*	.	.	.	.	.
	+ (19)	+ (17)	+ (21)	+ (4)	.	.	− (10)
Str. glycerinaceus	+ (1)	.	+ (1)	.	+ (1)	.	.
Str. liquefaciens	+ (4)	.	+ (1)	.	.	+ (4)	.
Str. lactis	− (21)	.	− (21)	− (4)	.	.	+ (23)

* The negative results were obtained with the ring test. Incubation of optimal proportions was not carried out.

Figures in brackets refer to the number of strains tested.

The above sera gave no precipitates with extracts of Lancefield groups A, B, C, E, F, G, H, K, L and M.

Reactions on blood agar

The importance of a standard technique for the observation of haemolysis was emphasized by Brown (1919), who studied various factors and conditions affecting the reaction of streptococci on poured horse-blood agar. Some confusion has arisen regarding the haemolytic activity of various strains of streptococci, and this may be due partly to the indiscriminate use by some workers of blood from animals other than the horse.

In observing haemolysis with deep colonies we compared results with both horse and cow blood. Brown's (1919) technique was followed throughout except that nutrient agar* was used as a basic medium instead of veal infusion agar. Duplicate plates were prepared with 5% defibrinated horse and cow blood. The inoculum was adjusted to give 100–200 colonies on incubation. *Str. faecalis* and the haemolytic group D strains were incubated at 37° C. and *Str. lactis* at 30° C. Reactions were recorded after 24 hr. and after 48 hr. incubation. Plates were then transferred to the ice chest and examined after a further 24 hr.

The haemolytic group D strains gave very different appearances on the two bloods. On horse blood zones of haemolysis varying in diameter from 3 to 7 mm. were produced, whereas on cow blood haemolysis was very much suppressed and in some cases (e.g. 'Heasman') was completely absent.

The *Str. faecalis* and *Str. lactis* strains, with the exceptions recorded in Table 4, gave similar if not identical appearances on the two bloods. These strains were either inert or produced narrow, and often

* Agar 2%, peptone 1%, lemco 1%, NaCl 0·5%; *p*H 7·0.

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Table 4

Strain	Horse blood	Cow blood
	Str. faecalis	
J.M.A.1	Incomplete β; zones 2–3 mm.	Incomplete β; zones 1 mm.
J.M.A.2	Slight greening	As with horse blood
J.M.A.3	Greening; zones 1 mm.	,,
J.M.A.4	Slight greening	No change
J.M.A.5	Greening; zones 1 mm.	As with horse blood
J.M.A.7	No change	,,
J.M.A.8	Slight greening	,,
E.B. 54	,,	,,
E.B. 56	,,	,,
E.B. 57	,,	,,
E.B. 96	,,	,,
2703	,,	,,
370	Greening; zones 1 mm.	,,
775	,, ,,	,,
2707	,, ,,	,,
Str. faecium O.J.	α (green zone 1 mm.)	α (green zone 2 mm.)
Sh. 3	α	As with horse blood
Clarke	Greening; zones 1 mm.	Greening; zones 2 mm.
	Group D	
Carter	β; zones 6–7 mm.	Incomplete β; zones 1 mm.
SHC 136	β; ,,	β; zones 2 mm.
SHC 159	β; zones 3 mm.	No haemolysis
SHC 167	β; zones 6–7 mm.	Incomplete β; zones 1 mm.
Heasman	β; ,,	No haemolysis
Mahoney	β; ,,	β; zones 2 mm.
Henry	β; ,,	β; ,,
Spence	β; ,,	Incomplete β; zones 1 mm.
Black	β; ,,	No haemolysis
Rundle	β; ,,	Incomplete β; zones 1 mm.
Magill	β; ,,	β; zones 2 mm.
	Str. bovis	
*Haines	Slight greening	Clear zone next to colony and hazy periphery of greening
*Goddard	,,	Clear zone next to colony and hazy periphery of greening
*Elene	Clear zone next to colony and hazy periphery of greening	As with horse blood
	Str. lactis	
O.J.	Slight greening	As with horse blood
H 195	No change	,,
H 199	Greening; zones 1 mm.	,,
H 201	No change	,,
H 203	,,	,,
H 207	,,	,,
H 222	,,	,,
H 240	,,	,,
H 257	Greening; zones 1 mm.	,,
H 287	No change	,,
H 288	Greening; zones 1 mm.	,,
H 290	No change	,,
H 299	,,	,,
Rice	Greening; zones 1 mm.	,,
M 3	α (very narrow zones)	Greening; zones 1 mm.
M 4	Greening; zones 1 mm.	α
M 6	No change	Trace of bleaching
M 11	Slight greening	No change
M 14	α (very narrow zones)	Greening; zones 1 mm.
M 15	No change	No change
M 16	,,	Slight greening
M 17	α (very narrow zones)	Greening; zones 1 mm.
M 18	Slight greening	As with horse blood

* Before refrigeration colonies showed zones of greening.

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hazy, zones of greening, or slight bleaching (Davis & Rogers, 1939) in which the blood corpuscles remained intact. The results are given in Table 4.

(*d*) *Serological differentiation of* Str. lactis *from* Str. faecalis *and group D streptococci*

Precipitating sera were prepared from three strains of *Str. lactis*, but only one strain, *Str. lactis* O.J. gave a good group serum. The other two strains gave type sera only. The serum *Str. lactis* O.J. gave strong reactions with all (23) strains of *Str. lactis* tested (Table 1) and was group specific in that no reactions were obtained with representative members of Lancefield's groups A–M (with the exception of a slight cross with group F after standing in the ice chest for 18 hr.). This serum gave no precipitates with ten strains of *Str. faecalis* or with eleven strains of group D (Table 1). The sera for *Str. faecalis* 2703 and for group D 'Carter' gave no precipitates with twelve strains of *Str. lactis* either as ring tests or after incubation of a series of dilutions. Further, nine freshly isolated strains of *Str. lactis* tested against *Str. faecalis* and group D sera (ring test) gave negative results (Table 1). *Str. faecalis* J.M.A.4 serum was tested against only four strains of *Str. lactis* and these results were also negative (Table 1).

From the evidence presented here it is clear that *Str. lactis* is serologically distinct from *Str. faecalis* and does not fall into any of the present Lancefield groups.

The place of that important organism known as *Str. cremoris* may be mentioned here. Recent tests with fourteen strains of streptococci having the biochemical reactions usually associated with *Str. cremoris* have confirmed our statement made earlier (*Report*, 1937) that *Str. lactis* and *Str. cremoris* fall into the same group. The further relationship of *Str. cremoris* to *Str. lactis* will be dealt with in a later paper.

DISCUSSION

The comparisons of the cultural and biochemical characters of *Str. faecalis* and group D strains show that apart from the action on blood there is no salient difference between them, and precipitin tests have shown that these organisms fall into the same group. These observations accord with those of Graham & Bartley (1939). These authors maintain that because the varieties or types which they describe fall into the same serological group they should all be included under the one name *Enterococcus* (*Str. faecalis*), and deprecate the use of the name 'Group D haemolytic *Streptococcus*' as liable to cause confusion. We do not altogether agree with this opinion, since, as we shall show in this discussion, organisms with well-marked differential characters are included in group D. There is, so far as we know, no reason for objecting to names for well-defined species which happen to fall into the same serological group. There is, on the other hand, no reason for excluding non-haemolytic organisms of the same serological group from group D, since lack of the property of haemolysing blood is in itself insufficient reason for excluding an organism from a group.

In this connexion the organisms under the names *Str. glycerinaceus* and *Str. liquefaciens* must be considered. In respect of the former, our own results show that it differs from *Str. faecalis* only in fermenting glycerol strongly. This substance, it is to be noted, is also fermented by many strains of *Str. faecalis*, and there is therefore no adequate reason for according specific rank to this organism.

Str. liquefaciens is not rare. It occurs frequently, for example, in cheese (and other dairy products), and by some is considered to contribute something to the ripening process. Its chief characters of interest for the purpose of this discussion are that it is strongly proteolytic and that it is non-haemolytic. Four such strains have been noted in Table 1. As has been pointed out gelatin liquefying strains of the *Enterococcus* have been described in the literature and, as for the moment there is nothing to distinguish these from *Str. liquefaciens*, it seems that this organism would be adequately described as a variety: *Str. faecalis* var. *liquefaciens*.

Str. durans (Sherman & Wing, 1937) is another streptococcus with clearly defined characters. It is of practical interest because it often occurs in spray dried milk powder which, to our knowledge, has been condemned as unfit for human consumption on this account. This organism grows well in the region of 50° C., is haemolytic, does not ferment mannitol or several of the usual sugars, and does not liquefy gelatin. We have no precise observations to record at present, but it was felt that attention should be drawn to this organism, particularly as it very probably belongs to group D (Sherman, 1938). It is possible that the non-mannitol-fermenting bovine strain referred to by Graham & Bartley (1939) as *haemolyticus* may in fact be *Str. durans*.

Amongst our strains in Table 1 are four, SHC 159, SHC 167, 'Black' and 'Magill', which appear to conform to the description of *Str. zymogenes* (MacCallum & Hastings, 1899). All the organisms in this table are haemolytic and mannitol fermenting and fall serologically into group D. Both proteolytic and non-proteolytic strains appear, but they are all quite distinct from *Str. durans*. The seven haemolytic, non-proteolytic strains seem to be identical with the mannitol-fermenting, *haemolyticus* variety of Graham & Bartley which differs from *Str. faecalis* only in its haemolytic properties. Our own feeling is that these non-proteolytic varieties should be included under the name *Str. faecalis* var. *zymogenes*.

It is convenient at this point to deal with *Str. bovis*. Three of the strains acquired in the course of collecting *Str. faecalis* strains which were isolated from human faeces, appeared to be of the *Str. bovis* type. These organisms grew at 45° C., fermented raffinose but not mannitol, and did not grow at *p*H 9·6 or in 6·5 % salt. Two of the strains reacted with both *Str. faecalis* and group D sera, and the third gave partial reactions. At present we feel that discussion of the place of this organism which, on biochemical grounds, seems to be distinguishable from *Str. faecalis*, should await the preparation of a suitable specific serum.

We have already pointed out the striking similarity of the reactions of *Str. lactis* and *Str. faecalis*. Nevertheless, it seems that the evidence given in this paper shows that those differences which have been established by means of the tests we have used are sufficient to warrant the acceptance of the specific name *Str. lactis*. Thus it has been shown that *Str. lactis* consistently fails to grow in broth at 45° C., and does not grow in broth at *p*H 9·6, nor survives heating at 60° C. for 30 min. under the conditions of our experiments which have been defined. This last result does not accord with the observations of Sherman, who reported (1937) that *Str. lactis* usually survived 60° C. for 30 min. We have found, however, the results to be consistently negative. It will be evident on comparing the results of these same tests with *Str. faecalis* that the differentiation is clear cut. In respect of the tolerance to 6·5 % NaCl and to heating at 65° C. for 30 min. as proposed by Sherman & Stark (1934) our results have been conflicting and not reproducible. In our opinion therefore these tests cannot usefully be used to differentiate the two organisms under discussion. From the serological point of view it is also quite clear that *Str. lactis* and *Str. faecalis* are indeed different organisms, since no extracts of *Str. lactis* reacted with a *Str. faecalis* or group D serum, and no extracts of *Str. faecalis* or group D organisms reacted with a *Str. lactis* serum. As, moreover, *Str. lactis* sera do not react with extracts of any of the known Lancefield groups (A–M) it constitutes one of a new roup of streptococci which should be designated group N. *Str. cremoris* also falls into this group. Seelemann & Nottbohm (1940) have also established the serological identity

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of *Str. lactis* and have proposed the letter L. But L and M have already been annexed to groups of streptococci isolated mainly from dogs and described by Fry (1938) before the Royal Society of Medicine, and by Fry & Hare (1939) before the Pathological Society of Great Britain and Ireland. That these groups are accepted is evident from the Harvey Lecture of Lancefield (1940–1). As we appear to have been the first to prepare a group serum for *Str. lactis* (*Report*, 1937) it seems that we might claim to attach the group letter N. An objection which might have been urged against this course—namely, the lack of haemolytic power of these organisms—is, in the light of recent knowledge, no longer valid in that non-haemolytic varieties have been described in other Lancefield groups, e.g. group A, Colebrook, Elliot, Maxted, Morley & Mortell (1942); group B, Stableforth (1937); group C, Little (1939); group D, Graham & Bartley (1939).

The conclusions reached in this paper may be summarized thus:

Group D:	*Str. faecalis*,	? *Str. durans*
	Str. faecalis var. *zymogenes*	? *Str. bovis*
	Str. faecalis var. *liquefaciens*	
Group N:	*Str. lactis*	*Str. cremoris**

* Previous workers have given this organism specific rank. It certainly falls into group N, but its specificity is not yet clearly established.

SUMMARY

1. It has been shown on serological and biochemical grounds that *Str. faecalis* falls into Lancefield's group D. *Str. liquefaciens* is considered to be a variety of *Str. faecalis*, and *Str. glycerinaceus* (Orla Jensen) has insufficient claim to a separate name.

2. *Str. lactis* has, on biochemical and serological grounds, been clearly differentiated from *Str. faecalis* and the other members of group D.

3. Reasons are given for assigning *Str. lactis* to a new serological group 'N' into which *Str. cremoris* also falls.

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