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Biological Properties and Mouse Virulence of *Streptococcus agalactiae* and Lancefield's Group "B" Streptococci from Human Sources.*

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During a bacteriological study of cow mastitis[1] a large number of strains of *Streptococcus agalactiae* (Lancefield's group B) were isolated. In the course of an investigation of the bacteriology of excised tonsils Lancefield's group B streptococci were isolated from 6% of the tonsils examined.[2] We have occasionally isolated group B streptococci from other pathological conditions. Other workers have isolated group B streptococci from various pathologic processes in humans. The bovine udder has been suspected by some as the ultimate source of group B streptococci from human sources. Careful studies have been made by others[3,4] in an attempt to prove or disprove this point. Further investigation is necessary to find more evidence that might throw light on this problem.

Fifty strains of *Streptococcus agalactiae* (Lancefield's group B) isolated during the study of cow mastitis and 20 strains from excised tonsils and other human sources have been studied in detail and the results compared.

Methods. Carbohydrate and other media. Beef heart infusion broth (Difco) was used as a basic medium with Brom-cresol purple as indicator. The carbohydrate solutions were sterilized by filtration and then added to 5 cc of the sterile basic medium to give a final concentration of 1%. The sugar media were inoculated with one drop of an 18-hour growth of the streptococci in beef heart infusion broth. The aesculin medium was prepared according to Diernhofer[5] as cited by Plastridge *et al.*[6]

The methylene blue milk medium was prepared containing 1:5000 final dilution of the dye as recommended by Avery[7] and also containing the dye in 1:20,000 dilution. The hydrolysis of sodium hippurate was tested by growing the organisms in the original medium described by Ayers and Rupp[8] as well as in that recommended by Coffey and Foley.[9]

Grouping of streptococci. We used commercial grouping serums or antiserums prepared by us by immunizing rabbits with standard strains supplied by Dr. Lancefield. The extracts for the precipitin tests were made by Fuller's[10] method.

Results. The fermentation reactions of all the human and bovine cultures are given in Table I.

All the bovine organisms fermented lactose. Eight of the 20 human strains did not ferment this substance. All the human and bovine strains fermented dextrose, saccharose, maltose, levulose, trehalose and galactose. The great majority of the cultures fermented these substances overnight. All the human strains fermented dextrin, one bovine strain did not ferment this substance and 10 gave a doubtful reaction.

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1 Pomales-Lebrón, A., Baralt, J., and Morales-Otero, P., in press.

2 Pomales-Lebrón, A., Morales-Otero, P., and Baralt, J., in press.

3 Brown, J. H., *J. Bact.*, 1939, **37**, 133.

4 Simmons, R. T., and Keogh, E. V., *Australia J. Exp. Biol. and Med. Science*, **18**, 151.

5 Diernhofer, K., *Milch-wirstch. Forsch.*, 1932, **13**, 368.

6 Plastridge, W. N., Anderson, E. O., Brigham, G. D., and Spaulding, E. H., *Storrs Agric. Exp. Sta. Bull.* 195, 1934.

7 Avery, R. C., *J. Exp. Med.*, 1929, **50**, 463.

8 Ayers, S. H., and Rupp, P., *J. Infect. Dis.*, 1922, **30**, 388.

9 Coffey, J. M., and Folley, G. E., *Am. J. Pub. Health*, 1937, **27**, 927.

10 Fuller, A. T., *Brit. J. Exp. Path.*, 1938, **19**, 130.

TABLE I.
Fermentation Reactions* of Lancefield's Group "B" Streptococci Obtained from Human and Bovine (Mastitis) Sources.

No. of Bovine strains / Human strains	Lactose	Dextrose	Saccharose	Maltose	Salicin	Levulose	Trehalose	Glycerol	Dextrin	Galactose	Mannitol, Xylose, Dulcitol, Rhamnose, Adonitol, Erythritol, Sorbitol, Arabinose, Amigdalin
9/5	+	+	+	+	+	+	+	+	+	+	—
1/0	+	+	+	+	+	+	+	—	—	+	—
1/0	+	+	+	+	—	+	+	—	+	+	—
18/2	+	+	+	+	+	+	+	—	+	+	—
1/0	+	+	+	+	±	+	+	—	±	+	—
1/0	+	+	+	+	—	+	+	—	±	+	—
1/0	+	+	+	+	±	+	+	—	+	+	—
3/0	+	+	+	+	±	+	+	—	±	+	—
8/5	+	+	+	+	+	+	+	±	+	+	—
7/0	+	+	+	+	+	+	+	±	±	+	—
0/7	—	+	+	+	+	+	+	+	+	+	—
0/1	—	+	+	+	+	+	+	±	+	+	—

Total No. of human strains examined—20.
Total No. of bovine strains examined—50
* Observations were made daily during a period of two weeks.

TABLE II.
Fermentation of Glycerol by Lancefield's Group "B" Streptococci of Human and Bovine Origin.

No. and % of strains	Bovine			Human		
	+	±	—	+	±	—
No.	9	15	26	12	6	2
%	18	30	52	60	30	10

+, Positive; ±, Doubtful; —, Negative.
Period of observation—2 weeks.

The fermentation of glycerol is summarized in Table II.

Simmons and Keogh[4] reported that all their human strains fermented glycerol. They stated, however: "Fermentation of glycerol was not apparent until the third to the sixth day; a few strains did not ferment until later, up to the 14th day." We found also that the fermentation of glycerol was much delayed. The positive strains took from 6 to 13 days to ferment this substance.

All our human strains fermented salicin overnight. Two of the bovine strains failed to ferment this substance and 3 gave a doubtful reaction after 2 weeks.

No strains fermented mannitol, xylose, dulcitol, rhamnose, adonitol, erythritol, sorbitol, arabinose or amigdalin.

Twelve human strains showed the same fermentation reactions of one or more of the bovine cultures.

None of the bovine strains and only 2 human strains reduced methylene blue in 1:5000 dilution. Twenty bovine strains and all the human strains reduced this dye in 1:20,000 dilution.

All the bovine cultures and 18 of the 20 human cultures hydrolyzed sodium hippurate. No strains were able to split aesculin.

Virulence for mice. Fifty bovine and 20 human strains were tested for virulence. The organisms were grown in 5 cc of tryptose phosphate broth (Difco). The 18-hour growth was used for inoculation. All the mice used came from the same colony and weighed from 16 to 20 g approximately. Two animals were inoculated intraperitoneally with 0.5 cc and 0.1 cc respectively of each culture. After death the heart's blood was cultured and if streptococci were present they were grouped by Lancefield's method[11] utilizing Fuller extracts.[10]

Only 2 bovine strains (4%) killed mice when 0.5 cc of the culture was given intraperitoneally. In both cases group B streptococci were recovered from the heart's blood.

[11] Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

None of the animals receiving 0.1 cc of the culture died during the 10-day period of observation. Thirteen human strains (65%) killed mice when 0.5 cc of culture was injected intraperitoneally.

Of these 13 strains 3 also killed when 0.1 cc was inoculated. All the animals died in from 24 to 48 hours after inoculation.

Summary and conclusions. The biological properties and mouse virulence of 70 Lancefield's group B cultures (50 bovine and 20 human) have been studied and compared. The fermentation reactions show that, aside from the inability to ferment lactose by 8 of the human strains and some difference in the fermentation of glycerol, dextrin and salicin, the reactions are very similar for both groups. In some instances the biological properties studied were identical for strains of bovine and human origin. It has been observed with the majority of strains that the differences between single bovine and human cultures were not greater than those between individual strains from the same source.

There is an indication that human strains are more likely to reduce methylene blue.

It is known that the mouse virulence of group B streptococci is low. Inoculation of the 70 cultures studied into mice gave results which indicated that the strains from human sources possessed a higher mouse virulence than the bovine strains.

In spite of minor differences between cultures from bovine and human origin, their similarity in other respects points to a close relationship between these organisms.

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Latex Rubber Capsule for Producing Hypertension in Rats by Perinephritis.

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In studies of dietary choices by hypertensive rats[1] it was necessary to produce large numbers of animals with graded and persistent hypertension. Constricting perinephritis was the method of choice for several reasons but the use of cellophane,[3,4] collodion[5] or silk[5,6] in young rats is technically difficult, the mortality is high, and the results are variable. Partial nephrectomy[2] could not be used because of the accompanying trauma and renal insufficiency. Simple compression of the renal artery had the disadvantage of technical difficulty coupled with later development of collateral circulation.

Enveloping the kidneys of young rats in molded pure gum latex capsules had previously been used to produce hypertension in a few animals in this laboratory.[7] This paper describes more complete experiments, indicating that this method can produce persistent hypertension ranging from 165 to 240 mm Hg at the end of 2 or 3 weeks in at least 70% of the total operated rats and in

1 Abrams, M., and Landis, E. M., to be published.

2 Chanutin, A., and Ferris, E. B., Jr., *Arch. Int. Med.*, 1932, **49**, 767.

3 Friedman, B., Jarman, J., and Klemperer, P., *Am. J. Med. Sci.*, 1941, **202**, 20.

4 Schroeder, H., *J. Exp. Med.*, 1942, **75**, 513.

5 Grollman, A., and Williams, J. R., Jr., *Am. J. Med. Sci.*, 1942, **204**, 73.

6 Kempf, G. F., and Page, I. H., *J. Lab. and Clin. Med.*, 1942, **27**, 1192.

7 Sobin, S., *Am. J. Physiol.*, 1946, **146**, 179.