THE BACTERIOLOGY OF BOVINE STREPTOCOCCUS MASTITIS

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In the May 1935 issue of this *Journal* there appeared an article by Gibson and Muir entitled "A study of the streptococci from fifty cases of bovine mastitis". Since the authors of that article do not appear to have fully appreciated the previous work on the subject, it has become necessary to state the position in the light of research carried out in various countries during the past few years.

It has long been known that mastitis in cattle may be caused by bacteria of different genera, while recognition of the fact that streptococci are principally concerned may be said to date from the publications of Nocard and Mollereau (1885, 1887). Since then the subject has been investigated by very many workers and the general conclusion has been reached that the most prevalent form of mastitis in milking cows, *i.e.* the chronic and contagious form, is caused by a group of streptococci to which the organism of Nocard and Mollereau belongs. At different times attempts have been made to define more clearly the characters of the group, one of the objects being to devise methods suitable for diagnosis. It is not proposed here to give a survey of the early literature, as this has been done elsewhere, *e.g.* Klimmer and Haupt (1930), Seelemann (1932), but reference should be made to a few outstanding points. In the early days reliance was placed on simple cultural peculiarities and fermentation tests. Thus, Savage (1906–7, 1907–8) isolated a large number of strains of streptococci from clinical cases of mastitis. Although on the whole the reactions in test media were somewhat variable, a type could be distinguished which possessed certain characters in common. The similarity of this organism to that of Nocard and Mollereau was recognised. Mejlbo (1924) examined the sugar fermentation reactions of 101 strains of streptococci isolated from milk samples from cows which had or were suspected of having mastitis. The strains were arranged in eight groups on the basis of fermentation results with twenty-eight test substances. An examination of Mejlbo's results, however, shows that seventy-one of his strains fermented lactose and sucrose and failed to ferment mannite, inulin or raffinose and in these respects belonged to a common group similar to that described by Savage and others. With the use of media containing blood a further advance was made Thus, Gminder (1912) in Germany used surface cultivation on blood agar and found that strains from four out of twenty-six samples of mastitis secretion gave haemolytic colonies, the rest

being of the *viridans* type. F. S. Jones (1918) in the United States used poured horse-blood agar plates and found that twenty-nine out of sixty-eight strains were beta-haemolytic. Klimmer, Haupt and Roots (1928) in Germany found mainly alpha-haemolysis and no true beta-haemolysis among 180 strains of mastitis streptococci. Diernhofer (1932*b*) in Austria found twenty-eight beta-haemolytic strains among 146 examined in ox-blood agar. S. J. Edwards (1934) records that in one series of 619 strains 144 gave non-haemolytic and 475 beta-haemolytic colonies in ox-blood agar.

As the outcome of their work on mastitis streptococci, Minett, Stableforth and Edwards (1929) and Edwards (1932) created four subdivisions or groups based on the physiological characters of the organism. Data relevant to these organisms and to *Str. lactis* are summarised in Table I.

Failure to reduce methylene blue in milk is regarded as an important characteristic of Group I. According to Klimmer and Haupt (1930) the proper designation for this group should be *Str. agalactiae* (Kitt, 1893). Strains of Group I may be beta-haemolytic or non-haemolytic. It is important, therefore, to observe that haemolysis among mastitis streptococci is not a differential character of importance, contrary to the assumption of Gibson and Muir. Reduction of methylene blue milk associated with failure to ferment salicin, mannite, inulin and aesculin, and with low production of acid in glucose broth are regarded as important features of Group II. The production of acid detectable by litmus in milk at $10°$ C. is regarded as an important character of Group III; reduction of methylene blue milk in association with fermentation of salicin, mannite and inulin are also significant characters. Streptococci of this group resemble *Str. lactis* in some respects, but the failure to grow in an aesculin medium containing bile salt, reduction of janus green and resistance to heat at $60°$ C. are important differential characters. Many strains of *Str. lactis* also reduce ammonium molybdate, which is unchanged by mastitis streptococci. Udder strains of the *Str. pyogenes* group ferment sorbitol and not trehalose, whereas strains of this group of human origin ferment trehalose but no sorbitol.

In the literature on *Str. agalactiae* there is much difference of opinion regarding the haemolytic properties, largely ascribable to differences in technique and to the failure of many authors to define their working conditions. The subject has been fully dealt with recently by Minett (1934). Soluble lysin production in 15 per cent. horse serum broth occurs frequently with beta-haemolytic Group I strains although it is low in comparison with that of the *Str. pyogenes* group. With Group I strains classified as "non-haemolytic", when the deep colonies in ox-blood agar number 300 or less, the zones about the colonies are unaltered or may be slightly green after 48 hours' incubation and there is no change on refrigeration. Surface colonies on heated blood agar for the most part show slightly green zones. With Group II cultures, well-isolated colonies are usually surrounded by a green zone and refrigeration may fail to produce any haemolysis. On more crowded plates the colonies are distinctly alpha-haemolytic, the clear zone being well marked and its width increased

Table I

Mastitis group	Haemolysis on ox-blood agar (deep colonies)	Growth in broth	Litmus milk 37°C	Litmus milk 10°C	Milk + methylene blue to 1:20,000 37°C	Final pH in 1% lactose broth	Fermentation Salicin	Mannite	Inulin	Aesculin	Sod. hip.	Janus green	Heat resistance 60°C 30 min.	Serological* characters	[pathogenicity]	Natural disease in cattle
I (Str. agalactiae)	a) non-haemolytic b) beta-haemolytic (narrow zone)	Broth clear or sl. turb., floc. dep.	AC sl. R	–	–	4·7 to 5·0	+ (usually)	–	–	–	+	–	–	Types a, b, c		Chronic (contagious)
II	Non-haemolytic	Fine dep., sl. turb.	A or A soft C part. R	–	R or R soft C	5·0 to 5·2	–	–	–	!	–	–	–	Heterogeneous but no type common to Groups I or III	rate	Acute or subacute (sporadic)
III	Non-haemolytic	Sl. diffuse, turb.	A or A soft C part. R	Sl. A	R or RC	4·7 to 4·9	+	+	+	+	+	–	–	Heterogeneous but no type common to Groups I or II		Acute or subacute (sporadic)
Str. pyogenes (udder group)	Beta-haemolytic (wide zone)	Sl. turb., floc. dep.	A or A soft C sl. R	–	–	5·0 to 5·2	+	–	–	–	–	–	!	Distinct from Str. pyogenes of human origin	,	Acute (sporadic)
Str. lactis	Non-haemolytic	Diff., turb.	ACR (rapid)	R	RC (rapid)	4·4 to 4·8	+	+ (usually)	–	+	+ or –	+	+	—		Very rare

A = clot, R = reduction.

The lytic characters refer to plates containing not more than 300 colonies.

With ps II and III the changes in litmus milk are inconstant and depend to some extent upon the number of organisms sown.

Obse ns cover a 5-day period.

Fern on medium was 1 per cent. peptone water, enriched with one-tenth of its volume of infusion broth and horse serum to a conc

Test sul s in concentration of 1 per cent. added to the sterile medium. Except with Str. lactis, the aesculin medium contained no on of 1 per cent.

* *Vide* Stableforth, A. W. (1932). Since 1932 types other than a, b and c have been defined in Group I.

by refrigeration. In the case of Group III cultures colonies are non-haemolytic, both in congested plates and in plates where they are well isolated.

The validity of the above groupings is substantiated in the first place by the results of serological analysis (Stableforth, 1932), and in the second place on clinical and epizoötiological grounds.

Stableforth has shown that the biochemical groupings are significant in that there is no antigenic overlapping between groups. Within each biochemical group there is more than one serological type, *e.g.* in Group I, three types have been reported and further types or subtypes will be described in a future article.

As previously mentioned, Group I streptococci cause the well-known chronic and contagious mastitis of milking cows, and owing to the wide distribution of this form in nature this group has received most attention. The clinical symptoms are similar whether the organisms are beta-haemolytic or not and the pathological changes consist of a gradually extending fibrosis which slowly destroys the milk-secreting tissue. Group II and *Str. pyogenes* cause acute or subacute disease in which contagion plays no obvious part and in which the organisms show no great tendency to persist for long periods in the milk. The reaction of the udder tissue in these cases is apparently sufficient to prevent an enduring carrier state. The reality of Group II infections and their independence of the contagious form due to Group I organisms was proved in animals belonging to four herds by Minett, Stableforth and Edwards (1932). Many of the cases in these herds were so severe that the udder quarter involved was speedily destroyed, and in one case the udder was proved to be infected prior to the first calving. From one of the four herds—a self-contained one— Group I infections were eventually eliminated, as was proved by the failure to discover Group I streptococci by cultural examination of the milk of individual cows every few months for a space of about $3\frac{1}{2}$ years (Minett, Stableforth and Edwards, 1933). The absence of Group I infections, however, did not bring about the disappearance from this herd of streptococcus mastitis; cases occurred from time to time, but these were due to either Group II or Group III streptococci. Group III streptococci are a less important cause of disease, but the group is not less valid on this account. The occurrence in natural disease of streptococci with characters similar to those of Groups II and III has also been proved by Diernhofer (1930, 1932a) and he found the locally isolated strains to be identical with representative cultures of these two groups sent from this Institute. Bendixon (1933) in Denmark among 120 streptococcus strains isolated from cases of mastitis found that 103 belonged to Group I while, of the remaining seventeen, seven were considered to belong to Group III. The pathogenicity of Group II and Group III strains for the cow's udder has also been established experimentally (Diernhofer, 1930; Edwards, 1932). Mastitis streptococci, to which the name *Str. pyogenes* may be given, have now been recognised in a number of different countries. In their haemolytic characters and in certain other respects they resemble *Str. pyogenes* of human

origin, but, as stated, they can be differentiated by the sorbitol-trehalose test (P. R. Edwards, 1932, 1933; Plummer, 1934; Minett, 1935) and also by group precipitin tests (Lancefield, 1933; P. R. Edwards, 1934). Finally, at the time of milk-borne epidemics it has occasionally been proved that mastitis in cows is caused by true *Str. pyogenes* of human origin.

Consultation of the original literature cited would show the considerable body of evidence which goes to support the position as set out above. In the work of Gibson and Muir the streptococci were classified according to Holman's system with the result that no two strains were found to be identical in all respects. The technique was cultural and biochemical—not serological—and a number of strains from human and animal sources were tested for comparison. Before the authors' conclusion as to the diversity of strains can be accepted a number of considerations arise. In the first place, Gibson and Muir make the fundamental error of regarding bovine mastitis as "a single clinical condition". This is as incorrect as it would be to regard enteritis, meningitis or encephalitis in a similar light. Moreover, as stated above, even if attention is confined to the streptococcus form of the disease, it would be quite wrong both on clinical and on epizoötiological grounds to speak of it as a single condition. A similar error has been the cause of great and pardonable confusion in the past and it is indeed the key to the situation now being considered. It would have been helpful if some particulars had been available regarding the clinical characters of the cases from which their strains were derived, but this was not possible since the cultures were obtained after death from the purulent milk of udders. It should also be observed that this method of collecting material would tend to reduce the proportion of *Str. agalactiae* strains, since udders containing grossly altered secretion were selected for examination and *Str. agalactiae* often produces mild and chronic infection only. In the second place, with regard to the results of the bacteriological tests used, the characters of the individual strains are not given, and it is, therefore, impossible in all cases to form an independent opinion as to how strains might have been distributed among the *Str. agalactiae* or other mastitis groups. It may be mentioned, however, that three of the strains fermented aesculin, even in the presence of bile salt, thirteen reduced methylene blue in milk at a concentration of 1 in 20,000 and nine of those belonging to the alpha and gamma series and described as *Str. faecalis* fermented mannite. These characters are definitely not those of *Str. agalactiae*, but no attempt was made to place such strains in groups corresponding to those of previous workers on the subject. Some of the results obtained are peculiar. For example, representative strains of *Str. agalactiae* forwarded from this Institute are arranged as α, α′, β and γ types, according to their action in ox-blood agar. The type of haemolysis in this medium and the production of lysin in serum broth were checked by Dr Stableforth before the cultures were despatched and none was of the gamma type; moreover, a recent examination has shown no change in haemolytic characters. Further, the failure

to ferment trehalose by eighteen haemolytic strains out of twenty-three of human origin is extraordinary in view of the conclusions of other workers. It is not possible to assess the meaning of the results with litmus milk, since changes in this medium are liable to vary according to the size of the inoculum, the amount of milk and the calibre of the tubes used (S. J. Edwards, 1932).

Gibson and Muir state that there is much greater diversity among organisms of the group "*Streptococcus mastitidis*" than in the group *Str. haemolyticus* of human scarlatina, but in view of the above-mentioned characters of mastitis streptococci this observation loses its point. At the same time, it would not be denied that within the *Str. agalactiae* group itself fine physiological differences may exist, but there is no evidence that such differences indicate fundamental biological variation; they cannot be correlated with serological or clinical dissimilarity. On the other hand, serological differences among streptococci of the same biochemical group can be related to differences in clinical type of disease; this has been shown to be the case with *Str. pyogenes* infections in man, and there is evidence to be published later indicating that the same is true for the *Str. agalactiae* group. These facts, of course, do not imply that biochemical methods in the gross are not of value; in the case of mastitis their value has already been shown as a means of controlling or eliminating streptococcus infections in herds of cows.

So much has to be said regarding the main part of the article under discussion. The opportunity must also be taken to correct a serious misrepresentation by the authors in their short summary of the literature on the subject. Thus, Diernhofer (1930) is said to have expressed the opinion that cases of mastitis due to *Str. agalactiae* are comparatively rare. Actually, Diernhofer examined 284 strains of streptococci isolated from the milk of living cows with mastitis and all these cultures were similar to one another and possessed the characters of *Str. agalactiae*. A small number of strains with other characters were met with and Diernhofer's view of the matter is expressed as follows: "Es gibt ausser dem gelben Galt[1] noch andere Streptokokkenmastitiden, die allerdings viel seltener sind." Quotation is made from a review article by Minett (1932) but several lines (dealing, it should be noted, with Groups II and III streptococci) are omitted after the words "Other strains gave haemolysis of the type alpha or alpha prime" (p. 238), so giving a totally false impression of the work of S. J. Edwards (1932), reference to which follows. The reference given as "Minett and co-workers (1930)" should read "Minett and Stableforth (1931)", while the few lines which follow do not express the position accurately. On p. 251 the work of P. R. Edwards concerning the value of the sorbitol-trehalose test for differentiation of streptococci is misrepresented. This author in his publication (1932) is particularly careful to point out that this test should be used for actively haemolytic hippurate-negative strepto-

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[1] The chronic catarrhal form of mastitis spreading by contagion was first referred to as "gelber Galt" by Swiss authors. It implies a condition in which the milk secretion is changed to a yellowish fluid and is greatly reduced in quantity.

cocci only and is not applicable to other groups of streptococci. Gibson and Muir used the test without such discrimination and their conclusion that the test is not of value for differentiation is, therefore, fallacious.

Gibson and Muir set out to investigate mastitis streptococci "by the methods of medical bacteriology", the object being "to throw some light on the difficult problems of classification within this bacterial group". As a reason for the inception of their work, it is stated that "previous workers on the bacteriology of bovine streptococcal mastitis have classified the strains isolated by methods unfamiliar to medical workers. The latter, accustomed to a sharp differentiation into alpha, beta and gamma according to the action upon blood, have been confused by a classification which ignored this criterion". This reason cannot be accepted as adequate. Solid media containing blood to enable a distinction to be drawn between alpha, beta and gamma types have been made not only by ourselves, but also by veterinary workers in other countries, *e.g.* the United States, Germany, Austria and Denmark. When the results of their work are considered, it must be said with regret that Gibson and Muir have failed to achieve their object and that their publication is calculated to introduce confusion into a subject which was becoming clear.

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