

# A COMPARISON OF THE STREPTOCOCCI FROM MILK AND FROM THE HUMAN THROAT

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The present comparative study of throat and milk streptococci was undertaken with the object of establishing a method for distinguishing between organisms isolated from these two sources. Much confusion has existed as to the relationships within this genus. The introduction of the biometric study of the streptococci according to their reaction in various carbohydrate mediums by Gordon (1904-1905) and the subsequent extension of the study by Houston (1905-1906) seemed to give a method of solving this obscure problem. The English results have been obtained by the use of litmus added to the mediums and the reactions recorded as positive or negative according to the color of the litmus, without titration. If the mediums were not neutral at the start, it is obvious that they must have classified many organisms as fermentors which have only very slight fermentative properties and really belong to the non-fermenting group. Winslow has pointed out that "they include a much greater percentage of positive records than the American ones."

TABLE 1.—PERCENTAGE OF CULTURES GIVING MORE THAN 1.1 PER CENT.  
ACID AT 37 C.

Fermented. .....	Per cent. Throat Cultures.	Per cent. Milk Cultures.
Dextrose only .....	22.2	6.3
Dextrose and lactose.....	20.4	38.0
Dextrose, lactose and saccharose	33.1	57.7

TABLE 2.—PERCENTAGE OF CULTURES GIVING MORE THAN 0.5 PER CENT.  
ACID AT 20 C.

Fermented. .....	Per cent. Throat Cultures.	Per cent. Milk Cultures.
No fermentation .....	50.0	0
Dextrose only .....	40.0	32.5
Dextrose and lactose.....	1.8	31.0
Dextrose, lactose and saccharose	0	31.0

Winslow introduced the method of titrating the cultures with phenolphthalein as an indicator, and at present this procedure seems to give a substantial basis for fixing the relationships within this group.

We examined seventy pure strains of streptococci isolated from fresh specimens in our laboratory. Cultures taken from sore, inflamed, or

otherwise unusual throats have been grouped as "abnormal." The small number of cultures used renders our generalizations more or less tentative, and it is our intention to substantiate the work, using larger numbers. Quantitative acid production was studied in six carbohydrate mediums: dextrose, maltose, lactose, saccharose, raffinose and mannite. Two tubes of a 1 per cent. carbohydrate broth were inoculated<sup>1</sup> at the same time, one tube being placed at 20 C. and the other at 37 C. for seventy-two hours. Five c.c. were then drawn off with a pipet, diluted with 45 c.c. of distilled water and titrated against twentieth normal sodium hydroxid, using phenolphthalein as an indicator. Blank tubes were incubated with the

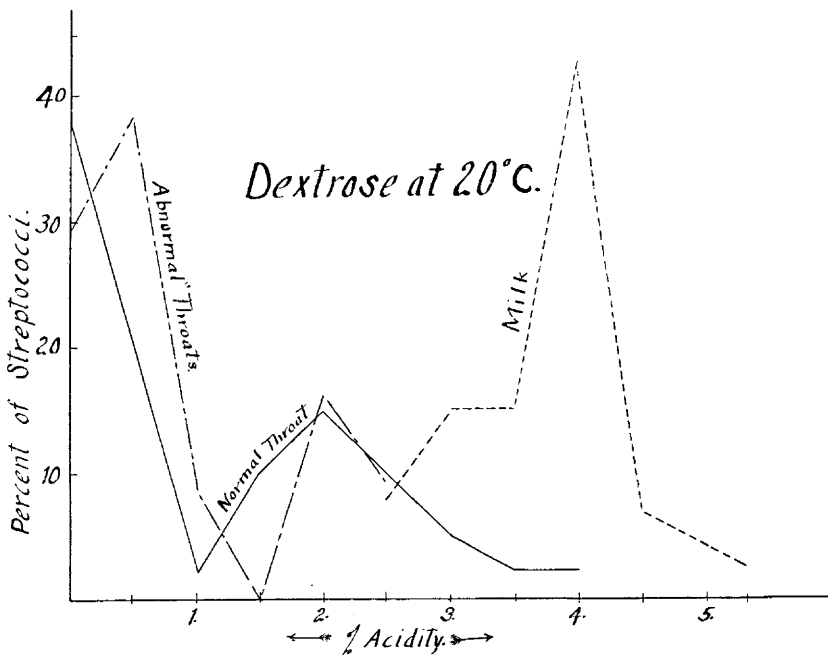


Chart 1.—Showing percentage of acidity and streptococci from abnormal throats, normal throats and milk cultured in dextrose at 20 C.

inoculated tubes and these were titrated at the same time. The difference between the blanks and the cultures was recorded as the amount of acid produced. We have omitted the maltose results in classifications tables, as the figures follow the lactose figures fairly closely. With the sugars that are fermented at all at 37 C., the first reaction mode was found to fall at or beyond 1.2 per cent. acidity expressed as normal acid, and we have used this as the dividing point between a positive and a negative reaction.

1. The inoculations were made by transferring two standard loops full of the growth found in the water of condensation on North's gelatin agar medium.

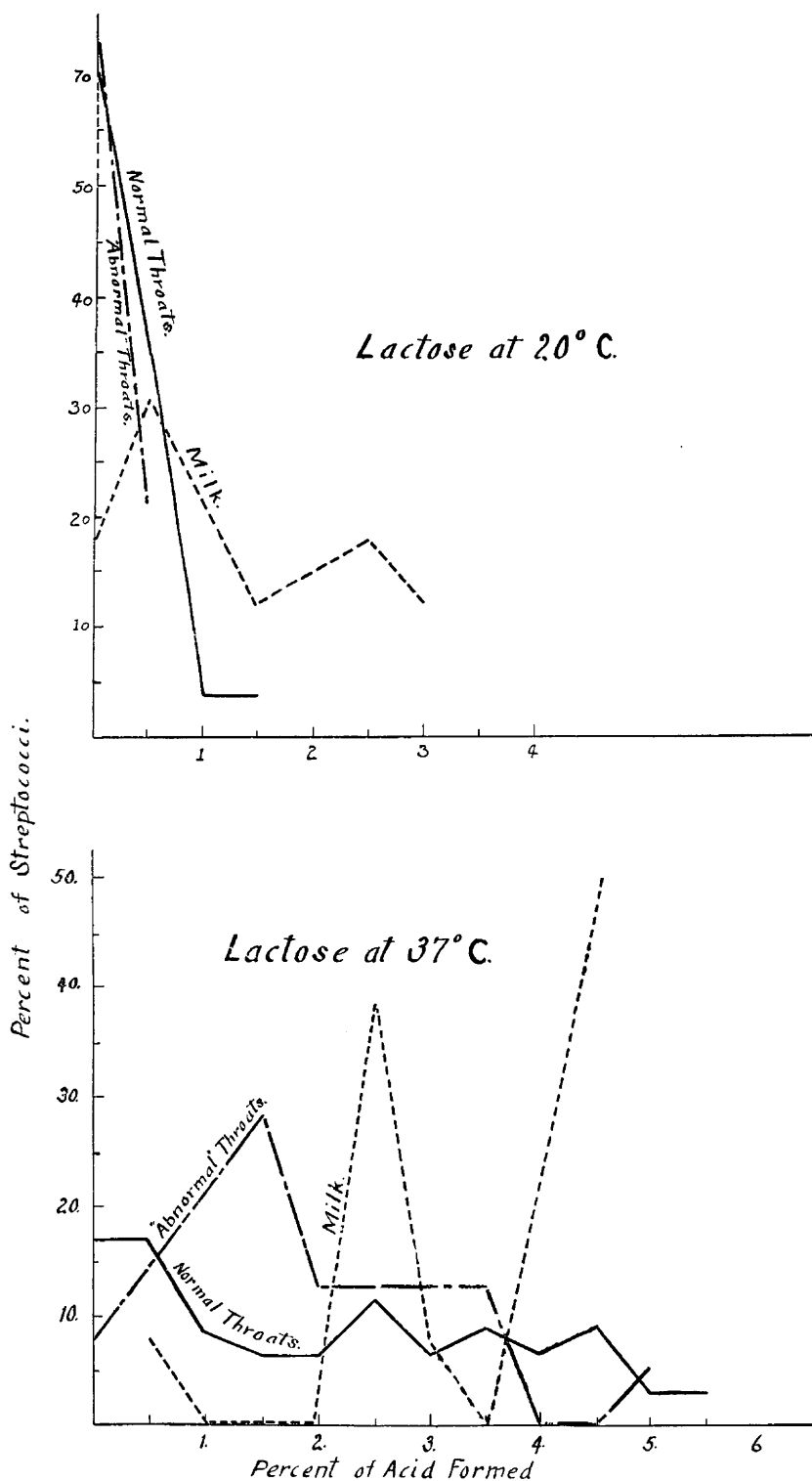


Chart 2.—Showing percentage of streptococci and acidity from normal and abnormal throats and milk cultured in lactose at 20 and 37 C.

On this basis, all but four cultures fermented one or more of the mediums used. It was found that 83 per cent. of these might be placed in three groups according to their fermenting capacity.

When incubated at 20 C. the first reaction mode falls at 0.5 per cent. acidity and on this basis the milk cultures fall into the same general groups as noted above, but the throat streptococci show 50 per cent. not fermenting at all, and 40 per cent. fermenting dextrose only.

It will be noted that the carbohydrates were attacked progressively in the order of the complexity of the molecular structure, the single sugar

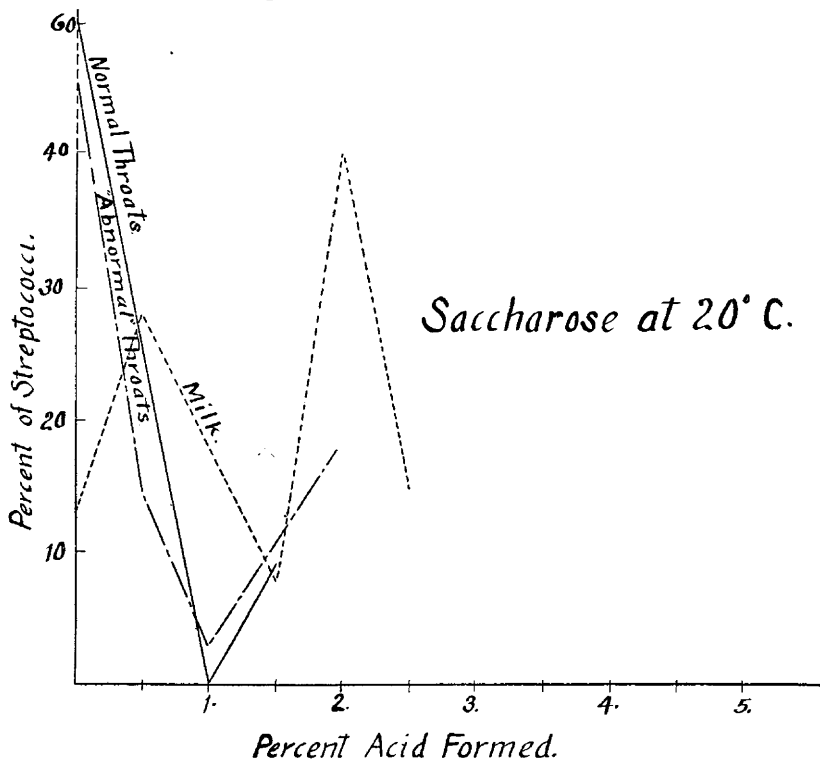


Chart 3.—Showing percentage of acidity and streptococci from normal and abnormal throats and milk cultured in saccharose at 20 C.

being most easily fermented, then the disaccharids, the trisaccharid and the alcohol being too complex to be used except by a very few cultures. It is seldom that a strain reacts with a sugar lower in the scale of fermentability while failing to attack all the simpler, more available ones, as is shown from a study of the tables at the end of this paper. Andrews and Horder suggested this grade of availability in 1906, referring to the phenomenon as "a set of gymnastic exercises in chemical decomposition." The colon group shows a similar metabolic relationship toward the carbohydrates but with them the size rather than the structure of the molecule

seems to be the controlling factor. Howe (1911) has aptly called this order of availability the "metabolic gradient." It suggests a very important advance in the biometric method.

A consideration of the reactions in the individual sugars brings out the following facts:

*Dextrose*: The normal throat strains seem to show the greatest fermentative properties, their mode coming at 6.0 per cent. acid when grown at 37° C. The milk cultures show a very specific reaction, all falling into a group giving from 3.5 per cent. to 5.5 per cent. acid. Reference to

TABLE 3.—STREPTOCOCCI ISOLATED FROM NORMAL THROATS

Sample No.	Long or Short Chain Form	Reaction	Percentage of Acid Formed in—											
			Dextrose.		Lactose.		Maltose.		Saccharose.		Raffinose.		Mannite.	
			37° C.	20° C.	37° C.	20° C.	37° C.	20° C.	37° C.	20° C.	37° C.	20° C.	37° C.	20° C.
1	Short	G +	7.3	3.5	.3	0	..	..	3.0	.1	1.1	0	0	0
2	Long	G +	3.3	1.9	3.7	0	.9	0	1.8	.1	.2	.1	.1	0
3	Long	G +	3.8	2.4	4.6	.3	1.6	.1	2.6	0	—1	—1	0	0
4	Short	G +	6.1	1.8	6.1	.2	2.7	.2	2.1	0	.3	0	0	.2
5	Short	G +	6.5	1.3	2.0	.2	.9	0	1.6	0	0	0	0	0
6	Short	G +	5.0	2.1	5.4	0	1.7	0	.1	1.4	.1	0	.2	.1
7	Short	G +	3.6	1.1	4.1	0	.6	.1	.8	0	.3	0	0	0
8	Long	G +	4.3	1.7	—1	—	1.5	1.1	1.1	1.3	1.3	0	.2	.2
9	Short	G +	5.7	1.7	—5	0	..	..	1.9	.1	1.1	0	0	0
15	Short	G +	4.2	2.6	0	.2	1.6	.4	.1	0	—1	.2	.1	0
16	Short	G —	.8	.9	.3	.6	..	..	0	.2	—3	0	0	0
17	Short	G —	4.2	2.2	4.3	0	..	..	0	0	1.7	0	.1	0
18	Long	G +	3.1	1.8	3.7	0	0	0	0	0	0	0	0	0
19	Long	G +	2.7	1.2	3.4	.5	0	0	0	0	—1	0	0	0
20	Short	G +	4.5	3.8	..	..	..	..	..	..	1.4	..	..	..
21	Short	G +	6.0	3.4	..	..	..	..	..	..	1.2	0	..	..
22	Short	G +	5.9	3.0	.1	.1	.9	.1	0	0	.1	0	0	0
24	Short	G +	6.0	.5	..	..	..	..	.8	.5	.2	0	..	..
25	Short	G +	6.0	1.6	4.1	0	2.9	.2	—1	0	1.0	0	.1	0
26	Short	G +	6.2	1.4	2.2	0	2.9	.3	0	.2	.1	0	0	0
27	Short	G +	6.3	.3	2.5	0	2.5	.1	2.1	0	.1	0	0	0
28	Short	G +	6.0	.2	3.0	0	2.8	0	1.4	0	—1	.1	.1	0
29	Short	G +	5.8	2.4	0	0	..	..	3.2	0	.9	.1	0	0
32	Short	G +	4.5	0	1.5	0	1.4	0	1.5	.8	1.1	0	0	0
33	Short	G +	5.1	0	.5	0	.5	.2	.9	0	.1	.3	0	0
34	Short	G +	2.0	.1	.2	0	.5	0	0	0	.4	0	0	0
35	Short	G +	4.8	.3	2.5	2.2	2.5	2.2	3.5	.1	.2	.1	0	0
36	Short	G +	3.8	.2	.6	.2	.6	0	.1	0	.2	.1	.2	0
38	Long	G +	4.0	0	1.7	0	1.3	.2	1.1	1.1	0	.1	0	0
43	Short	G —	3.8	.1	3.3	0	0	0	1.5	0	0	0	0	0
44	Short	G +	1.5	0	3.1	0	2.9	0	2.0	.2	.1	0	0	0
45	Short	G —	5.4	0	0	0	..	..	.9	0	0	0	0	0
46	Short	G +	..	..	..	..	..	..	..	..	..	..	0	0
47	Short	G +	5.8	0	2.4	0	2.3	.2	2.6	0	.4	0	0	0
48	Long	G +	0	0	.6	.1	.7	.3	0	0	.8	0	0	0
49	Short	G +	4.6	.5	1.2	0	1.5	0	2.4	0	1.1	0	0	0
50	Short	G +	6.0	0	2.6	0	2.5	.2	1.9	0	.1	0	0	0
51	Long	G +	5.4	0	0	0	1.2	0	.2	.1	0	0	0	0
52	Long	G +	3.8	—3	1.6	.1	1.5	0	1.1	.1	.2	0	0	.1
53	Long	G +	3.5	0	0	0	2.1	0	.4	0	.6	0	0	0
54	Short	G +	1.1	0	.1	0	.4	0	.8	0	.1	0	0	0
55	Long	G +	1.7	0	.7	0	0	0	.4	0	.1	0	0	0

Chart 1 shows the 20 degree results plotted. Only about 10 per cent. of the throat strains give over 2.5 per cent. acid, while all of the milk cultures give a higher percentage than this.

*Lactose:* We find here a fairly even distribution of the throat strains at 37 C., while the milk streptococci all give high acidity. At 20 C. lactose proves much more available for the milk than for the throat cultures, as is clearly shown in Chart 2.

TABLE 4.—STREPTOCOCCI ISOLATED FROM "ABNORMAL" THROATS

Sample No.	Long or Short Chain Form	Reaction	Percentage of Acid Formed in—											
			Dextrose.		Lactose.		Maltose.		Saccharose.		Raffinose.		Mannite.	
			37 C.	20 C.	37 C.	20 C.	37 C.	20 C.	37 C.	20 C.	37 C.	20 C.	37 C.	20 C.
68	Long	G +	5.5	1.2	0	0	1.8	.4	1.9	1.3	1.4	0	0	0
69	Long	G +	6.2	0	4.7	0	..	..	2.1	1.7	.2	0	0	0
30	Short	G +	5.2	2.2	.3	.3	2.0	1.4	2.0	1.8	.1	.2	.1	0
31	Long	G +	2.3	.6	.1	0 0	..	..	0	.1	0	0	0	0
37	Short	G +	4.3	1.7	..	..	..	..	..	..	0	.1	0	0
57	Long	G —	3.3	1.9	2.7	2.4	1.7	.8	0	0	.2	0	.3	0
58	Long	G +	1.5	.1	1.4	.1	1.8	.8	.9	.1	.4	.1	.2	0
59	Long	G +	1.5	.3	1.5	0	.8	0	2.0	0	.3	0	.2	0
60	Short	G +	3.1	0	2.9	0	2.8	0	2.8	0	0	0	.1	0
61	Short	G +	1.8	.1	1.8	0	1.9	.2	1.7	0	1.5	0	0	0
62	Short	G +	3.2	0	3.5	0	2.8	0	3.1	0	0	0	0	0
63	Long	G +	1.6	.1	1.6	.1	1.7	0	1.0	0	.3	0	0	0
64	Short	G +	1.9	.3	1.9	0	2.1	.9	.2	0	1.7	.1	.2	0
65	Short	G +	3.4	0	3.2	0	2.8	.8	0	0	1.5	0	0	0
66	Long	G +	..	..	1.5	0	1.7	0	.1	0	.3	0	.1	0

TABLE 5.—STREPTOCOCCI ISOLATED FROM MILK

Sample No.	Long or Short Chain Form	Reaction	Percentage of Acid Formed in—											
			Dextrose.		Lactose.		Maltose.		Saccharose.		Raffinose.		Mannite.	
			37 C.	20 C.	37 C.	20 C.	37 C.	20 C.	37 C.	20 C.	37 C.	20 C.	37 C.	20 C.
10	Long	G +	4.1	3.4	4.0	0	1.6	0.2	2.0	1.1	1.6	0	.4	0
11	Long	G +	4.1	4.0	.4	0	2.0	1.0	1.0	.4	.2	0	0	0
12	Long	G +	4.4	3.9	4.1	1.5	2.3	1.1	2.3	2.1	.1	0	.1	0
13	Long	G +	3.2	3.9	4.2	4.2	1.3	.5	2.1	2.1	0	.2	.1	0
14	Long	G +	4.3	3.8	4.5	.3	2.1	1.1	1.8	2.0	0	.2	.1	0
23	Long	G +	4.0	5.8	2.2	2.4	1.9	1.9	0	0	—1	0	0	0
39	Long	G +	5.4	4.8	2.6	2.6	1.6	1.9	0	0	0	.1	0	0
40	Long	G +	5.2	4.1	2.5	2.5	1.7	1.4	.1	.1	0	0	0	0
41	Long	G +	4.5	2.8	2.3	1.2	2.0	1.0	.7	2.0	0	.2	0	0
42	Long	G +	4.9	2.9	2.4	.3	2.2	.8	1.6	1.9	.1	.1	0	0
56	Long	G —	3.2	2.4	2.5	2.2	1.5	0	0	.1	0	0	0	0
67	Short	G +	1.2	1.2	2.6	2.6	.7	1.7	..	..	.1	.1	0	0
70	Long	G —	4.6	1.2	4.5	.4	2.3	1.2	2.1	.3	0	0	0	0
71	Long	G +	4.5	4.0	4.2	.5	2.0	1.0	2.0	2.0	0	0	0	0
72	Long	G +	4.2	3.5	4.3	.5	2.4	1.0	2.1	2.0	.1	.2	0	0
73	Long	G —	4.8	3.6	4.4	0	2.2	1.3	2.2	1.9	.1	.1	0	0

*Maltose:* The throat cultures show a distinct reaction mode, falling between 1.0 and 1.5 per cent. acid. As in the case of lactose, the streptococci from milk are most adaptable as regards their temperature relations.

*Saccharose:* In this sugar the milk cultures readily produce 2 per cent. acid at 20 C., while the throat strains seldom give more than 0.5 per cent. Reference to Chart 3 shows strikingly this distinction. No other features worthy of particular note are brought out by the 37 degree reactions.

*Raffinose:* This trisaccharid was rarely fermented and when a reaction was present it was very feeble as shown by reference to the tables.

*Mannite:* Only a single culture was capable of producing as much as 0.4 per cent. acid with the alcohol mannite. It is obviously of little diagnostic value with throat or milk streptococci.

The results from the cultures isolated from "abnormal" throats showed no essential differences from the normal throat streptococci.

No clue to the relationship of the forms studied was obtained from either morphologic or staining characteristics.

#### CONCLUSIONS

Our work leads us to make the following tentative conclusions:

1. Streptococci from the human throat and from fresh milk very generally ferment one or more of the sugars, dextrose, lactose, maltose and saccharose, attacking them most readily in the order named. They do not generally ferment raffinose or mannite.

2. The streptococci of the sore throat and the normal throat show no cultural differentiation in relation to the carbohydrates used. Virulence tests might have separated the two groups.

3. The throat streptococci do not readily ferment at 20 C. any of the sugars used, while the milk organisms attack the same sugars and to the same extent at this temperature as at 37 C. This is, perhaps, the most valuable differential feature between chained cocci from these two sources.

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