FURTHER STUDIES ON THE DIFFERENTIATION OF HUMAN AND ANIMAL STRAINS OF HEMOLYTIC STREPTOCOCCI[1]

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Received for publication September 2, 1932

The differentiation of hemolytic streptococci of human and animal origin is obviously a subject of great practical importance in the field of public health and hygiene. This problem has occupied the time of a number of workers and has been the subject of many publications. Since hemolytic streptococci have been found so generally in milk and milk products, most of these investigations were efforts to distinguish between human and bovine strains. While our knowledge concerning bovine strains of hemolytic streptococci has advanced rapidly, as a result, study of the streptococci from other species of animals has been neglected. No method has as yet been discovered by which it is possible to effect a final separation of human and animal strains.

Ayers (1916), Ayers, Johnson and Davis (1918), and Avery and Cullen (1919) demonstrated that many hemolytic streptococci of bovine origin could be distinguished from human strains by the final hydrogen-ion concentration produced in glucose broth. Ayers and Rupp (1922) found that the members of this group of bovine streptococci were able to hydrolyze sodium hippurate, while human strains did not attack this substance. Avery (1929) further showed that the high-acid-producing strains of bovine origin could be distinguished from human strains by the reduction of methylene blue by the former. Brown (1920) studied a

[1] The investigation reported in this paper was made in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

series of hemolytic cultures from bovine sources and found that the majority could be distinguished from human strains by final acidity in glucose broth, action on blood plates, and hemolysis in a fluid medium. Jones (1920) in the study of hemolytic streptococci from milk observed two groups, one a high-acid-producing type identical with mastitis streptococci, the other a low-acid-producing type more or less similar to human strains. The low-acid-producing type consistently failed to ferment salicin.

Brown, Frost and Shaw (1926) studied a large number of cultures isolated from certified milk, septic sore throat, and various pathological conditions in human beings. These cultures were examined for capsule production, hemolysis in fluid media, pathogenicity for mice, hydrolysis of sodium hippurate, and fermentative characters. The authors concluded that it was possible to distinguish human strains from bovine strains by these tests. The cultures were divided into nine groups on the basis of their reactions. One group was hemolytic in a fluid medium, failed to hydrolyze sodium hippurate, was pathogenic for mice, did not produce an acidity exceeding pH 4.8 in glucose broth, and generally produced acid from lactose, sucrose and salicin, but not from mannitol. These cultures were considered to be of human origin. This group was composed of cultures from infections in man, from cows thought to be responsible for epidemics of septic sore throat, and in a few instances from cases of mastitis in cows that had no connection with human disease. None of these cultures were from certified milk. The strains from certified milk all differed in one or more particulars from the human strains, and were considered to be of bovine origin.

The papers cited above indicate that bovine and human strains can in most instances be distinguished from each other with a fair degree of accuracy. However, certain strains of hemolytic streptococci are found in cows and other species of animals that are indistinguishable from human cultures by the criteria that have been used. Seeleman and Hadenfeldt (1930) studied certain strains of hemolytic streptococci from cases of mastitis and came to the conclusion that *Str. pyogenes* from cows is probably identical with *Str. pyogenes* of human origin. Hergesell (1931), in

studying the cultures described by Seeleman and Hadenfeldt, also came to the conclusion that they were identical with streptococci of human origin. Smith (1929) studied cultures from cows, horses and guinea pigs. These were largely low-acid-producing strains which failed to hydrolyze sodium hippurate. They were markedly hemolytic in fluid media and were pathogenic for guinea pigs. A detailed study of the cultural, biochemical, and serological characters of these strains failed to reveal any method whereby they could be distinguished from human cultures.

In an earlier paper (Edwards, 1930) attention was called to the fact that there was a large group of animal strains which could not be distinguished from human cultures by the methods that had been used previously. These strains were found to be present in cows and in other species of domestic animals. They produced a low acidity in glucose broth, failed to hydrolyze sodium hippurate, and were actively hemolytic in fluid media, thus possessing the characters ordinarily attributed to human strains. A large majority of these cultures could be differentiated from human streptococci by their action on sorbitol and trehalose. The human strains produced acid from trehalose, but failed to ferment sorbitol. The majority of the animal strains fermented sorbitol, but did not attack trehalose. In addition the animal strains as a rule produced a slightly higher acidity in glucose broth than did the human strains. The present paper is a continuation of this study, in which a larger number of strains is included and in which other morphological and biochemical characters are considered.

MATERIAL AND METHODS

The material to be reported here is based upon a study of 173 strains of animal, and 75 of human, origin. The sources of the strains are given in table 1. They were all actively hemolytic, low-acid-producing streptococci which failed to hydrolyze sodium hippurate. They all belonged to the group which has long been designated as *Str. pyogenes*.

The following characters are reported in the present paper: Acid-production from glucose, reduction of methylene blue, pres-

ence of capsules, and fermentative reactions. While a large number of fermentable substances have been tested, only those which are usually employed in the study of streptococci, and others that are of value in differentiation, are included.

TABLE 1

Source of cultures

Animal strains:	
Equine, os uteri	100
Equine, aborted fetuses	6
Equine, septicemia and arthritis	12
Equine, rhinitis	11
Equine, pneumonia	3
Equine, wound infection	1
Equine, *Str. equi* from Strangles	8
Bovine, septicemia	3
Bovine, placenta	1
Bovine, milk	2
Swine, septicemia	2
Swine, abortion	5
Swine, arthritis	1
Chicken, femur	11
Fox, pneumonia	2
Guinea pig, adenitis	4
Rabbit, septicemia	1
Human strains:	
Throat cultures, sore throat and scarlet fever suspects	22
Scarlet fever, stock cultures	22
Broncho pneumonia	9
Erysipelas	9
Mastoiditis	6
Puerperal fever	3
Meningitis	1
Peritonitis	1
Pleuritis	1
Smallpox	1

The methods used in testing for hemolysis, hydrolysis of sodium hippurate, final acidity in glucose broth, and fermentation of sugars have been previously described (Edwards, 1930). The presence of capsules was determined in moist india ink preparations of cultures grown in meat-infusion broth containing 20 per cent ascitic fluid.

In testing for the reduction of methylene blue the following medium was used:

Beef infusion	950 cc.
Casein digest	50 cc.
Bacto peptone	10 grams

The reaction was adjusted to pH 8.0 before sterilization. After eighteen hours incubation at 37.5°C., 0.1 cc. amounts of the cultures were used to inoculate tubes of the same broth to which sufficient sterile aqueous solution of methylene blue had been

TABLE 2

	NUMBER OF CULTURES	pH IN GLUCOSE BROTH	HYDROLYSIS OF SODIUM HIPPURATE	HEMOLYSIS	REDUCTION OF METHYLENE BLUE	CAPSULES	SALICIN	MANNITOL	LACTOSE	SORBITOL	TREHALOSE
Human	75	5.8	−	+	72− 3+	65− 10+	+	70− 5+	71+ 4−	−	+
Animal:											
Type A	159	5.2	−	+	−	+	+	−	+	+	−
Type B1	5	5.0	−	+	+	−	+	−	−	−	+
Type B2	1	5.0	−	+	+	−	+	−	+	−	+
Str. equi	8	5.0	−	+	−	+	+	−	−	−	−

added to give a final dye concentration of 0.000025 molar. The inoculated tubes containing the methylene blue were incubated at 37.5°C. for seventy-two hours, and observed at intervals of twenty-four hours for evidence of reduction. Cultures were recorded as positive which caused a complete decolorization of the medium except for a faint ring of blue at the surface of the broth.

RESULTS

The results of the tests are given in table 2. The animal streptococci were divided into four groups, on the basis of their reactions. The designations given these types by Ogura (1929)

in his work on equine streptococci were retained, since the same groups were observed among streptococci from various species of animals that Ogura found in horses. The first and largest group (type A) was composed of the strains which fermented sorbitol and failed to produce acid from trehalose. Type B was made up of cultures which, like the human strains, fermented trehalose but did not attack sorbitol. Type B was further divided into two types, B1, which did not produce acid from lactose, and B2, which fermented this sugar. The fourth type or group is designated as *Streptococcus equi*.

There was a difference in the final hydrogen ion concentration produced by the human and animal strains in glucose broth. The figures given in the table record the averages for the four different types. While there was a distinct difference in the acidity produced by the human and animal strains, this character could not be relied upon for differentiation, since there was considerable overlapping of the individual strains of different groups.

Str. equi attacked neither lactose, sorbitol nor trehalose. The number of strains (8) studied here is small. Holth (quoted by Jensen, 1911), Adsersen (1915), and Ogura (1929), together, examined more than 250 cultures of *Str. equi* and found them absolutely constant in their biochemical characters. Of the animal strains other than *Str. equi* studied here, 159, or 96 per cent (type A), fermented sorbitol, but did not attack trehalose. All of the human strains, on the contrary, produced acid from trehalose, but failed to ferment sorbitol. Six of the animal strains (types B1 and B2) gave the same reactions in sorbitol and trehalose as the human. One of the six produced acid from lactose, while the remaining 5 strains failed to attack this substance.

Since the Type B (1 and 2) animal strains possessed the same fermentative characters as the human, other means were sought to separate them. The method that gave the most promise was the methylene blue reduction test. All of the Type B animal strains reduced this dye under the conditions of the experiments, while only 3, or 4 per cent, of the human strains acted upon it. None of the sorbitol-fermenting Type A cultures reduced the dye, nor did *Str. equi* affect it.

Among the animal strains, Type A and *Str. equi* regularly produced capsules, while Types B1 and B2 did not. Certain of the Type B strains were subjected to serial passage through animals, but even after several passages there was no evidence of capsule formation. Capsules were observed on a few of the human cultures. No special effort was made to induce capsule formation in this group. While capsule production served to distinguish the Type A animal strains and *Str. equi* from the Type B animal strains, it did not serve to set them apart sharply from those which were of human origin.

DISCUSSION

Of the 173 animal strains, 96 per cent could be distinguished from the human strains by their fermentative reactions. The cultures of the group of animal streptococci designated here as Type A were absolutely constant in the characters studied. While the human strains varied somewhat in their reactions, particularly in the fermentation of lactose and mannitol, the sorbitol-fermenting cultures of Type A were quite consistent in these respects. No Type A cultures were found which fermented mannitol, and all of them produced acid from lactose.

The action of the low-acid-producing, hemolytic streptococci on sorbitol and trehalose was extremely interesting. A total of 315 cultures of this group was examined. With the exception of *Str. equi*, which fermented neither of these substances, the cultures all acted upon one or the other, but no strain fermented both.

The failure of *Str. equi* to ferment either sorbitol or trehalose, as well as its lack of ability to produce acid from lactose, established it as a definite bacteriological entity. Certain non-lactose-fermenting strains found in human infections have been designated by various writers as *Str. equi*. The problem of the differentiation of this species has been complicated further by the failure of various workers to recognize other streptococci, which are associated with *Str. equi*, in the lesions of strangles. This has caused some confusion; however, when the characters re-

ported here are used in differentiations, *Str. equi* stands out as a definite species, and should be so regarded.

While it was not possible to separate absolutely the Type B animal strains from those of human origin, the methylene blue reduction test very nearly served to differentiate them completely. All of the animal strains reduced the dye under the conditions of the experiments, while only 3 human strains gave this reaction. These tests were repeated several times and the results found to be constant. The dye reduction test was, of course, a quantitative one, a 0.000025 molar concentration of the dye being used in the differentiation. When as much as 0.00001 molar solution was employed all of the cultures caused visible reduction. When, on the other hand, a concentration of 0.00005 molar was used none of the strains reduced it completely. The medium used in these tests favored a luxuriant growth of the various organisms.

While the question of identity or exact relationship of the human to the Type B (1 and 2) strains may still remain in some doubt, it is quite probable that they are distinct and that the Type B strains are not in reality strains of human streptococci that have been transferred to animals. The Type B strains in general were of low virulence, and were found sometimes in tissues and organs that showed no pathologic changes. They were found associated with organisms of Type A or *Str. equi* in pathologic conditions in horses. It is probable that these cultures are usually saprophytic.

Another point of importance was the constant presence of capsules on the Type A animal strains. These capsules were large, prominent and easily demonstrated. As has already been stated, this group of animal strains is indistinguishable from human streptococci by the methods in general use. The Type A strains were indistinguishable from *Str. epidemicus* as characterized by Brown, Frost and Shaw (1926) and Pilot, Hallman and Davis (1930). The Type A animal strains not only formed capsules, but under appropriate conditions of culture formed large, spreading, mucoid colonies. This character usually has been considered peculiar to *Str. epidemicus*. While it was not intended to enter into a detailed discussion of the characters of

Str. epidemicus at this time, the writer is of the opinion that these animal strains are not the organism of septic sore throat. However, since we have received a number of cultures labelled *Str. epidemicus*, which were identical with the Type A animal strains, it is evident that they are often mistaken for this organism.

Opinions concerning capsule formation by the hemolytic streptococci may be greatly changed in the future. Brown and Kindwall (1928) demonstrated that cultures of non-encapsulated streptococci acquire capsules under appropriate treatment. Tunnicliff (1931) observed that the smooth forms of scarlet fever and erysipelas streptococci are encapsulated. Capsule production, or ability to form capsules under proper conditions, may be a much more common property of the hemolytic streptococci than has been generally supposed.

The question of infection of man with animal streptococci and the infection of animals with human streptococci is of great importance. The writer's opinion, based on the examination of more than 300 strains, is that the sorbitol-fermenting streptococci, which compose 95 per cent of the animal strains studied here, rarely, if ever, infect man. However, it has been demonstrated that human streptococci may at times infect animals. Jones and Little (1928) have demonstrated that cows may be infected with scarlet fever streptococci and transmit the disease to consumers of the milk. Furthermore, *Str. epidemicus*, which has long been considered as being of human origin, is known to infect cows and cause a severe mastitis (Brown and Orcutt, 1920). We have examined several strains from cows that were thought to be responsible for human epidemics and have found them to be of the human type. Infection of animals with human streptococci presents a situation which is the reverse of the condition prevailing in the Salmonella group where animal paratyphoids frequently infect man, while animals are rarely, if ever, infected with the human types.

SUMMARY AND CONCLUSIONS

In the study of 248 strains of hemolytic streptococci of human and animal origin, it was demonstrated that a large majority of

the strains that were of animal origin, namely 96 per cent, could be distinguished from the human cultures by their action on sorbitol and trehalose. The animal strains fermented sorbitol, but did not produce acid from trehalose. The human strains, on the contrary, fermented trehalose, but did not attack sorbitol. The small group of animal strains which resembled the human cultures in fermentative reactions all reduced methylene blue under suitable conditions. Under the same conditions only 4 per cent of the human strains reduced the dye. *Str. equi* was differentiated from all other human and animal strains by its inability to ferment lactose, sorbitol, or trehalose.

The sorbitol-fermenting animal strains (Type A) and *Str. equi* regularly produced capsules. The sorbitol-fermenting strains possessed the characters usually attributed to *Str. epidemicus.*

The sorbitol-fermenting strains of animal origin rarely, if ever, infect man. However, human streptococci are occasionally found in mastitis of cows.

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