

A BIOMETRICAL STUDY OF MILK STREPTOCOCCI.*†

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A comparative study of the fermentative reactions of milk streptococci was begun in February, 1911, at the suggestion of Professor C.-E. A. Winslow, and, with occasional helpful criticism from him, completed early in June, 1911. Through the courtesy of Dr. William H. Park of the New York Research Laboratory, 100 strains of streptococci were obtained from milk plates made in the routine milk examination conducted by Dr. Schroeder. Most of these plates were made from samples obtained at the farms and dairies, and shipped on ice to the Research Laboratory. But one strain was used from each plate, making, presumably, 100 sources for the 100 strains isolated. Fishings from the agar milk plates supplied by Dr. Schroeder were grown in milk, transferred to agar streaks and then again to milk, next to slant agar for two or three days (until a good growth was secured), and then transferred to the special media. These included eight of Gordon's¹ nine test substances: neutral red for reduction; milk for coagulation; and for acid formation, lactose, saccharose, salicin, raffinose, mannit, and inulin. Sugar-free broth was used in making 1 per cent media of the last six substances. Microscopic examination (methylene blue) was made at every stage. Only those strains were used which produced on agar the characteristic veil-like growth, and which showed in liquid media chains of four or more cocci. Chains of 40 were the longest observed. With a few exceptions the early stages of isolation, owing to lack of incubator space, were conducted at room temperature; slow-growing organisms were reincubated at 37°C. This latter temperature was used for all the agar slants and for all the special media. After three days at 37°C. these special media (with controls) were titrated to determine the amount of acid formed. Five c.c. of liquid and 45 c.c. of distilled water were

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¹ Supplement, *Thirty-third Annual Report, Local Government Board*, 1903-4.

titrated in the cold against $\frac{n}{20}$ NaOH, using phenolphthalein (three drops) as an indicator. Two controls were titrated with every lot, and their average results subtracted from those recorded for the inoculated tubes. As a color standard the pink in the top line of the color chart frontispiece of Winslow's *Systematic Relationships of the Coccaceae* was used. Each agar tube used in inoculating the special media was reincubated (for three days) and then examined microscopically. Purity in this agar tube was taken to indicate purity in the eight subcultures previously made from it.

The 31 strains first isolated were not transferred directly to the special media, owing to an unavoidable delay in the preparation. They were subcultured on agar for one week, but good growths were obtained before transferring to the test substances mentioned. In this lot, however, are found all of the 11 strains fermenting salicin only; these will be mentioned later.

RESULTS.

The results for the 100 strains are given below (Table 1). The first column gives the series numbers, arranged in order of isolation. The records of acidity represent, as stated above, the difference between the value obtained for each culture and two controls incubated at the same time under the same conditions. Coagulation of the milk and reduction of neutral red are indicated by the plus sign; the lack of either by a minus sign.

In the second table the results, except those for milk and neutral red, are grouped together in percentage acidity classes. In each test substance there were two types of organisms: one yielding little or no acid, or even causing an alkaline reaction; and a type yielding between 1.5 (approximately) and 8.5 per cent acid. The exact intermodal point for each substance is indicated by an asterisk.

It is of course impossible to consider a different intermodal point for each substance; therefore 1.5 per cent has been selected as a basis for comparison, and all strains falling below 1.5 per cent acid are considered non-fermenters. The acid-forming strains are indicated by a plus sign (Table 3), the non-acid strains by a blank. It will be observed that on this basis a large number of strains

TABLE I.

SERIES NUMBER OF STREPTOCOCCI	PERCENTAGE NORMAL ACID FORMED IN						COAGULA- TION OF MILK	REDUC- TION OF NEUTRAL RED
	Lactose	Saccha- rose	Salicin	Raffinose	Mannit	Inulin		
1.....	-0.4	4.0	4.0	0.1	1.0	0.1	-	-
2.....	2.5	3.9	5.7	0.2	2.4	3.4	-	-
3.....	0.1	0.7	0.2	0.5	-0.1	-0.3	-	-
4.....	0.2	0.3	2.8	0.4	-0.5	-0.4	-	-
5.....	2.5	2.3	4.8	0.6	2.5	3.3	-	-
6.....	0.3	-0.9	4.4	-0.1	-0.4	-0.3	-	-
7.....	0.6	-0.8	3.6	0.2	-0.4	-0.1	-	-
8.....	4.0	3.9	6.3	0.1	1.5	-0.1	-	-
9.....	3.6	0.3	7.6	0.3	-0.7	-0.1	+	+
10.....	4.5	0.2	6.2	-0.4	-0.3	-0.3	+	+
11.....	-0.1	0.1	-0.1	0.2	-0.3	-0.5	-	-
12.....	3.0	2.8	5.0	0.5	3.6	4.5	-	-
13.....	4.6	3.3	5.8	-0.7	-0.4	0.2	-	-
14.....	-0.2	0.3	4.3	1.5	-0.4	-0.1	-	-
15.....	0.3	0.5	3.5	0.2	-0.4	-0.1	-	-
16.....	0.6	1.7	4.1	0.2	0.6	-0.6	-	-
17.....	2.7	2.3	2.3	5.1	3.3	0.0	+	+
18.....	3.0	2.5	1.5	3.7	0.9	1.4	+	+
19.....	2.6	3.1	4.1	0.5	3.7	4.5	-	-
20.....	0.1	0.0	3.2	0.2	-0.4	0.0	-	-
21.....	0.4	-0.8	3.5	0.4	-0.2	-0.1	-	-
22.....	0.2	0.0	4.6	0.2	-0.2	-0.4	-	-
23.....	0.1	0.3	3.6	0.5	-0.2	-0.4	-	-
24.....	-0.2	-0.3	2.6	0.7	-0.3	-0.5	-	+
25.....	0.1	0.2	3.7	0.3	0.0	-0.3	-	-
26.....	3.3	2.3	5.7	0.5	2.1	4.1	-	-
27.....	2.8	2.0	4.9	0.5	3.7	4.5	-	-
28.....	4.1	3.8	6.6	0.2	1.5	-0.4	-	-
29.....	0.3	0.9	-0.2	0.2	0.0	-0.1	-	-
30.....	0.8	0.8	4.5	0.6	-0.1	-0.2	-	-
31.....	4.8	0.8	6.6	-0.4	-0.2	-0.4	+	+
32.....	3.3	3.6	0.0	0.2	-0.3	-0.3	+	+
33.....	2.8	3.1	3.3	0.3	3.9	2.8	-	-
34.....	3.3	4.1	3.8	0.2	4.2	2.9	-	-
35.....	3.8	3.6	5.0	0.0	3.5	3.2	-	-
36.....	2.8	4.0	4.0	-0.6	3.1	2.7	-	-
37.....	2.5	3.6	1.9	-0.1	2.5	0.0	-	-
38.....	3.1	0.2	2.4	-0.2	2.6	-0.2	+	+
39.....	2.4	3.6	3.0	0.4	2.6	2.3	-	-
40.....	4.2	4.6	2.3	-0.2	0.2	-0.2	+	+
41.....	2.2	2.8	3.4	0.4	0.3	1.5	+	+
42.....	2.4	3.4	3.3	-0.1	2.6	0.0	-	-
43.....	3.2	3.8	3.0	0.2	0.2	2.1	-	-
44.....	2.6	3.4	3.2	-0.4	3.2	-0.5	+	+
45.....	4.2	4.8	5.5	-0.2	2.2	-0.5	+	+
46.....	3.8	4.7	4.1	1.3	0.5	0.2	+	+
47.....	2.0	0.1	0.7	0.1	0.5	0.4	-	-
48.....	0.6	0.1	1.1	0.1	0.3	0.2	-	-
49.....	1.4	0.3	0.2	0.7	0.3	0.2	-	-
50.....	3.5	1.3	4.3	0.4	0.7	0.6	+	+
51.....	3.2	2.5	3.9	3.9	3.7	3.2	+	+
52.....	3.6	3.9	3.7	3.3	3.7	3.8	+	+
53.....	3.4	3.1	0.5	3.3	-0.3	2.6	+	+
54.....	3.4	4.1	4.1	3.5	4.1	2.8	+	+
55.....	4.8	-0.3	2.5	0.3	0.5	0.2	+	+
56.....	4.4	0.1	3.3	0.3	0.3	0.2	+	+
57.....	2.4	2.9	0.5	0.5	0.5	0.0	+	+
58.....	3.4	3.5	1.5	0.9	0.1	2.0	+	+
59.....	3.4	3.4	5.0	3.6	3.9	0.6	+	+
60.....	3.2	0.1	0.3	0.0	0.6	0.6	-	-
61.....	1.0	5.4	4.8	0.6	0.1	0.8	-	-
62.....	0.8	5.3	4.3	1.1	0.1	0.4	-	-
63.....	5.0	0.3	7.5	0.2	0.4	0.4	+	+
64.....	4.6	6.0	3.7	0.1	5.9	0.2	+	+
65.....	4.4	6.3	5.2	0.4	0.3	0.0	+	+
66.....	2.6	4.0	-0.3	0.1	0.6	0.2	+	+
67.....	2.9	3.4	3.1	0.6	0.6	3.0	+	+
68.....	4.5	5.2	-0.4	0.3	0.5	0.0	+	+
69.....	5.2	0.2	6.7	0.5	3.2	0.1	+	+

TABLE I—Continued.

SERIES NUMBER OF STREPTOCOCCI	PERCENTAGE NORMAL ACID FORMED IN						COAGULA- TION OF MILK	REDUC- TION OF NEUTRAL RED
	Lactose	Saccha- rose	Salicin	Raffinose	Mannit	Inulin		
70.....	6.3	8.4	0.5	5.5	0.5	—0.1	+	+
71.....	3.7	4.5	5.5	0.5	4.4	5.9	—	+
72.....	2.5	3.2	0.8	0.3	0.6	0.9	—	—
73.....	3.2	3.4	4.2	0.1	0.7	4.8	—	—
74.....	0.6	3.9	5.2	0.5	0.4	3.5	+	—
75.....	2.9	3.3	2.8	0.6	0.9	3.0	+	—
76.....	2.3	3.3	4.8	0.6	0.9	3.8	+	—
77.....	2.5	3.3	5.5	0.7	1.1	3.6	+	—
78.....	2.5	3.8	5.2	0.6	0.7	0.9	+	+
79.....	2.6	4.0	4.7	0.5	0.6	3.2	+	—
80.....	4.6	5.2	6.5	0.3	0.6	0.2	+	—
81.....	4.8	5.1	5.7	0.3	0.5	0.4	+	—
82.....	4.1	0.7	0.5	0.9	0.7	0.4	+	—
83.....	3.8	2.3	4.5	0.3	0.6	4.1	+	—
84.....	6.0	1.7	0.3	0.7	0.5	0.2	+	+
85.....	3.6	1.0	4.9	0.0	0.5	4.0	—	—
86.....	2.7	5.1	5.6	0.3	2.8	0.6	—	—
87.....	0.2	0.7	0.6	0.3	1.1	0.6	—	—
88.....	2.5	4.8	4.9	0.4	1.3	3.8	+	—
89.....	4.2	0.8	6.2	0.4	0.4	0.7	+	+
90.....	2.7	4.8	0.7	3.1	0.2	1.1	—	—
91.....	1.8	4.2	0.5	2.8	0.4	2.8	+	—
92.....	2.7	4.0	2.2	0.8	0.4	4.9	—	—
93.....	1.4	4.8	4.2	0.0	0.6	3.9	+	—
94.....	2.6	4.5	0.4	2.6	1.1	5.8	—	—
95.....	2.7	4.9	5.0	0.4	1.4	5.1	+	+
96.....	2.6	3.3	2.7	3.9	0.5	5.2	+	+
97.....	4.7	1.8	6.4	0.4	0.6	5.3	+	+
98.....	2.5	4.5	1.2	3.7	0.5	5.3	+	+
99.....	4.2	1.4	3.9	1.0	0.7	0.6	—	—
100.....	2.9	5.3	3.6	0.6	1.3	1.0	—	—

ferment lactose, saccharose, and salicin. The order of fermentability is salicin, 82 per cent; lactose, 76 per cent; saccharose, 66 per cent; inulin, 38 per cent; raffinose, 13 per cent; and mannit, 27 per cent. Inulin has a high record for a substance heretofore described as not fermented by streptococci.

TABLE 2.
MILK STREPTOCOCCI GROUPED IN PERCENTAGE ACIDITY CLASSES.

Classes	Number of Strains in Each Class																			
	—0.9	—0.4	.1	.6	1.1	1.6	2.1	2.6	3.1	3.6	4.1	4.6	5.1	5.6	6.1	6.6	7.1	7.6	8.1	
	0.5	0.0	.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	
Lactose.....	0	4	11	7	2*	2	13	18	15	7	11	7	1	1	0	0	0	0	0	
Saccharose.....	3	3	15	9	2*	4	6	3	15	16	8	6	7	1	0	0	0	0	1	
Salicin.....	0	5	10	3	4	1*	5	6	10	10	12	13	6	6	5	2	1	1	0	
Raffinose.....	2	12	54	16	2	1	0*	2	3	6	0	0	2	0	0	0	0	0	0	
Mannit.....	2	18	28	18	7	1*	5	5	5	7	3	0	1	0	0	0	0	0	0	
Inulin.....	5	27	16	11	3	1*	2	9	6	8	5	2	3	2	0	0	0	0	0	

These milk streptococci vary greatly in the number of substances they are able to ferment. This same table (Table 3) shows that the 100 strains fall into 20 groups, based upon the number and

kind of substances fermented. Four strains were able to ferment all of the test media. Fifteen strains fermented five, the largest being the group of 12 strains that fermented all except raffinose. Twenty-seven strains fermented four substances, the largest group in the lot consisting of 15 strains fermenting lactose, saccharose, salicin, and inulin. In the 14 strains which were able to ferment but three of the special media, the largest group consists of nine strains fermenting lactose, saccharose, and salicin; none of the 14 affects raffinose. In the 19 strains which fermented but two substances, chiefly lactose, saccharose, and salicin, inulin is no longer represented, and raffinose by but two (probably aberrant) strains. Fifteen strains fermented but one of the special media, lactose or salicin; most of them—11 strains—fermented salicin. Six strains failed to ferment any of the six test media.

TABLE 3.
MILK STREPTOCOCCI GROUPED IN CLASSES ACCORDING TO THE NUMBER OF SUBSTANCES FERMENTED.

Number (and Percentage) of Strains		Lactose	Saccharose	Salicin	Raffinose	Mannit	Inulin
4	4	+	+	+	+	+	+
15	12	+	+	+	..	+	+
	2	+	+	+	+	+	..
	1	+	+	+	+	+	..
27	15	+	+	+	+
	8	+	+	+	..	+	..
	2	+	+	..	+	..	+
14	9	+	+	+	..	+	..
	2	+	+	+
	1	+	+
19	8	+	+	+
	5	+	+	+
	4	+
	1	+	+
15	11	+
	4	+
6	6
Total.....	100	76	66	82	13	27	38

The negative and positive results are both of interest in this connection. The inability of an organism to use one or more of the substances is as definite a character as the fermentative power itself. Thus, we may say that the 12 strains recorded in the second

line of the third table form a group or type of streptococci which are characterized (1) by the power to ferment lactose, saccharose, salicin, mannit, and inulin, and (2) by the inability to ferment raffinose. Similarly, in the fifth line we find a second group of 15 strains characterized (1) by the ability to use lactose, saccharose, salicin, and inulin, and (2) by the inability to use raffinose and mannit. These reaction combinations are much more significant than the mere number or kind of substances fermented. In the 100 milk streptococci tested 20 such combinations or groups (Table 3) occur. They vary greatly in size (one to 15 strains), but the larger groups may be taken as types of the streptococci to be found in milk.

It is possible that the longer period on agar mentioned earlier in connection with the first 31 strains isolated is correlated with the presence of the group fermenting salicin only. These 11 strains grew well in salicin (2.6–6.6 per cent acid). The other 20 strains represent nine of the reaction groups given in the third table. It is therefore probably fair to include the 11 salicin fermenters in this report. An additional reason for including them is that, later, occasional strains about which I felt uncertain with regard to their purity were delayed a similar period and transferred to the special media with the next batch; none of these delayed strains fermented salicin only.

The coagulation of milk (incubated for three days at 37° C.) proved unsatisfactory as a diagnostic character. About half the strains (48 per cent) caused coagulation. There is some indication that the time of year is correlated with these results; e.g., by the middle of April 40 strains had been isolated and but nine of these coagulated milk, while the balance isolated between that time and June 1 contained 39 strains which coagulated milk. In considering milk in connection with the 20 reaction groups (Table 3) we find (1) that the number of these groups is practically doubled, and (2) that the groups are consequently too small to be helpful in indicating types of milk streptococci. Houston feels that coagulation is greatly affected by the temperature used in sterilizing the milk. In view of this unsatisfactory condition of affairs, this qualitative reaction with milk is not considered in the balance of this paper.

As shown by the first table the neutral red (after three days at 37° C. under anaerobic conditions) gave no reduction in 85 per cent of the strains. Reduction was but slight in several of the remaining strains, and since this test has recently been rejected by Houston also as not sufficiently diagnostic, it will not be discussed further in this paper.

Morphological characters, length of chain, degree and regularity of staining, and the presence of occasional abnormal units in a chain do not seem to be correlated with the fermentative powers. One illustration will be sufficient. Eight of the strains used contained chains of 20 cocci or more, even at the last examination from the reincubated slant agar. These eight fall into six different groups of those given in Table 3 with regard to the substances fermented; every substance except mannit was fermented by one or more strains; and a wide range was found in the amount of acid formed: a range of 4.7 per cent in lactose, 5.4 per cent in saccharose, 6.0 per cent in salicin, 2.7 per cent in raffinose, 0.8 per cent in mannit, and 3.2 per cent in inulin.

COMPARISONS WITH EARLIER METHODS AND RESULTS.

The English bacteriologists—Gordon, Andrewes and Horder, and Houston—have done a great deal of work on the fermenting powers of the streptococci. They, however, used the qualitative method, with litmus as the indicator. Houston¹ has recently worked on the streptococci with reference to the possibility of discovering the source of the streptococci found in the London water supply. His reaction groups are indicated by names made ingeniously of the first letters of the test media; e.g., a lamirasacsal organism or type ferments *lactose*, coagulates *milk*, and ferments *raffinose*, *saccharose*, and *salicin*. Since milk is not considered in this paper, it has not been possible to use these convenient names for indicating the reaction groups.

In Houston's² work with milk streptococci, 172 strains, he obtained a higher percentage fermenting lactose and saccharose; his figures for the other substances are lower. The exact percentages

¹ *Fifth Research Report, Metropolitan Water Board, London, 1910.*

² *Report to the London County Council on the Bacteriological Examination of Milk, July 11, 1905.*

follow: lactose (H)[†] 97 per cent, (B)[†] 76 per cent; saccharose (H) 90 per cent, (B) 66 per cent; salicin (H) 60 per cent, (B) 82 per cent; raffinose (H) 19 per cent, (B) 29 per cent; mannit (H) 20 per cent, (B) 27 per cent; and inulin (H) 21 per cent, (B) 38 per cent. When discussing this whole question last summer (1911), Houston said he discarded during isolation all strains which did not ferment lactose. The equine streptococci rarely ferment lactose. He also used the Conradi-Drigalski medium for isolation. These two facts doubtless partly explain these and other differences in our results. Omitting Houston's results with neutral red and milk, and rearranging his reaction groups, we find that he has 16 groups to my 20. In his 16 groups I find but 12 of my groups, and but 74 per cent of my strains.

His figures for equine, bovine, and human fecal streptococci indicate that my milk strains may be of human origin. The human fecal strains may be compared with my milk strains as follows: lactose (H) 76 per cent, (B) 76 per cent; saccharose (H) 86 per cent, (B) 66 per cent; salicin (H) 92 per cent, (B) 82 per cent; raffinose (H) 32 per cent, (B) 29 per cent; mannit (H) 24 per cent, (B) 27 per cent; and inulin (H) 4 per cent, (B) 38 per cent. These compare rather favorably, except for inulin. His largest reaction groups for human strains (when regrouped as above) contain 26, 23, 21, and 11 per cent of his strains; these correspond respectively to 24, 20, 4, and 8 per cent of my milk strains. These results really correspond more nearly to my milk records than to Houston's own milk records. Houston concluded that a certain proportion of milk streptococci are not derived from either human or bovine sources. It is more probable that the qualitative results cannot be so minutely compared. At any rate, while Houston's results with milk differ so much from mine, it is not advisable to lay too much emphasis upon the closer resemblance between his human and my milk strains.

His conclusion that milk streptococci compared with the human strains are more often positive for lactose and inulin, and negative for salicin and raffinose, is but partly supported by my results. Houston's work with bovine, sewage, and water streptococci adds

[†] H indicates Houston's figures; B, my own.

but little, directly or indirectly, in further explanation of my results. In his figures for bovine streptococci, groups containing 75 per cent and 13 per cent correspond to smaller groups of 2 and 21 per cent respectively in my milk strains. In the mixed city sewage where, except for the predominance of equine strains one might expect greater likeness to the results with milk streptococci, both representing the same possible mixed sources, we find still greater differences; the two largest groups, 49 per cent and 44 per cent, are represented by but 1 per cent of the milk strains. The commonest type isolated from London city water fermented (qualitatively, of course) lactose, saccharose, salicin, and raffinose, and is represented by but 4 per cent of my strains.

In 1910 Winslow and Palmer¹ published a comparative study of intestinal streptococci from the horse, the cow, and man. Dextrose, lactose, mannit, and raffinose were used. The results were estimated quantitatively. To test the application of these results to milk streptococci the present study was begun. Dextrose, which seemed to be fermented by practically all the strains, was omitted in my series, and salicin, mannit, and inulin added. For comparison Winslow and Palmer's percentage acidity classes² are reprinted with my milk results (Table 4).

It will be noticed that in the amount of acid formed the milk strains differ greatly, the highest values being remarkably high; in lactose, the highest record was 6.3 per cent, salicin, 7.5 per cent, and in saccharose, 8.4 per cent. The highest for the fecal streptococci (Winslow and Palmer) was 3.6 per cent in lactose. This was reached by but one of their 300 strains, or 0.3 per cent. In the 100 milk strains 3.6 per cent acid was equaled or exceeded by 28 per cent of the strains in lactose, 40 per cent in saccharose, and 56 per cent in salicin. As stated earlier, sugar-free broth was used in making my special media. It averaged 1.6 per cent acid (1.2–2.2 per cent to phenolphthalein) in the controls. This high initial acidity accentuates the difference in the acid values, instead of

¹ *Jour. Infect. Dis.*, 1910, 7, p. 1

² These have been rearranged from Winslow and Palmer's published tables showing the percentage of acid formed by each strain, as a few mistakes were found in their table of percentage acidity classes. To avoid decimals the approximate per cent of strains is given for those obtained from the cow and man.

explaining it. Were it not that some of my records are also lower than those of Winslow and Palmer, this high acid production might be considered to be due to the fact that I used milk as a medium during the isolating period.¹ In this connection it must be remembered that in any milk samples, the streptococci have, of course, grown in milk some time before isolation. In all other respects the method (incubation period, color limit for phenolphthalein reaction, titration in the cold, etc.) was the same as that used by Winslow and Palmer.

TABLE 4.
COMPARATIVE TABLE OF PERCENTAGE ACIDITY GROUPS.

	-1	0	+1	2	3	4	5	6	7	8
<i>Lactose</i>										
Horse		66	26	4	2	1	0	1		
Cow		31	16	1	0	9	9	23	0	
Man		17	20	2	4	14	10	20	10	
Milk		4	11	7	2	13	18	15	7	
<i>Raffinose</i>										
Horse		54	42	1	0	2	1			
Cow		42	30	0	3	7	13	3	1	
Man		29	65	2	1	0	1	20	1	
Milk		2	12	54	16	2	1	0	2	
<i>Mannit—</i>										
Horse		62	36	0	1	1				
Cow		62	31	2	3					
Man		40	28	5	12	10	0	1		
Milk		2	18	28	18	7	1	5	5	
<i>Saccharose—</i>										
Milk	3	3	15	9	2	4	6	3	15	16
<i>Salicin—</i>										
Milk		5	10	3	4	1	5	6	10	16
<i>Inulin—</i>										
Milk	5	27	16	11	3	1	2	9	6	8

These high records are even more remarkable if the high initial acidity is taken into consideration. Later work will be done with broth controls to see how much the milk affects the acid-forming powers of the streptococci. If the acid results are interpreted as the point at which acidity checked the growth, it is necessary to add the initial acidity to these records; the highest records are then lactose, 8.1 per cent; salicin, 9.0 per cent, and saccharose, 10.1 per cent.

¹ Hilliard and Stowell (*Science*, N.S., 1912, 35, p. 223) have stated that milk streptococci are "much more facultative than throat strains in relation to the temperature at which they are grown."

With these higher acidity values obtained for the milk streptococci occurs a shifting of the intermodal point between the non-acid and the acid-forming groups. Instead of 0.5 adopted by Winslow and Palmer, it is about 1.5 per cent for my milk strains. The results with these fecal streptococci have been recharted here

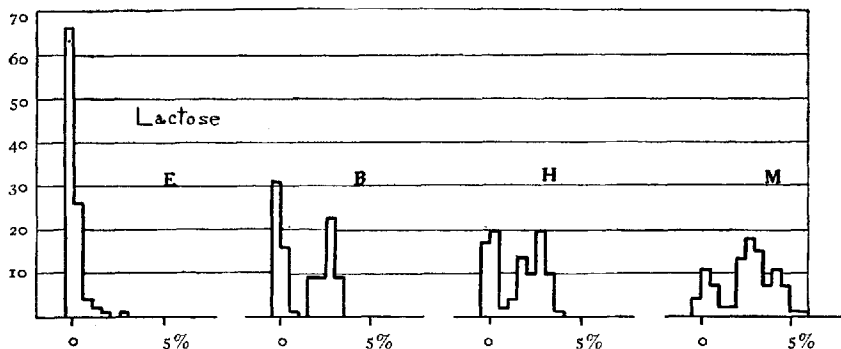


CHART 1.—Acid-producing power of streptococci in lactose. E, equine; B, bovine; H, human; and M, milk.

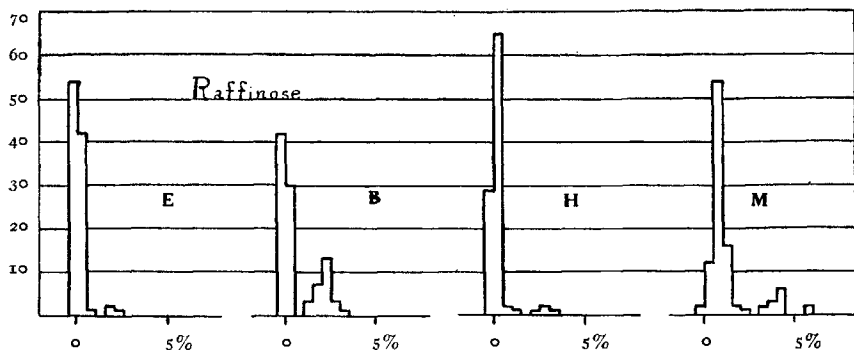


CHART 2.—Acid-producing power of streptococci in raffinose. E, equine; B, bovine; H, human; and M, milk.

for further comparison with my milk streptococci (Charts 1, 2, and 3). In lactose, saccharose, and salicin, for which we have comparable data, it will be noted that the milk charts are more like the human ones in lactose and mannit; in raffinose, the resemblance is nearer the bovine than the human.

Charts are also given for the acid formed in inulin, salicin, and

saccharose (Chart 4). It is unfortunate that similar ones for the fecal streptococci are not available, because (1) inulin has been considered not fermented by streptococci; and (2) salicin and

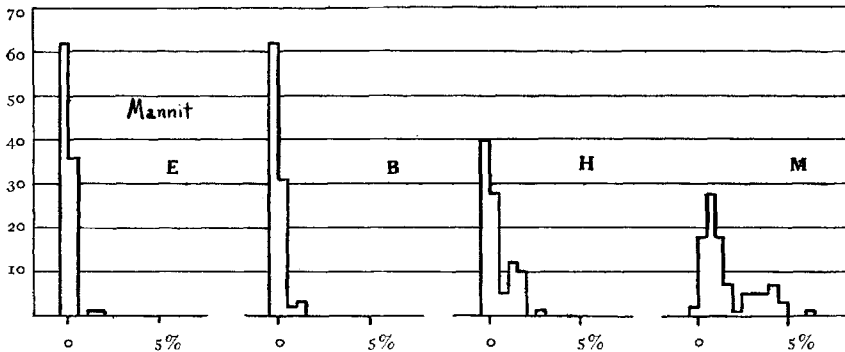


CHART 3.—Acid-producing power of streptococci in mannitol. E, equine; B, bovine; H, human; and M, milk.

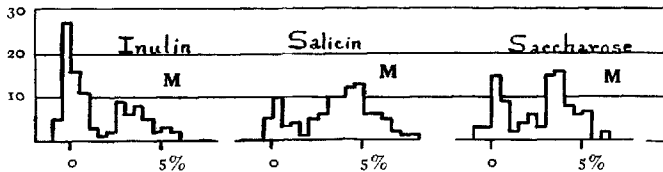


CHART 4.—Acid-producing power of milk streptococci in inulin, salicin, and saccharose. M, milk.

saccharose are much like lactose in the proportion of acid and non-acid strains, and it is possible that they may resemble lactose in diagnostic value.

SUMMARY.

Morphological characters are not correlated with fermentative powers. Milk and neutral red are not sufficiently diagnostic to aid in determining the sources of streptococci. Lactose, saccharose, salicin, raffinose, mannitol, and inulin seem to have significant fermentative reactions. Saccharose, salicin, and inulin should be tested with human, bovine, and equine streptococci. The milk streptococci form a large number of groups when classified with regard to their effect upon the six test substances. The milk

streptococci are characterized by unusually high fermentative powers. The incomplete data at hand indicate that the milk strains are most like the human strains; there is less likeness between the milk and the bovine strains; they show practically no resemblance to the equine strains.

It is proposed to continue this quantitative comparison of fecal (human, bovine, and equine) and milk streptococci in these and other media, in the hope that *complete* quantitative comparisons will give a method of determining the source of streptococcal pollution of milk.