The origin of some of the streptococci found in milk / by L.A. Rogers and Arnold O. Dahlberg, Dairy Division, Bureau of Animal Industry.

REPRINT FROM

JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

VOL. I WASHINGTON, D. C., MARCH 25, 1914 NO. 6

THE ORIGIN OF SOME OF THE STREPTOCOCCI FOUND IN MILK

By L. A. ROGERS and ARNOLD O. DAHLBERG

THE ORIGIN OF SOME OF THE STREPTOCOCCI FOUND IN MILK

By L. A. Rogers and Arnold O. Dahlberg,
Dairy Division, Bureau of Animal Industry

INTRODUCTION

In the higher plants and animals we are accustomed to associating species with a more or less definite habitat. Certain animals are found only in certain localities. One species of trees may be found only on a particular type of soil. A still narrower limit of distribution is found in some of the parasitic fungi which grow only on closely related host plants. Zoologists or botanists find the types on which they base their descriptions in the natural habitat of the organism. This relation has not always existed in the published descriptions of bacteria. The association of a natural group with a particular habitat has been more or less incidental, except perhaps with the pathogenic bacteria, and even with some of these it is not impossible that the pathological conditions under which they are found may not be the true habitat of the species. The colon group, while it is frequently found in water and milk, has its natural habitat in the intestinal tract of warm-blooded animals. Winslow found that certain chromogenic cocci were associated with the skin of animals.[1] Some of the English bacteriologists have pointed out that the streptococci from horse manure, for instance, have a set of physiological reactions which differentiates them from those from saliva or pathological conditions.[2] It is only through a knowledge of the habitat and the study of sufficient cultures to establish a type that true bacterial species can be determined. If we were to write a description of the German people we would go to Germany, not to an American city where German immigrants live.

Countless descriptions have been written of bacteria isolated from milk until we have come to consider certain types as peculiar to this medium. The bacteria found in milk, however, are a heterogeneous collection, and the true types of milk bacteria are to be sought in the sources from which milk is contaminated. Esten has suggested that the streptococci or lactic-acid bacteria of milk come originally from the mouth of the cow.[3] The feces of the animal must, unfortunately,

[1] Winslow, C. E. A., and Winslow, Anne R. Systematic relationships of the Coccaceæ. ed. 1, 300 p., illus. New York, 1908.

[2] Andrewes, F. W. Report on the micro-organisms present in sewer air and in the air of drains. 36th Ann. Rpt. Local Govt. Bd. [Gt. Brit.], 1906–07, Suppl. Rpt. Med. Off., p. 183–204. 1908.

[3] Esten, W. M. Bacterium lactis acidi and its sources. Conn. Storrs Agr. Expt. Sta. Bul. 59, 27 p., 5 fig. 1909.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.

Vol. I, No. 6
Mar. 25, 1914
A—3

be considered as a possible source of bacteria in milk, among which would undoubtedly be found members of the lactic group. Kinyoun and Dieter believe that the presence in milk of cocci which form chains in lactose bile at 37° C. is presumptive evidence that the milk is contaminated with feces.[1] It is the more common practice, however, to consider this type as the indication of the presence in the herd producing the milk of one or more cows with infected udders.

The mouth is known to contain streptococci, and the habit of cows of licking their flanks and udders provides a more or less direct connection between the mouth and the milk pail. Each of these sources may be considered as the normal habitat of bacteria. Under these conditions they persist for indefinite generations, adapting themselves to their environment until it is reasonable to suppose the characters acquired become sufficiently fixed to have at least varietal significance.

The study of streptococci originating within such circumscribed limits is of interest in addition to its taxonomic importance, in the light it may cast on the origin of some of the bacteria in milk and the significance from the hygienic standpoint of the presence of certain types.

In this paper are recorded the results of a study of streptococci representing three of the possible sources from which this group may find its way into milk. The morphology of this collection was studied with the hope that this would give some basis for a division into varieties. The ability of these cultures to utilize a number of carbohydrates and alcohols was determined. On the basis of these fermentations several groups are established, each of which is made up of a large number of identical cultures constituting the type about which are grouped similar cultures, but which varied from it in one or two reactions. The probable relation of one of these groups to well-known species is pointed out.

THE CULTURES STUDIED

A collection of streptococci were obtained from milk, from bovine feces, from the mouths of cows, and from the udders of cows. With the exception of those from milk an effort was made to make the cultures as representative as possible. The procedure of isolating the milk cultures followed that usually employed in the laboratories of boards of health. Small portions of the milk were added to lactose-bile tubes which were incubated at 37° C. Tubes showing streptococci in distinct chains on microscopical examination were plated on lactose agar and the chain-forming cocci subcultured. In this way 42 cultures were isolated from 25 samples of milk and cream collected at Washington or at the creamery at Troy, Pa. No two samples came from the same farm. A few cultures were obtained through the courtesy of Dr. Kinyoun and Mr. Dieter from lactose-bile tubes in the laboratory of the health department of the District of Columbia. These cultures, therefore, did not

[1] Kinyoun, J. J., and Dieter, L. V. A bacteriological study of the milk supply of Washington, D. C. Jour. Amer. Pub. Health Assoc., v. 2, no. 4, p. 262–274. 1912.

represent the normal streptococci of milk but rather those which would usually be distinguished as indicating contamination from infected udders or fecal sources.

Fifty-one cultures were isolated from 19 samples of milk obtained by milking directly into sterile test tubes. The cows from which these samples were obtained represented all gradations of infected udder from occasional evidence of garget to acute mammitis. Part of these were in the Dairy Division herd at Beltsville, Md., and the remainder in the herd on the Naval Academy farm at Annapolis, Md. One hundred and fourteen cultures came from 56 samples of cow manure obtained, with the exception of a few from Troy, Pa., at the Dairy Division farm and at the dairy of the Government Hospital for the Insane at Washington. Thirty-nine cultures were made from the mouths of animals at the Dairy Division farms. With the exception of one culture obtained from the mouth of a mule, all of these cultures were of bovine origin. In Table II the origin of the culture is indicated by M for milk, U for udder, F for feces, and B for mouth. The sample from which the culture was secured is indicated by a number following the letter. For instance, "F15" represents sample of feces No. 15. This will enable the reader to determine the origin of each culture and the number of cultures from each sample.

MORPHOLOGY OF THE CULTURES

While it is generally recognized that there is little morphological basis for subdivisions of the streptococci, reference is frequently made to certain types of cells. Stowell, Hilliard, and Schlesinger,[1] in selecting streptococci from milk for comparison with those isolated from the human throat, rejected diplococci and the oval-chained form which they designate as the *Streptococcus lacticus* of Kruse or the *Bacillus lactis acidi* group, respectively. In selecting our cultures no attention was paid to morphology beyond determining that it was a coccus apparently dividing in one plane, with the exception of those from milk, which were not accepted if they did not form chains of at least 8 or 10 cells. The morphology of nearly all cultures was determined by examination of specimens stained with gentian violet. Camera-lucida drawings were made using a Leitz 3 mm. objective and No. 18 ocular, a combination which gave a magnification of 2,400 diameters at the ocular, or 4,800 diameters on the drawing board. Sufficient light to give a clear image was obtained by using a special arc light with a copper-sulphate ray filter.

Preliminary studies showed that the medium on which the culture was grown had an appreciable influence on both the size and the form of the cell. This is shown in figure 1, which is reproduced from camera-lucida drawings of typical cultures grown on various media. Milk gave quite

[1] Stowell, E. C., Hilliard, C. M., and Schlesinger, M. J., A statistical study of the streptococci from milk and from the human throat. Jour. Infect. Diseases, v. 12, no. 2, p. 144-164. 1913.

uniformly smaller cells and less tendency to chain formation than broth or agar. The cells at *a* are from culture *lo* on milk, *b* on broth, and *c* on agar, all incubated 48 hours at 37° C. The difference between the distinctly rod-shaped cell found on agar and the small round cell obtained from milk is marked. That differences in size of cells are not due entirely to differences in the medium is shown by the chain at *h*. This combination of small and large cells in a single chain is not unusual in broth, a medium in which there is a marked tendency to form enlarged and abnormal cells. In some cultures the transition from normal cells to those of monstrous size and form was so rapid that it was difficult to obtain preparations showing what could be considered normal cells. The most satisfactory preparations were obtained in incubating broth cultures until a distinct cloudiness was obtained, centrifuging the culture, siphoning off the broth, and washing the sediment with sterile water. After centrifuging again the water was siphoned off, and a preparation made from the sediment. This gave a clear field suitable for examination under a high-power microscope.

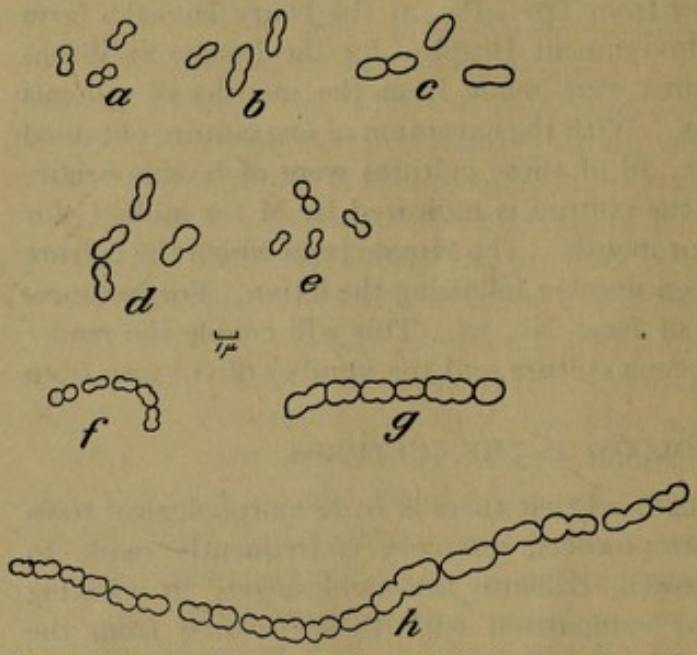

FIG. 1.—Cells of streptococci, showing variation in size and morphology. *a*, culture *lo* on milk; *b*, culture *lo* on broth; *c*, culture *lo* on agar; *d*, culture *li* on lactose bile; *e*, culture *li* on broth; *f*, culture *gm* on milk; *g* and *h*, culture *gm* on broth.

Various types of cells which were found in this collection are shown in figure 2. It will be observed that much of the variation in these types is in size only or in chain formation. The slender-pointed cells shown at *F* were peculiar to the cultures obtained from the mouth of animals, but the cultures from this source were not confined to this type. In Table II the letter under the heading "Morphology" refers to figure 2, although it is obvious that in many cases the assignment to a particular type can be only an approximation. The variation of the morphology is so great and so easily affected by the environment that it was not considered in the final arrangement of groups. It should be stated, however, that among the udder cultures the tendency to chain formation was much more marked and more constant than among all other cultures.

METHODS OF DIFFERENTIATION

When morphological distinctions are lacking, we are forced to use the physiology of the organism as a basis of classification. No single system

of characters can be adopted for all classes of bacteria. The significant characters will be found for each group only by a study of its normal activities and the utilization of those functions which show the nature, limitations, and relationship of the group. The striking characteristic of the streptococci is their ability to form acids from carbohydrates and related substances, and this peculiarity has been very generally utilized for purposes of classification. The voluminous literature bearing pro and con on the constancy and the value of these tests has been reviewed fully in various papers and need not be taken up here. It may be safely asserted, however, that the fermentative ability is as constant and as significant for purposes of classification as the characters adopted by those who reject the fermentation tests as too variable. For instance, Davis, who rejects the sugar fermentations as untrustworthy, divides the

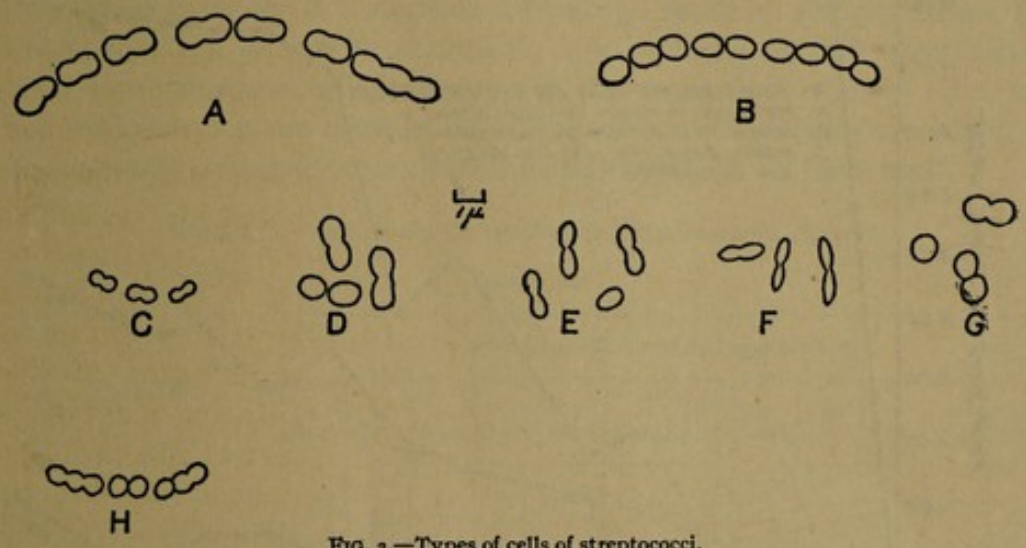

FIG. 2.—Types of cells of streptococci.

streptococci into five groups on the basis of hemolysis, green colonies on blood agar, capsule, solubility in bile, inulin fermentation, experimental arthritis, and experimental endocarditis.[1]

For purposes of classification, we have used the liquefaction of gelatin and the fermentation of dextrose, saccharose, lactose, raffinose, starch, inulin, mannite, and glycerin. Adonite and dulcite were tested, but as they were fermented by only one or two of these cultures they were of no value. The liquefaction of gelatin was determined by inoculating the surface of the gelatin tube with a few drops of a broth culture and measuring the liquefaction after 30 days at 20° C.

The fermentation of the test substances was determined in a medium made as follows:

	Per cent.
Beef extract	0.4
Peptone	1.0
Dibasic potassium phosphate	.5
Test substance	2.0

[1] Davis, D. J. Interrelations in the streptococcus group with special reference to anaphylactic reactions. Jour. Infect. Diseases, v. 12, no. 3, p. 386. 1913.

The cultures were incubated for seven days at 30° and titrated cold against twentieth-normal sodium hydrate with phenolphthalein as an indicator. From the results so obtained is subtracted the titration of a blank, and the result is expressed as the percentage of normal acid. Some objection may be raised against the use of 30° C. as an incubation temperature rather than the more common one of 37°. The lower temperature was adopted because practically all streptococci will grow at this temperature, while a few grow at 37° slowly or not at all.

The fermentation produced by the streptococci is in almost all cases so marked that there is very rarely any question about the presence or

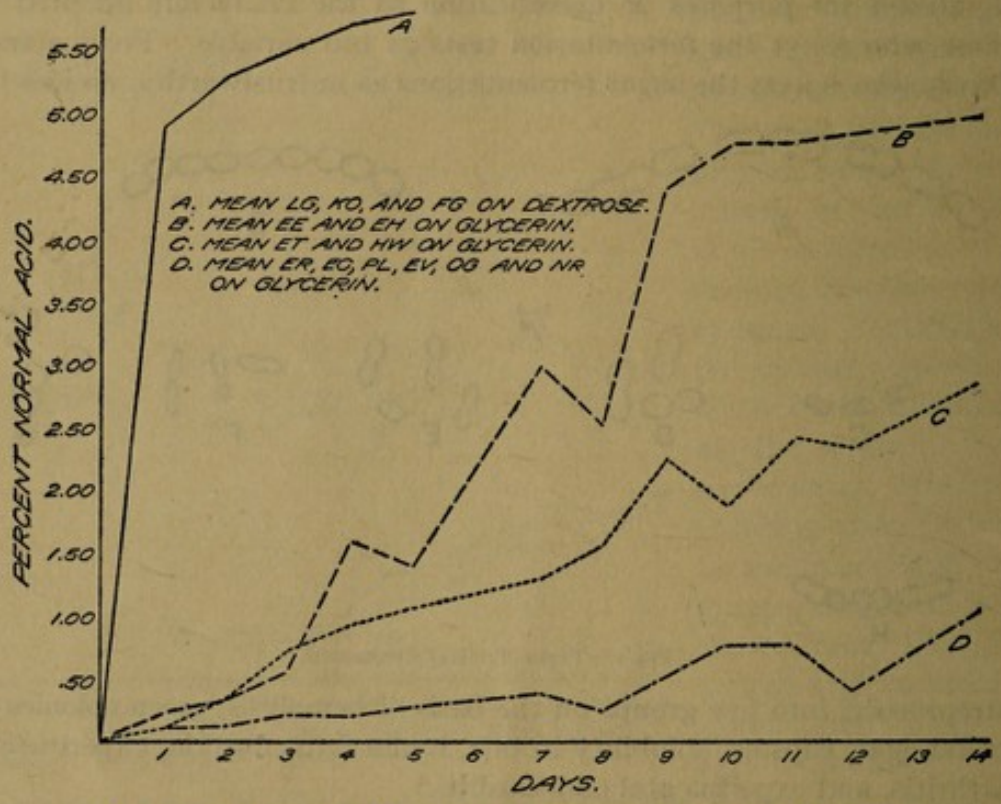

FIG. 3.—Curve showing the typical rate of fermentation of dextrose and glycerin.

absence of the fermentation. Of all the substances we have used glycerin forms an exception to this rule. The fermentation proceeds slowly and in seven days may be slightly above or slightly below 1 per cent normal acid, the point selected as marking the line between fermentation and no fermentation. This is illustrated by Table I, which shows the progressive rate of fermentation by typical cultures. Three cultures fermenting dextrose are included to show the usual course of the fermentation in the more easily fermented sugars. Each titration was made from a separate tube. A study of this table shows that the 12 cultures may be divided into three quite distinct types on the basis of the rate of fermentation of glycerin. This is shown more clearly in figure 3, in which the average titrations for each of the three types are plotted. Two of these cultures fermented the glycerin with comparative

rapidity and after three days there was no question that the cultures were able to utilize glycerin. Those represented by the curve *D*, on the other hand, produce only a very slight increase in acidity, which even at the end of 14 days is only slightly above 1 per cent normal.

Between these two is a third group in which there is a slow but distinct increase in acidity. At seven days the acidity is above 1 per cent normal. While an error may be introduced in some cases by drawing the line between fermentation and no fermentation of glycerin at 1 per cent normal, it is believed that in these results this error will be slight. These results illustrate the value of the exact results obtained by titration which we have always used in preference to the simpler and more rapid way of determining the change of reaction with litmus in solution in the broth or with litmus papers.

We also consider it a decided advantage to allow sufficient time for the completion of the fermentation, thus securing an end point rather than some intermediate and varying determination. Seven days are not sufficient for the completion of the glycerin fermentation, but it is undoubtedly ample for other test substances which we have used.

TABLE I.—*Progressive fermentation of dextrose and glycerin.*

DEXTROSE.

Culture No.	Percentage of normal acid produced in—											
	1 day.	2 days.	3 days.	4 days.	5 days.	7 days.	8 days.	9 days.	10 days.	11 days.	12 days.	14 days.
lg	4.6	5.2	5.2	5.6	5.5	.....	.....	.....	.....	.....	.....	.....
ko	4.6	5.0	5.2	5.5	5.5	.....	.....	.....	.....	.....	.....	.....
fg	5.2	5.8	6.2	6.1	6.5	.....	.....	.....	.....	.....	.....	.....

GLYCERIN.

ec	0.15	0.19	0.31	0.34	0.44	0.55	0.58	0.91	0.53	0.96	0.78	1.21
ee	.25	.23	.80	1.67	1.31	3.65	2.73	4.96	4.85	4.96	.....	5.43
eh	.22	.46	.35	1.57	1.54	2.40	2.38	3.96	5.06	4.61	3.74	4.68
er	.15	.14	.37	.37	.96	.86	.28	.63	1.83	.92	.33	1.13
et	.05	.59	.82	.95	1.09	1.30	1.49	2.16	1.73	2.18	2.30	2.63
ev	.15	.09	.34	.17	.00	.40	.45	.66	1.03	.96	.70	1.18
hw	.20	.11	.67	.95	1.07	1.35	1.67	1.38	2.08	2.71	2.45	3.15
nr	.15	.09	.14	.00	.00	.25	.02	.31	.43	.76	.21	.....
ny	.20	.29	.04	.00	.05	.03	.00	.01	.00	.21	.00	.....
og	.11	.09	.02	.07	.09	.05	.02	.11	.48	.46	.03	.....
om	.15	.00	.09	.11	.09	.06	.00	.21	.13	.19	.00	.00
pl	.05	.09	.22	.32	.24	.36	.31	.71	.53	.76	.66	.78

THE CORRELATION OF PHYSIOLOGICAL CHARACTERS

The complete results of the tests made on this collection of cultures are presented in Table II. The reduction of neutral red and the curdling of milk are given in the table, but are not used in the correlations. Adonite and dulcite are necessarily excluded, since these cultures almost without exception fail to ferment these two substances.

TABLE II.—*Physiological characters of all cultures.*

Culture No.	Origin.	Morphology.	Chains.		Liquefied gelatin.	Neutral red reduction.	Milk curd.	Percentage of normal acid.									
			Milk.	Broth.				Dextrose.	Adonite.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Dulcite.
					mm.												
ec	M1	G	....		0	−	+	4.1	0	0.1	4.3	0	1.3	0	0	0.6	0.1
ed	U1	G	....		30	+	+	1.7	.1	4.0	4.0	0	.4	0	2.4	.8	0
ee	U1	G	....		28	+	+	2.5	0	4.1	4.2	.1	.2	.2	2.9	.8	.1
eh	U1	G	....		27	+	+	2.6	0	3.6	3.8	.2	.2	0	1.8	.9	0
ej	U2	B	....	+	0	−	+	2.7	0	2.7	4.6	0	0	.1	0	.1	.1
en	M2	G	....	−	0	−	+	4.4	0	0	4.5	.1	.2	.1	2.3	.6	0
eo	M3	GE	....	−	50	−	+	4.3	.1	4.7	5.1	.2	.4	.5	4.4	1.7	.2
eq	M4	GE	....	−	0	−	+	4.1	.1	4.8	4.8	0	.2	.1	4.0	2.1	.1
er	M5	G	....	−	0	−	....	4.6	0	.2	5.4	0	.2	0	.1	.7	0
et	M6	G	....	−	0	−	+	6.5	0	4.1	5.1	.1	.1	.2	3.9	.9	0
eu	M7	G	....	−	0	−	+	6.6	0	.4	5.0	0	0	0	2.4	.4	0
ev	M7	G	....	−	0	−	+	3.0	0	.3	5.0	0	0	.1	2.1	.3	0
ew	U1	G	....	−	0	−	−	4.9	0	3.9	3.6	0	0	0	1.5	.7	0
ez	U1	G	....	−	19	−	−	4.7	0	3.0	3.6	.1	.1	0	.9	.6	0
fa	U1	G	....	−	55	−	+	2.0	.2	3.4	3.2	.2	.3	.4	.8	.8	.2
fc	U2	D	....	−	0	−	−	1.8	0	0	.4	0	0	0	0	0	.2
fg	U2	EG	....	−	49	−	+	6.6	0	.4	5.6	0	.3	.2	6.2	2.0	.2
fi	M8	.....	−	....	0	−	−	5.3	0	5.6	5.6	.2	.1	0	3.9	0	.1
fj	M9	C	−	−	0	−	+	5.6	0	5.3	4.9	.1	0	0	3.5	0	0
fk	M10	.....	−	....	0	−	+	7.1	.2	.2	5.7	.3	0	.1	0	.5	.2
fl	M11	.....	−	....	0	−	+	5.0	0	.4	5.2	.4	.2	.2	4.3	1.3	.1
fn	M12	.....	−	....	.....	−	−	5.1	0	5.5	5.3	0	.2	0	4.2	0	0
fo	M12	G	....	−	0	−	....	5.5	.1	5.6	4.5	0	0	0	4.4	.1	0
fp	M8	E	....	−	0	−	....	5.9	.1	5.8	4.7	0	.2	0	4.3	.4	0
fr	M13	G	−	−	0	−	+	5.3	0	4.8	5.5	.3	0	.1	4.3	1.2	.1
ft	M9	.....	−	....	0	−	+	5.4	0	5.1	5.3	0	.1	.1	2.7	.2	.1
fu	M10	G	−	−	0	−	+	7.0	0	.1	5.2	0	0	0	.1	.5	0
fv	M10	.....	−	....	0	−	+	6.6	0	.1	5.6	.1	.1	.1	0	.5	0
fw	M14	.....	....	....	0	−	+	5.4	0	4.8	5.2	.2	.1	.1	4.1	1.2	.1
fx	M15	C	....	−	0	−	....	5.3	0	5.4	4.6	0	0	0	3.8	.1	0
fy	M15	.....	.....	....	0	−	−	5.3	0	5.5	5.2	.2	.1	0	4.3	1.1	0
ga	M16	.....	.....	....	0	−	+	6.0	0	0	5.6	.2	0	0	.1	.1	0
gb	M16	.....	.....	....	0	−	+	6.5	.1	.1	5.4	0	.1	.2	.1	.4	.1
gc	U3	H	....	+	0	−	....	4.4	0	3.6	4.0	0	.1	3.8	0	0	0
ge	U3	.....	+	....	0	−	+	4.6	0	4.7	4.8	.3	.4	0	0	.1	1.9
gh	U4	.....	+	−	0	−	+	4.2	0	0	4.9	.1	0	.1	0	.1	0
gi	U5	H	....	+	.....	−	+	4.8	0	.1	4.3	0	.2	0	0	.1	0
gk	U5	B	+	+	0	−	+	4.5	.1	4.4	4.8	.1	0	.1	0	.3	0
gl	U5	.....	.....	.....	.....	−	+	4.6	0	0	4.3	0	0	0	.2	0	0
gm	U5	A	+	+	0	−	+	4.4	.1	4.7	4.9	.2	0	0	0	0	0
go	U6	.....	+	....	0	−	+	4.5	0	4.8	4.8	0	0	0	0	0	0
gp	U5	.....	+	....	.....	−	+	4.3	.3	0	4.2	0	.7	.2	0	.1	0
gt	f1	.....	−	....	0	−	+	4.7	.3	4.6	4.6	.1	.4	0	0	.3	0
gw	U6	.....	+	....	0	−	+	4.5	0	4.6	5.0	.1	0	0	0	0	.1
gx	U6	.....	+	....	0	−	+	4.5	.1	4.9	5.0	.1	0	.2	0	0	0
gz	U2	B	....	+	.....	−	+	4.7	0	0	4.4	0	0	0	0	0	0
ha	M17	E	−	−	0	−	+	6.4	0	0	5.1	.1	0	0	2.3	.3	0
hb	M17	E	−	−	0	−	+	6.4	.2	0	5.1	.1	0	.1	3.8	.4	.1
hc	M17	.....	−	....	0	+	+	5.5	.1	0	5.9	.1	.2	0	.2	.5	0
hd	M18	E	−	−	0	−	+	5.8	0	5.6	5.2	0	0	.1	3.9	.4	0
he	M18	E	−	−	.....	−	+	6.1	0	5.5	5.6	.1	0	0	3.4	.4	.4
hg	M19	G	....	−	.....	−	+	5.9	.2	.2	5.5	.1	.1	.1	.2	.7	0
hh	M19	.....	−	....	0	−	+	6.2	.1	0.1	5.5	.1	.2	.2	0	0.6	0
hj	M20	.....	−	....	0	−	+	6.8	0	0	4.7	.1	.2	0	0	.4	0
hk	M21	.....	−	....	.....	−	+	4.3	.2	.2	5.5	.1	.1	0	0	.5	.1
hl	M21	.....	−	....	0	−	+	7.1	.2	0	5.6	0	0	0	0	.5	.1
hm	M22	E	−	−	0	−	+	6.1	.4	0	5.3	.1	.1	.1	2.8	.3	0
hn	M22	.....	−	....	0	−	+	5.6	.2	0	6.3	.1	.1	0	2.7	.4	0
ho	F2	.....	−	....	0	−	+	5.7	0	6.0	5.5	5.3	0	.1	0	0	0
hq	F3	E	−	−	.....	−	−	6.4	.2	5.5	5.3	0	0	0	.1	.1	0
hr	F3	.....	....	....	.....	+	−	4.7	.....	5	5.0	.1	0	.1	0	0	.1
hs	F4	.....	−	....	0	−	+	5.8	.3	5.8	5.7	5.3	0	0	0	.1	0
hu	M23	E	−	−	.....	−	+	6.0	.1	5.5	5.2	.3	.3	0	4.6	0	0
hv	M23	E	−	−	0	−	+	6.2	.7	5.1	.7	0	0	0	5.0	1.5	.1
hw	M24	.....	−	....	0	−	+	5.3	.3	4.8	5.1	.2	.2	0	4.3	.5	0
hx	M24	.....	−	....	0	−	+	5.0	.1	4.7	5.2	.2	.2	.1	4.1	1.5	0
hy	F5	.....	−	....	0	−	+	5.7	.2	5.9	5.5	5.3	4.7	0	0	0	.1
hz	F5	.....	....	....	0	−	+	5.1	0	5.4	5.1	5.0	4.7	0	0	.1	0
ib	F6	.....	−	....	0	−	+	5.8	.2	6.4	5.8	5.1	0	0	4.3	.2	0
ic	F6	.....	−	....	0	−	+	5.7	0	6.7	5.6	5.2	0	0	4.3	.2	0
id	F7	.....	−	....	0	−	+	5.7	0	5.8	5.6	5.4	0	0	0	0.1	0
ie	F7	.....	....	....	0	−	+	5.7	0	6.0	5.4	5.3	0	0	0	0	0
if	F8	.....	−	....	0	−	−	4.9	0	5.5	5.4	0	0	0	0	0	0.1
ig	F8	.....	....	....	0	+	−	4.3	0	4.6	5.2	5.9	0	0.1	0	.1	0
ih	F9	G	....	−	0	−	....	6.1	.2	5.5	4.8	1.8	0	0	1.7	.2	0
ii	F9	.....	.....	....	0	−	−	7.7	.1	8.9	5.4	3.2	0.2	0	1.4	.3	0

TABLE II.—*Physiological characters of all cultures*—Continued.

Culture No.	Origin.	Morphology.	Chains.		Liquefied gelatin.	Neutral red reduction.	Milk curd.	Percentage of normal acid.									
			Milk.	Broth.	mm.			Dextrose.	Adonite.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Dulcite.
ik	M25	G	–	–	0	–	+	5.2	0.2	4.3	4.7	3.2	0.2	2.9	4.0	0.2	0.1
il	M25	.....	–	...	0	–	+	4.6	.2	4.4	4.7	3.5	0.2	3.2	3.7	.3	.1
im	F10	E	–	–	0	–	+	6.5	0	6.1	5.0	4.8	4.6	0	.3	.1	0
in	F10	.....	–	....	0	–	+	4.8	0.2	5.5	5.5	5.1	4.4	0	0	0	0
io	F11	.....	–	....	0	–	+	5.8	0	5.8	5.1	5.0	.2	0	0	.3	0
ip	F12	.....	–	....	0	–	....	5.4	.1	5.4	5.6	5.0	.1	0	0	.2	0
ir	F13	E	...	–	0	–	....	5.6	.2	7.6	5.7	4.5	.3	0	1.8	.2	0
is	F13	.....	–	....	0	–	+	4.8	.1	5.6	5.9	5.9	4.4	0	0	0	0
it	F13	.....	–	....	.....	–	+	5.5	.1	5.7	5.9	6.0	4.7	.1	0	0	.1
iu	F5	E	–	–	0	–	+	5.5	0	5.3	4.9	4.8	6.2	0	0	.4	0
iv	F5	.....	–	....	0	–	....	6.1	.2	6.2	5.4	.1	.2	0	5.3	.8	0
ix	F14	.....	–	....	0	–	+	5.5	.1	6.2	5.8	5.1	4.5	0	4.1	.2	0
jd	F15	D	–	–	0	–	+	6.9	0	6.0	5.2	4.7	6.2	0	0	0	0
je	F15	.....	–	....	0	–	+	4.3	.1	5.4	5.1	5.0	0	0	0	0	0
jf	F15	E	–	–	0	–	....	5.6	.2	6.3	5.5	5.3	0	0	0	0	0
jg	F16	.....	–	....	0	–	....	5.7	.1	5.8	5.5	5.0	4.4	0	0	0	0
jh	U7	.....	+	....	0	–	+	4.5	.2	4.2	5.1	0	.1	0	0	.1	0
ji	U7	.....	+	....	0	–	+	4.5	.1	4.6	4.9	0	.2	0	0	.1	0
jk	U2	.....	–	....	.....	–	+	4.7	0	4.4	4.6	0	0	.1	0	.1	0
jl	U5	.....	–	....	0	–	+	4.8	.1	4.9	4.4	.1	0	.1	0	.1	.1
jm	U5	.....	+	....	0	–	+	3.4	.1	4.8	4.5	.3	.1	.2	0	.2	.1
jn	U3	H	....	+	0	–	....	4.8	0	4.5	3.6	.2	.3	3.9	2.7	.2	0
jq	U8	.....	....	....	0	–	....	4.8	.1	3.8	0	.1	.2	.1	3.6	0	0
jr	U8	.....	....	....	0	–	–	4.5	.1	3.8	4.0	0	0	0	3.4	.1	.1
js	F17	D	–	–	0	–	+	6.4	.2	6.2	4.9	4.9	0	0	.3	0	0
jt	F17	.....	–	....	0	–	+	5.5	.1	5.8	5.3	5.2	0	.1	0	.1	0
ju	F17	G	–	–	0	–	+	5.4	.2	5.9	5.3	5.1	4.8	0	.2	.1	0
jv	F18	E	....	–	0	–	....	5.8	.1	4.9	5.2	4.9	3.9	6.4	0.1	.1	0
jw	F18	.....	....	....	0	–	–	4.9	0	5.1	4.9	5.5	.1	.2	.1	0	.1
jx	F18	.....	....	....	0	–	–	5.9	0	4.8	4.9	5.1	6.0	5.8	0	0	.1
jy	F19	.....	–	....	.....	–	–	3.5	0	5.3	5.4	6.0	.2	0	.3	.1	0
jz	F20	.....	–	....	0	–	+	5.4	.2	5.8	5.1	5.1	.1	0	0	.2	0
ka	F20	E	–	–	0	–	+	5.8	0	5.8	5.5	5.2	0	0	0	.1	0
kb	F20	.....	–	....	0	–	–	6.9	0	0	5.5	0	.2	.1	.1	0	.1
kc	F21	.....	–	....	0	–	....	5.5	.1	4.9	5.3	4.8	4.6	.1	0	.1	0
kd	F21	.....	–	....	0	–	+	5.4	.1	5.8	5.0	5.1	4.6	0	0	0	0
ke	F21	.....	–	....	0	–	+	6.1	.1	5.0	5.1	4.6	.1	0	0	0	0
kf	F22	.....	.....	.....	.....	–	+	5.9	.1	6.1	6.2	0	.2	.1	3.9	.1	0
ki	F23	C	–	–	0	–	+	6.1	.1	6.5	5.2	4.8	.1	0	0	0	0
kj	F23	E	–	–	0	–	+	5.9	.1	5.9	5.0	5.3	0	0	0	0	0
kk	F24	G	–	–	0	–	+	6.6	.2	0	5.0	4.4	6.3	0	.1	0	0
kl	F24	C	–	–	0	–	+	5.8	.1	5.9	5.2	4.3	.1	0	4.0	0	0
km	F24	H	–	+	0	–	+	6.6	.1	6.0	5.3	4.7	0	.2	4.1	0	0
kn	F25	E	–	–	0	–	....	5.9	.1	5.5	5.5	5.2	4.3	.1	0	.2	0
ko	F25	C	–	–	0	–	+	4.9	0	5.2	4.3	4.8	.1	.1	4.1	2.3	.1
kp	F25	C	–	–	0	–	+	6.5	.2	5.4	4.9	4.8	6.2	0	.1	0	0
kq	F26	.....	–	....	0	–	+	5.8	.2	5.9	5.4	5.1	0	0	0	.1	0
kr	F26	E	–	–	0	–	+	6.1	.2	6.4	5.4	4.6	6.5	0	0	.1	0
ks	F26	D	–	–	0	–	+	5.8	.2	6.1	5.5	5.1	0	0	0	.1	.1
kt	F27	D	....	–	0	–	....	7.3	.2	5.9	5.7	.2	.2	0	2.2	.3	.1
ku	F27	D	....	....	0	–	+	7.5	.2	7.2	4.8	.5	.2	.3	1.7	.1	0
kv	F28	.....	+	....	0	–	+	5.8	0	3.8	5.7	5.0	0	.2	0	.1	0
kw	F28	E	–	–	0	–	+	5.9	0	6.2	5.4	4.9	.1	.1	0	.1	0
ky	F29	.....	–	....	0	–	+	5.2	.1	5.7	5.4	5.1	3.8	5.9	.1	.1	.1
la	F30	.....	....	....	0	–	–	5.5	.1	4.8	5.0	4.6	5.9	6.2	0	.1	0
lb	F31	E	....	–	0	–	....	5.9	0	5.1	5.0	5.7	5.6	0	.1	0	0
lc	F31	.....	–	....	0	–	....	6.0	.1	5.4	4.7	0	0	0	0	0	0
ld	F31	.....	....	....	0	–	....	5.4	0	5.5	5.3	0	0	0	0	.1	.2
le	F32	C	....	–	0	–	+	5.1	0	4.3	5.0	5.2	6.3	5.8	0	0	0
li	F33	.....	–	....	0	–	+	4.9	.1	5.8	5.2	4.6	.2	0	0	.2	0
lg	F34	G	–	–	36	–	–	5.1	.5	4.8	3.5	.5	.5	.7	4.6	2.3	.1
lh	F35	G	....	+	0	–	+	6.2	0	6.5	6.0	4.5	6.1	6.4	4.5	.1	0
li	F35	C	–	+	0	–	+	6.6	0	6.4	1.7	4.7	.1	0	4.3	.1	0
lk	F36	E	–	–	0	–	+	5.2	0	5.2	5.0	4.6	6.1	6.1	0	.1	0
ll	F36	H	–	+	0	–	+	6.3	0	4.9	4.7	4.6	.2	.1	.2	0	0
lm	F36	H	–	+	0	–	+	6.5	0	5.0	5.0	4.8	0	0	.1	0	0
ln	F37	D	....	–	0	–	+	5.7	0	5.0	4.6	4.4	6.0	6.4	.1	0	0
lo	F37	.....	–	....	.....	–	+	5.4	.1	5.2	5.6	4.3	6.0	.2	0	0	0
lp	F37	E	....	–	0	–	....	6.1	0	4.9	5.1	4.9	5.9	6.3	.1	.1	0
lq	F38	E	....	+	0	–	+	6.3	0	5.2	4.9	4.8	6.4	.1	0	0	0
lr	F39	.....	–	....	.....	–	+	6.6	0	6.3	5.2	4.8	0	0	.2	0	0
ls	F39	E	+	+	0	–	+	6.8	0	6.2	5.1	5.0	0	0	3.9	0	0
lt	F39	E	–	–	0	–	+	6.5	0	6.4	4.7	4.4	0	.2	0	0	0
lu	F40	E	....	–	0	–	+	5.6	0	5.2	4.7	5.3	5.3	.1	.1	0	0
lv	F40	G	–	–	0	–	+	5.8	0	5.3	5.6	6.0	6.2	0	0	0	0
lw	F40	.....	–	....	.....	–	+	5.7	0	5.2	5.8	5.3	6.1	.2	3.6	0	0

TABLE II.—*Physiological characters of all cultures*—Continued.

Culture No.	Origin.	Morphology.	Chains.		Liquefied gelatin.	Neutral red reduction.	Milk curd.	Percentage of normal acid.									
			Milk.	Broth.				Dextrose.	Adonite.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Dulcite.
					mm.												
lx	F41	E	....	—	0	—	....	6.2	0	5.5	5.2	5.2	6.1	0.1	0	0	0.1
ly	F41	E	—	—	0	—	....	6.8	.1	5.3	5.1	0	6.3	.1	0	.2	.1
lz	F41	E	....	—	0	—	....	6.5	.1	5.1	5.0	5.0	6.3	0	.1	.1	0
ma	F42	C	—	—	0	—	+	6.2	0	4.3	5.4	3.4	.2	2.9	4.4	.4	0
mc	F43	E	....	—	0	—	....	6.5	0	5.3	4.6	5.2	6.0	6.2	0	.1	0
md	F43	.....	.....	.....	.....	—	—	5.6	0	5.0	4.6	5.3	6.2	6.2	.1	.1	0
me	F44	C	—	—	0	—	+	6.6	0	5.2	4.8	5.1	7.0	0	0	0	0
mf	F45	C	....	—	0	—	+	6.6	.2	5.3	5.0	5.5	4.6	.1	0	0	0
mg	F46	B	—	+	0	—	+	5.4	0	4.8	4.6	4.6	6.5	.2	.1	0	0
mh	F46	E	—	—	0	—	+	5.9	0	5.4	4.6	4.3	0	0	.1	0	0
mi	F38	E	—	—	.....	—	+	6.4	0	5.1	5.0	4.5	6.5	.1	.6	0	0
mj	F38	E	—	—	0	—	+	6.3	0	5.1	4.6	4.9	.1	0	0	.2	0
mk	F47	D	....	—	0	—	....	6.4	0	4.8	5.1	5.0	6.2	.2	.2	0	0
ml	F47	.....	.....	.....	.....	—	—	6.0	0	5.0	4.9	4.9	6.1	5.6	0	0	0
mm	F47	C	....	—	0	—	....	6.4	0	4.7	5.3	5.2	6.3	5.8	0	.2	.1
mn	F48	E	—	—	.....	—	+	5.6	0	5.9	5.5	.1	.1	.3	2.5	0	0
mp	F48	—	—	—	0	—	+	5.7	0	5.9	5.0	0	.1	.2	3.0	.4	0
mq	F49	C	....	—	0	—	....	6.2	0	5.3	5.3	5.5	6.2	6.5	0	.2	.1
mr	F49	E	....	—	0	—	....	6.6	0	5.1	4.8	5.1	6.2	5.7	.1	0	0
ms	F50	E	....	—	0	—	....	6.4	0	5.1	4.9	0	6.1	0	0	0	0
mt	F50	A	....	+	0	—	....	6.1	0	5.2	5.0	5.0	6.4	6.2	0	0	0
mu	F51	A	....	+	0	—	....	6.0	0	5.1	5.0	5.0	6.4	0	0	0	0
mv	F51	H	....	—	0	—	....	5.9	.2	5.1	4.7	5.2	6.2	6.0	0	0	.1
mw	F52	E	....	—	0	—	....	5.7	0	5.0	4.8	5.4	6.2	5.5	.7	0	0
mx	F52	.....	....	....	0	—	—	5.9	.1	3.8	5.0	0	.1	0	0	.1	0
my	F53	.....	....	....	0	—	—	5.5	.1	5.2	4.9	4.9	6.1	5.9	.1	0	.4
mz	F53	E	....	—	0	—	....	5.8	.2	3.9	4.8	0	.2	.1	.2	.1	0
na	F53	E	....	—	0	—	....	6.5	0	5.0	5.2	5.4	5.3	0	0	0	0
nb	F54	.....	....	....	0	—	—	5.0	0	5.1	4.7	0	0	0	.1	0	0
nc	F54	.....	....	....	.....	—	—	4.4	.2	5.1	4.9	0	0	0	0	.1	0
nd	F55	.....	—	....	0	—	+	5.6	0	5.9	5.4	5.3	4.5	0	0	0	.1
ne	F55	.....	—	....	0	—	+	5.9	.2	6.0	5.4	5.5	.2	.2	0	.2	0
nf	F56	.....	....	....	0	—	+	5.8	.1	5.1	5.1	0	.2	.2	0	0	.1
ng	F56	.....	....	....	0	—	....	4.7	.1	5.2	5.5	0	0	0	0	.2	.1
ni	U9	H	....	—	0	—	+	4.6	0	4.6	3.1	.1	.2	0	.1	.3	0
nj	U9	.....	....	....	0	—	+	4.9	0	5.2	5.0	.2	0	.1	0	.3	.1
nk	U9	B	....	+	0	—	+	5.1	.1	4.8	5.1	.2	.2	0	.1	.1	.1
nl	U10	G	....	—	35	—	+	7.2	.1	4.7	5.1	.2	.4	.3	4.9	1.8	.1
nm	U10	G	....	—	24	—	+	7.4	0	5.1	4.3	.2	.5	.1	5.1	1.8	.3
nn	U10	H	....	+	32	—	+	7.4	.2	4.4	5.1	.2	3.0	.2	5.2	1.7	.2
no	U11	G	....	—	0	—	+	4.4	0	4.7	3.8	0	.5	0	.2	.3	.1
np	U12	H	....	+	0	+	+	5.8	0	0	.4	0	.....	0	4.5	.4	0
nq	U13	H	....	+	0	—	+	5.3	0	4.1	4.7	.1	0	.1	0	2.0	0
nr	U14	B	....	+	0	—	+	6.4	0	5.6	1.5	0	0	0	5.5	.5	.1
ns	U14	E	....	—	0	—	+	6.4	0	5.5	5.1	0	0	0	4.8	.3	.1
nt	U15	H	....	—	0	—	+	5.8	0	5.1	4.8	.2	0	0	.1	1.7	0
nu	U16	H	....	+	0	—	+	3.8	0	4.4	4.7	.2	0	.1	.3	0	.1
nv	U16	H	....	+	0	—	+	5.9	0	4.7	4.8	.2	.1	0	.2	.1	0
nw	U16	H	....	+	0	—	+	5.4	0	4.4	4.7	.3	1.1	0	.1	.9	0
nx	U17	H	....	+	0	—	+	5.9	.2	4.0	4.5	.2	0	.1	.4	.4	.2
ny	U17	H	....	+	0	—	+	5.2	.1	.1	4.5	.3	.2	0	.2	.5	.1
nz	U17	H	....	+	0	—	....	6.0	0	4.5	4.6	.3	0	.1	.1	0	.1
oa	B1	C	....	—	0	—	—	5.7	.4	4.4	5.2	5.6	4.8	5.5	.1	0	.2
ob	B1	.....	.....	.....	0	—	+	4.4	0	5.3	4.9	4.4	4.1	0	0	0	.2
oc	B2	H	....	—	0	—	+	4.3	0	5.4	4.7	4.5	.1	0	0	0	.1
od	B2	F	....	—	0	+	+	5.7	0	4.6	4.5	7.1	0	3.0	4.0	.2	0
oe	B3	F	....	—	0	+	+	5.4	0	4.9	5.0	4.1	0	3.3	3.8	.1	0
of	B3	F	....	—	0	—	+	5.8	0	4.5	4.6	4.0	0	3.3	3.9	1	0
og	B4	F	....	—	0	—	+	5.4	0	4.0	5.1	3.9	0	3.0	3.8	.5	0
oh	B5	E	....	—	0	—	+	6.1	0	6.2	5.5	.1	.1	0	4.6	.3	0
oj	B6	E	....	—	0	—	+	5.9	0	5.8	5.0	1.7	0	3.7	3.9	.2	0
ol	B7	E	....	—	0	—	+	5.7	.2	4.4	5.4	3.8	0	0	3.8	.3	0
om	B7	E	....	—	0	—	+	5.4	0	1.4	4.8	4.3	.3	0	3.9	.4	.1
on	B8	G	....	—	0	—	+	6.1	.1	.2	5.4	0	0	0	4.3	.4	.1
oo	B8	E	....	—	0	—	+	6.4	.1	.2	5.5	0	.1	0	4.2	.2	.1
op	B9	E	....	—	0	—	+	6.0	0	.2	5.3	0	.1	0	4.5	0	0
oq	B9	E	....	—	0	—	+	6.3	.1	.2	5.5	.2	0	0	4.4	.1	.1
or	B10	E	....	—	0	+	+	6.1	0	6.1	5.2	.1	.2	0	4.6	.2	.2
os	B10	G	....	—	0	—	+	6.6	0	7.1	5.5	6.2	5.0	0	.1	.1	.2
ot	B11	E	....	....	0	—	....	6.0	0	6.0	5.4	0	.1	0	4.4	.1	.2
ou	B11	E	....	—	0	+	+	6.1	0	5.9	2.0	.1	0	0	4.6	.3	.2
ov	B12	G	....	—	0	—	—	6.9	.1	5.9	5.1	.1	0	0	0	.3	.2
ow	B12	G	....	—	0	—	—	6.8	.1	5.9	5.1	.1	.1	0	3.8	.2	.2
ox	B13	G	....	—	0	—	—	6.3	.1	6.1	5.3	0	0	.1	4.0	.3	.2

TABLE II.—*Physiological characters of all cultures*—Continued.

Culture No.	Origin.	Morphology.	Chains.		Liquefied gelatin.	Neutral red reduction.	Milk curd.	Percentage of normal acid.									
			Milk.	Broth.				Dextrose.	Adonite.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Dulcite.
					mm.												
oy	B13	G	....	—	0	—	—	6.6	0.1	5.8	5.6	0.1	0	0	3.7	0.2	0.1
oz	U18	B	..,.	+	0	—	+	4.9	.1	.4	4.5	.1	.1	.1	0	0	.2
pa	U18	.....	....	....	0	—	+	5.2	0	.1	4.3	0	0	0	0	.1	.1
pb	U19	.....	....	....	0	—	+	4.9	0	4.6	5.3	.2	0	0	0	.2	.2
pc	U19	.....	....	....	0	—	+	5.0	0	5.2	5.3	.1	0	.2	.1	.1	.1
pd	B14	E	....	—	0	—	+	4.8	.2	6.2	4.5	.1	0	0	3.9	.2	0
pe	B14	E	....	—	0	—	+	4.3	0	4.1	4.6	0	0	0	3.5	.1	0
pf	B15	E	....	—	0	—	+	5.3	0	4.4	5.0	3.8	0	2.8	3.7	.2	0
pg	B15	E	....	—	0	—	+	5.4	0	4.5	5.0	3.9	0	2.9	3.6	.3	0
ph	B16	G	....	—	0	—	+	6.0	.2	1.8	4.5	.1	0	0	4.3	.1	.2
pi	B16	G	....	—	0	—	+	5.9	.3	1.8	4.7	.1	0	0	4.7	.2	.1
pj	B17	E	....	—	0	—	+	4.7	0	4.1	4.7	0	.1	0	3.5	.1	0
pk	B17	E	....	—	.....	—	+	4.6	0	4.2	4.8	0	0	0	3.9	.2	.3
pl	B18	E	....	—	0	—	+	5.5	0	4.2	5.0	3.9	0	2.8	3.7	.5	.1
pm	B18	E	....	—	0	—	+	5.6	0	4.2	4.8	4.0	0	2.8	3.8	.4	0
pn	B19	G	....	—	0	—	+	4.8	0	4.1	4.6	3.3	0	0	3.6	.2	0
po	B19	E	....	—	0	—	+	4.8	0	4.3	4.8	3.2	0	0	3.8	.1	.1
pq	B20	E	....	—	0	—	+	5.2	0	4.2	4.8	0	.2	.2	3.6	1.6	.2
pr	B20	E	....	—	0	—	+	5.4	.1	4.3	4.8	0	0	.3	3.5	.1	.2
ps	B21	E	....	—	0	—	+	6.2	.1	4.2	5.1	0	0	.1	2.9	0	0
pt	B21	E	....	—	0	—	+	6.2	0	5.3	4.9	0	0	.2	2.9	0	0

In one particular our results do not agree with the conclusions reached by Stowell, Hilliard, and Schlesinger[1] and by Howe and others in that the "metabolic gradient" which they establish, in our opinion, can be correct only for the particular group under consideration, since the number of cultures utilizing any particular carbohydrates or similar compound is dependent on the peculiarities of the cultures as well as on the composition or the configuration of the test substance. While in a general way our cultures follow the scheme outlined by Stowell, Hilliard, and Schlesinger, this arrangement may be varied, as will be pointed out later, by varying the source from which the cultures are obtained. In one group of our collection a much larger percentage of cultures give a fermentation with mannite than with raffinose; in others the conditions are reversed. In no case did we obtain a higher percentage of positive results with mannite than with inulin, although both Winslow and Stowell, Hilliard, and Schlesinger put inulin above mannite. Dulcite may be considered as one of the more difficultly fermented alcohols, and yet in our work on the colon group we found that dulcite was fermented most frequently, not by the more active group but by the one which otherwise showed weak fermentative ability. With adonite the conditions were reversed.

There is among all acid-forming bacteria and especially among the streptococci considerable variation in the maximum amount of acid produced. Winslow has shown that this may be a valuable aid in dis-

[1] Stowell, E. C., Hilliard, C. M., and Schlesinger, M. J. A statistical study of the streptococci from milk and from the throat. Jour. Infect. Diseases, v. 12, no. 2, p. 144–164. 1913.

tinguishing cocci of different species.[1] Stowell, Hilliard, and Schlesinger, in the paper already quoted,[2] have pointed out the marked difference in this regard between streptococci from milk and those from the human throat. In Table III is shown the distribution of cultures according to their source and the quantity of acid formed in dextrose broth. This is also shown graphically in figure 4. The mode for the culture from the mouth falls over 6.5 per cent, while that for the udder organisms is over 5.0 per cent, and that for those from feces is 5.5 per cent. The mode for each group is sharply defined, especially those for the udder and feces groups. On the assumption that the cultures obtained from milk may have come originally from any of the other sources, we would expect the curve representing the milk cultures to spread over the space occupied

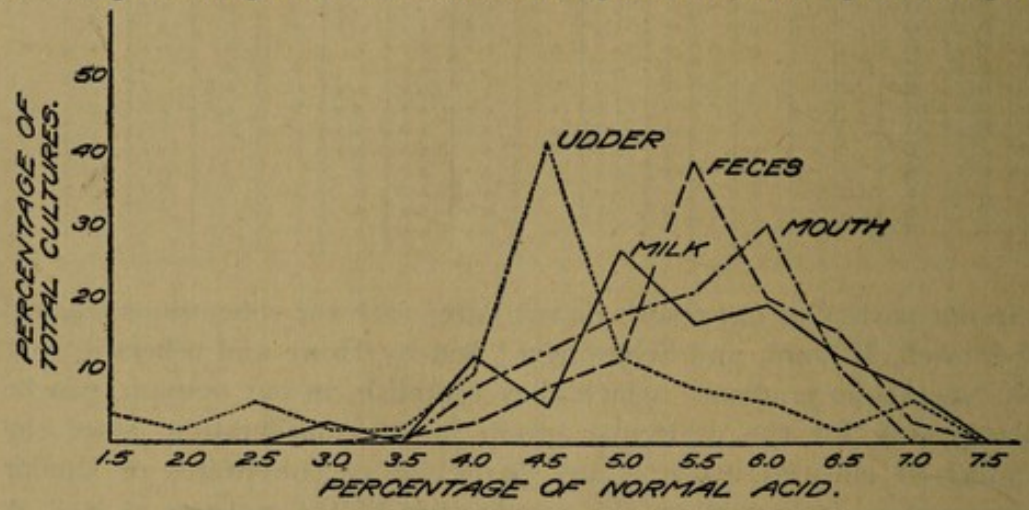

FIG. 4.—Frequency curves showing acid formation in dextrose broth.

by the other curves. This is true in a general way, but the curve for the milk cultures has a mode falling between that for the udder and the feces cultures. It should be remembered that the milk cultures were not selected promiscuously but from bile tubes incubated at 37° C.

TABLE III.—*Distribution of cultures according to the percentage of normal acid produced in dextrose broth.*

Source.	Total number of cultures.	Below 1.0.	1 to 1.5.	1.5 to 2.0.	2.0 to 2.5.	2.5 to 3.0.	3.0 to 3.5.	3.5 to 4.0.	4.0 to 4.5.	4.5 to 5.0.	5.0 to 5.5.	5.5 to 6.0.	6.0 to 6.5.	6.5 to 7.0.	7.0 to 7.5.	Above 7.5.
Milk:																
Number	42	0	0	0	0	0	1	0	5	2	11	7	8	5	3	0
Per cent		0	0	0	0	0	2.38	0	11.90	4.76	26.19	16.67	19.05	11.90	7.14	0
Udder:																
Number	51	0	0	2	1	3	1	1	5	21	6	4	3	1	3	0
Per cent		0	0	3.92	1.96	5.88	1.96	1.96	9.80	41.18	11.76	7.84	5.88	1.96	5.88	0
Feces:																
Number	114	0	0	0	0	0	0	1	3	9	13	44	23	18	3	0
Per cent		0	0	0	0	0	0	0.88	2.63	7.89	11.40	38.59	20.17	15.79	2.63	0
Mouth:																
Number	39	0	0	0	0	0	0	0	3	5	7	8	12	4	0	0
Per cent		0	0	0	0	0	0	0	7.69	12.82	17.95	20.51	30.77	10.26	0	0

[1] Winslow, C. E. A., and Winslow, Anne R. Systematic relationships of the Coccaceae. ed. 1, 300 p., illus. New York, 1908.

[2] Stowell, Hilliard, and Schlesinger. Op. cit.

ACTION ON LITMUS MILK

Late in the course of the investigation it was noticed that there were distinct differences in the action of different cultures on the litmus in milk and that this difference was in some relation to the source of the cultures. Some cultures decolorized the litmus promptly, leaving a white curd, with the exception of a pink ring at the top, which slowly extended downward. Other cultures produced a curd which remained pink throughout for an indefinite period. This action was recorded for the cultures then available, and the results are given in Table IV. It will be noticed that while the ability to reduce litmus is characteristic of the mouth cultures it is almost entirely lacking in the cultures from

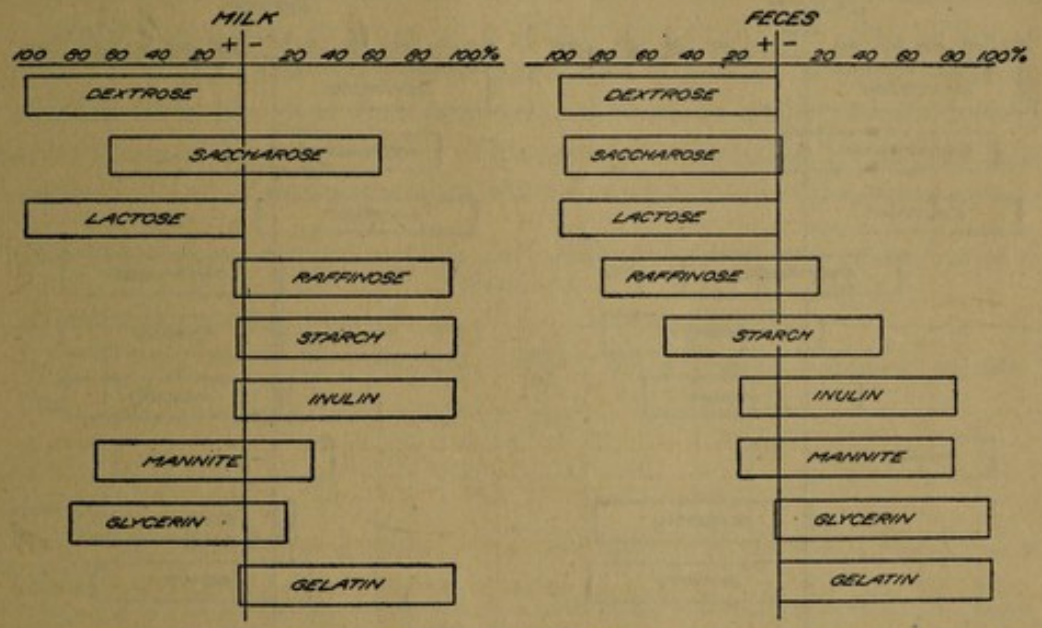

FIG. 5.—Graphic representation of the characters of cultures of streptococci from milk and from bovine feces.

the udder. The number of cultures in the two other groups in which this character was recorded is too small to permit conclusions, but there may be observed a tendency in the milk cultures to agree with those from the udder.

TABLE IV.—*Distribution of cultures according to action on litmus in milk.*

Cultures recorded from—	Number of cultures.	Cultures reducing litmus.		Cultures failing to reduce litmus.
		Number.	Per cent.	
				Per cent.
Milk	17	4	23. 53	76. 57
Feces	16	6	37. 50	62. 50
Udder	29	2	6. 89	93. 10
Mouth	35	29	82. 86	17. 14

THE FERMENTATION OF TEST SUBSTANCES

In Table V the cultures are arranged on the basis of fermentation or nonfermentation of eight test substances. In this table all reactions of 1 per cent or over are counted as positive and those falling below as negative. The results given in this table are arranged in a form more easily studied in figures 5 and 6. In these diagrams all positive results are plotted to the left of a vertical line and the negative results to the right. The udder organisms are characterized by the general lack of ability to ferment the test substances. They fail almost without exception to ferment raffinose and the polysaccharids, but show some tendency to attack the two alcohols. On the other hand, the 114 cultures

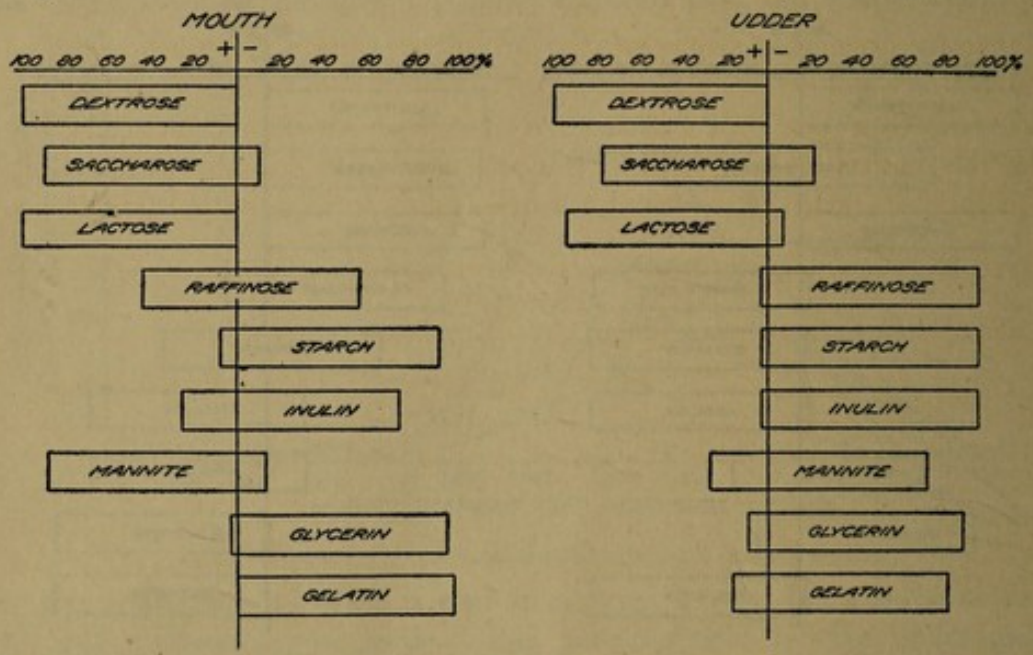

FIG. 6.—Graphic representation of the characters of cultures of streptococci from the mouths of cows and from infected udders.

from bovine feces fail almost entirely to utilize the alcohols, but show exceptional activity in fermenting the more complex sugars and the polysaccharids.

TABLE V.—*Fermentation of test substances.*

Origin of culture.	Dextrose.		Saccharose.		Lactose.		Raffinose.		Starch.		Inulin.		Mannite.		Glycerin.	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Milk:																
Total.....	42	0	21	21	42	0	2	40	1	41	2	40	29	13	34	8
Percentage of total....	100	0	50	50	100	0	4.8	95.2	2.3	97.7	4.8	95.2	69.0	31.0	80.9	19.1
Udder:																
Total.....	51	0	40	11	48	3	0	51	2	49	2	49	14	37	6	45
Percentage of total....	100	0	78.4	21.6	94.3	5.7	0	100	4	96	4	96	27.4	72.6	11.6	88.4
Feces:																
Total.....	114	0	112	2	114	0	93	21	60	54	20	94	21	93	2	112
Percentage of total....	100	0	98.2	1.8	100	0	81.5	18.5	52.5	47.5	17.6	82.4	18.5	81.5	1.8	98.2
Mouth:																
Total.....	40	0	35	4	39	0	17	22	3	36	10	29	34	5	1	38
Percentage of total....	100	0	89.7	11.3	100	0	43.6	56.4	7.7	92.3	25.6	74.4	87.2	12.8	2.6	97.4

The cultures from the mouth differ from those from the udder in the higher percentages of raffinose, inulin, and mannite fermenters and in less action on glycerin and gelatin. They are sharply differentiated from the feces organisms in their general failure to ferment starch and the much higher percentage of mannite fermenters.[1]

The milk cultures are distinguished by the comparatively small number of saccharose fermenters, the failure to ferment raffinose, starch, and inulin, and the active fermentation of both mannite and glycerin.

THE LIQUEFYING CULTURES

It will be noted that with the exception of a few obtained from milk, all of the liquefying cultures came from the udder. If we consider the 11 gelatin-liquefying cultures as a group we obtain the data given in Table VI, which shows that the liquefaction of gelatin is not an isolated variation from the type but is correlated with an ability to utilize the alcohols, mannite, and glycerin. This peculiar correlation between gelatin liquefaction and glycerin fermentation was also noticed in the colon group.

TABLE VI.—*Comparison of liquefying and nonliquefying cultures of streptococci from the udder.*

Item.	Gelatin.	Dextrose.		Saccharose.		Lactose.		Raffinose.		Starch.		Inulin.		Mannite.		Glycerin.	
		+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Number of cultures.	+	11	0	10	1	11	0	0	11	1	10	0	11	9	2	6	5
Per cent...	.....	100.0	0	90.90	9.09	100.0	0	0	100.0	9.09	90.90	0	100.0	81.81	18.19	54.54	45.46
Number of cultures.	−	43	0	33	10	40	3	0	43	2	41	2	41	7	36	0	38
Per cent...	.....	100.0	0	76.74	23.26	93.02	6.98	0	100.0	4.65	95.35	4.65	95.35	16.28	83.72	0	100.0

The characters of the 11 cultures included in Table VI agree very closely with the ' Group C" of the article by the writers on the lactic-acid bacteria.[2] If we divide the udder cultures into gelatin-liquefying and nonliquefying groups, we obtain figure 7, in which the cultures are arranged as in figures 5 and 6. This gives two groups in each of which the cultures show distinctive characters and remarkable uniformity.

We have, then, at least three sharply defined varieties: Two from the udder, of which one has weak fermentative ability, attacking only dextrose, saccharose, and lactose, with an occasional culture-producing acid from mannite, inulin, or starch, and a second less numerous type, which liquefies gelatin and ferments dextrose, saccharose, lactose, mannite, and usually glycerin; and one from bovine feces, character-

[1] Since this paper was written, a communication by C. A. Fuller and V. A. Armstrong entitled "The differentiation of fecal streptococci by their fermentative reactions in carbohydrate media" has appeared in the Jour. of Infect. Diseases, v. 13, no. 3, p. 442–462, Nov., 1913. The characteristics of their cultures from bovine feces agree in all essential particulars with those found by the writers.

[2] Rogers, L. A., and Davis, B. J. Methods of classifying the lactic-acid bacteria. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 154, 30 p., 6 fig. 1912.

ized by its active fermentation of sugars and polysaccharides and general failure to ferment the alcohols, mannite and glycerin. The group from the mouth has certain distinctive characters, but is not as clearly defined as the other three groups. It will need additional study before it can be described as a distinct variety.

If we consider the milk cultures individually, we find that two of them, *ik* and *il*, clearly belong with the feces group. The one which liquefies gelatin has the characters of the typical liquefying udder culture. The remaining 39 cultures may be placed with the nonliquefying udder organisms. If, however, we assume that the fermentation of mannite and glycerin places the nonliquefiers with the liquefiers, an assumption

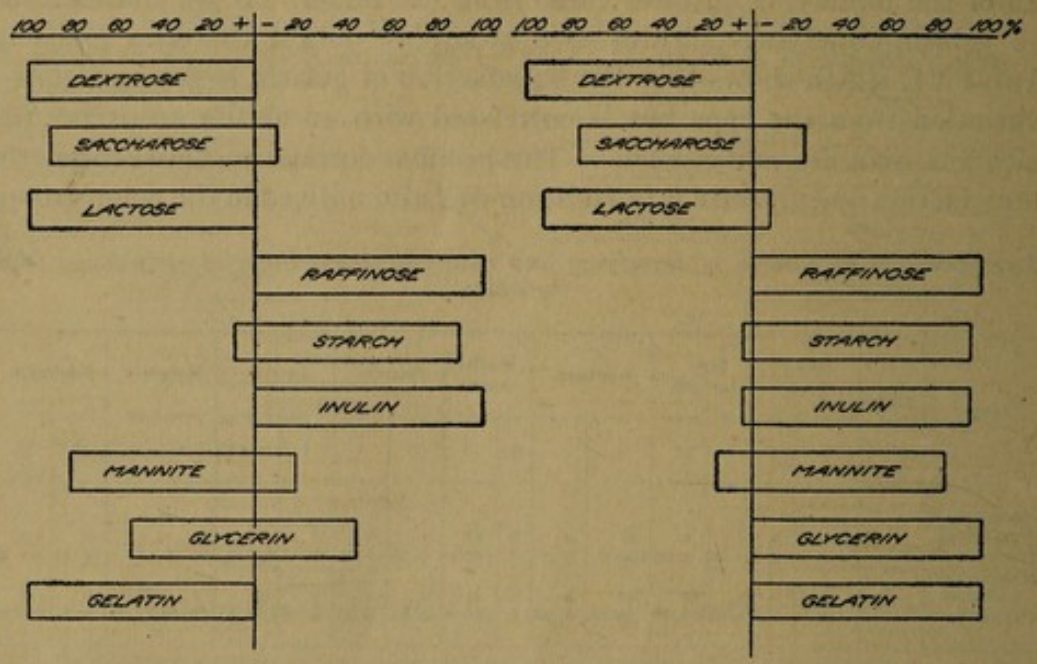

FIG. 7.—Diagram showing the fermentation reactions of two types of udder cultures of streptococci.

based on the possible variation of the liquefying power, we obtain a division of the milk cultures as shown in Table VII and figure 8. Thus, we obtain two groups agreeing very closely with those into which we were able to separate the udder cultures. This points very strongly to the infected udders rather than to the feces as the source of chain-forming cocci growing in lactose broth at 37° C.

TABLE VII.—*Two possible groups of the milk cultures.*

Significant characters.	Total number of cultures.	Gelatin. +	Dextrose. +	Saccharose. +	Lactose. +	Raffinose. +	Starch. +	Inulin. +	Mannite. +	Glycerin. +
Gelatin +	8	1	8	7	8	0	0	0	8	8
Mannite +										
Glycerin +										
..per ct.		12.5	100	87.5	100				100	100
Gelatin −	32									
Mannite +		0	32	13	32	0	1	0	18	0
Glycerin −										
..per ct.			100	40.6	100		3.1		56.3	

The same test applied to the mouth cultures would show that almost any individual culture could be included in the feces group. However, almost any mouth culture would be an exceptional, not a typical, feces culture. A culture fermenting saccharose, lactose, raffinose, and mannite could be either from the mouth or from feces, but there is a high probability that it would be of buccal origin. On the other hand, a culture fermenting saccharose, lactose, raffinose, and starch, but failing to ferment mannite or glycerin, would almost certainly be of fecal origin.

RELATION OF THESE GROUPS TO NAMED VARIETIES

It would be difficult to identify all of these groups with previously described species. Until the work of Gordon, few cultures were described

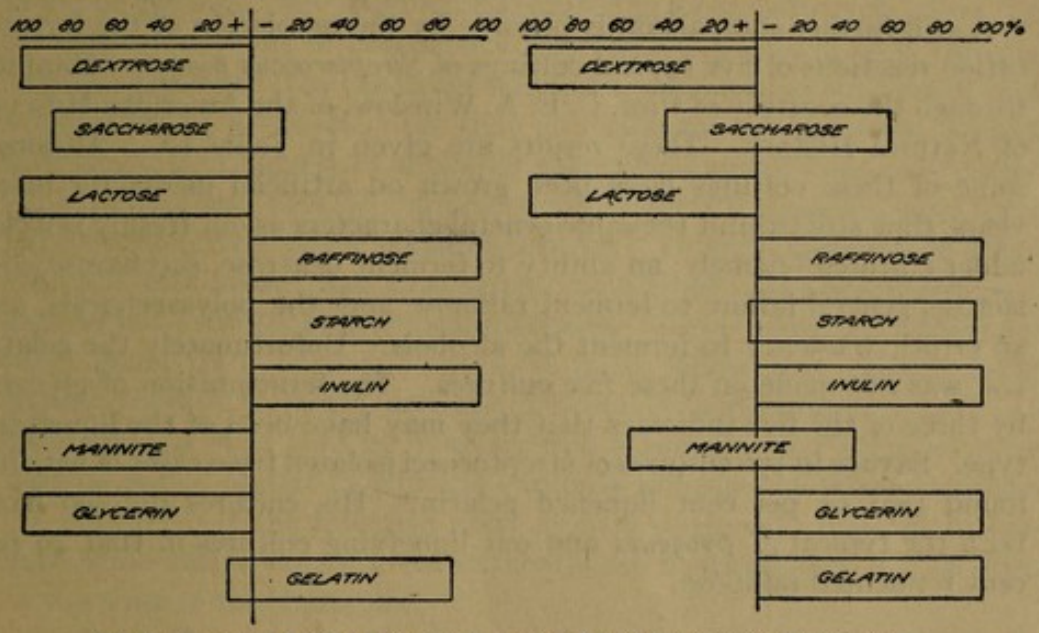

FIG. 8.—Diagram showing a possible grouping of the milk cultures of streptococci.

on the basis of the fermentation of a large number of test substances, and in only a very few cases have the cultures been obtained from a definite source. An exception may be made of the pathogenic bacteria in which the cultures described have been selected from definite and very similar sources. Among the streptococci we have an example in the pus-forming organism generally described as *Streptococcus pyogenes*. In Table VIII are compiled the typical reactions given for *Streptococcus pyogenes* by three investigations. The reactions given by Andrewes and Horder are compiled from a large number of cultures, and those given by Gordon are from a number of his own cultures.[1] Those given by Bergey are the reactions of a comparatively few typical cultures.[2] So

[1] Andrewes, F. W., and Horder, T. J., A study of the streptococci pathogenic for man. Lancet, v. 2, no. 11, p. 708–713; no. 12, p. 775–782; no. 13, p. 852–855. 1906.

Gordon, M. H. Report on an investigation of the fermentative characters of streptococci present on fauces during scarlet fever. 40th Ann. Rpt. Local Govt. Bd. [Gt. Brit.], 1910–11, Suppl. Rpt. Med. Off., p. 302–31, 1911.

[2] Bergey, D. H. Differentiation of cultures of streptococcus. Jour. Med. Research, v. 27 (n. s., v. 12), no. 1, p. 67–77. 1912.

far as it is possible to make comparisons, the reactions given agree very closely with our nonliquefying udder cultures.

TABLE VIII.—*Results of fermentation tests of Streptococcus pyogenes described in the literature.*

Authority.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Salicin.	Coniferin.
Our nonliquefying udder cultures...per cent..	76	93	0	4	4	16	16	.....	.....
Andrewes and Horder.....	+++	+++	−	.....	−	±	.....	±	−
Gordon.....			−	.....	−	.....	.....	±	.....
Bergey.....	+	+	−	.....	−	.....	.....	−	.....

A still further comparison is possible by the tabulation of the fermentation reactions of five typical cultures of *Streptococcus pyogenes* obtained through the courtesy of Prof. C. E. A. Winslow, of the American Museum of Natural History. These results are given in Table IX. Although some of these cultures have been grown on artificial media for many years, they still exhibit the same general characters as our freshly isolated udder cultures—namely, an ability to ferment dextrose, saccharose, and lactose, general failure to ferment raffinose and the polysaccharids, and an erratic tendency to ferment the alcohols. Unfortunately the gelatin test was not made on these five cultures. The fermentation of glycerin by three of the five indicates that they may have been of the liquefying type. Savage in 176 cultures of streptococci isolated from cases of mastitis found that 95 per cent liquefied gelatin.[1] His cultures differed from both the typical *S. pyogenes* and our liquefying cultures in that 49 per cent fermented raffinose.

TABLE IX.—*Results of fermentation tests of five cultures of Streptococcus pyogenes from American Museum of Natural History (New York) collection.*

Source.	Dextrose.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.
New York Post Graduate Medical College (fatal septicemia).....	3.90	3.85	3.60	0.20	0.18	0.23	0.35	0.59
Dr. Bien, Chicago, Ill. (abscess in erysipelas).....	5.45	4.75	3.25	0	.13	.18	2.55	2.34
Boston Board of Health (urine).....	3.85	4.05	.45	0	3.98	0	0	1.74
Johns Hopkins University.....	6.45	4.95	4.70	.20	.45	.08	4.91	1.67
Michigan Agricultural College.....	2.50	0	1.15	.05	0	.09	.23	.19

VARIATION FROM TYPE IN THE UDDER ORGANISMS

The trouble from infected udders at both the Beltsville and Annapolis farms was in the nature of an epidemic. The infection apparently spread from cow to cow and became so severe that at Annapolis one or

[1] Savage, W. G., Report upon the bacteriology and pathology of "Garget" (or mastitis) in cows. 37th Ann. Rept. Local Govt. Bd. [Gt. Brit.], 1907–8. Suppl. Rept. Med. Off., pp. 359–424. 1909.

more cows were rendered useless. There was no apparent connection between the two epidemics except that they occurred at about the same time. We may assume that these epidemics originated in one of two ways, either of which must admit more or less variation in physiological reactions from the original type. It may be possible that the udders of one or more cows may have become infected by some of the streptococci coming originally from the mouth, intestines, or other sources. Under the influence of its new environment this organism may have acquired pathogenic properties sufficient to produce the symptoms observed in mammitis. Heinemann has shown that pathogenicity is a property readily acquired when ordinary streptococci are grown in animals.[1] If these infecting organisms came from the mouth, the intestines, or the milk they must have acquired in a comparatively short time an entirely new set of biochemical reactions in addition to a variation in pathogenicity. On the other hand, we may assume with much more appearance of reasonableness that the infection spread from a single infecting cell or aggregate of similar cells which already possessed pathogenic powers and general characters identical with those we have found to be characteristic of the udder organisms. This assumption is in accord with the established fact that streptococci from pathological lesions in general have similar biochemical reactions. If the infection in these two cases came from various sources, it must follow that growth under similar conditions would produce uniform fermentation reactions in a short time, a view held by Walker, who maintains that these reactions may be varied almost at will and can only indicate the latest habitat of the culture.[2] If the infection came from a single cell, there must have been some variation, since the fermentation reactions were not identical at the time of this isolation.

In Table X are shown the varieties of nonliquefying udder cultures and the number occurring in each of the two herds. There were seven varieties in all. The most numerous one ferments dextrose, saccharose, and lactose only and occurred 24 times, equally divided between the two herds. The next most numerous variation differed from the first in failing to ferment saccharose and occurred 8 times. A third variation fermented mannite in addition to dextrose, saccharose, and lactose and occurred 4 times. The remaining four varieties evidently occur only once or twice in every 40 cultures. Viewed from any standpoint it is evident that these organisms are subject to variation from the type, but these variations are not of sufficient magnitude or frequency to detract from the value of the physiological reactions as a means of establishing true species.

[1] Heinemann, P. G., The pathogenicity of *Streptococcus lacticus*. Jour. Infect. Diseases, v. 4, no. 1, p. 87–92. 1907.

[2] Walker, E. W. A., On variation and adaptation in bacteria, illustrated by observations upon streptococci, with special reference to the value of fermentation tests as applied to these organisms. Proc. Roy. Soc. [London], s. B, v. 83, no. 567, p. 541–558. 1911.

TABLE X.—*Variation from type in nonliquefying udder cultures.*

Significant characters.								Number of cultures from herd at—		Total number of cultures.
Dextrose.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Beltsville.	Annapolis.	
+	+	+	−	−	−	−	−	12	12	24
+	+	−	−	−	−	−	−	6	2	8
+	+	+	−	−	+	+	−	2	2	4
+	+	+	−	−	−	−	+	1	1	2
+	+	−	−	−	−	+	−	0	1	1
+	+	+	−	−	+	+	−	1	0	1
+	+	+	−	−	+	+	−	1	0	1

SUMMARY

A collection of cultures of streptococci was made consisting of 42 cultures from milk which formed chains in lactose bile at 37° C., 51 cultures from infected udders, 114 cultures from bovine feces, and 39 cultures from the mouths of animals.

The morphology varied under different conditions and could not be correlated with the source of the culture, except that the udder cultures had a more marked tendency to chain formation than those from other sources.

The ability of these cultures to liquefy gelatin and to form acid from dextrose, lactose, saccharose, raffinose, starch, inulin, mannite, glycerin, dulcite, and adonite was determined. Only one or two cultures utilized adonite or dulcite.

When glycerin was attacked, the fermentation proceeded slowly, failing to reach its maximum in 14 days, in contrast to the fermentation of the sugars, in which the maximum was reached in two or three days.

A high percentage of the udder cultures failed to give the characteristic reduction in litmus milk.

Twelve cultures liquefied gelatin; one of these came from milk and 11 from infected udders.

The cultures from feces were characterized by their activity in fermenting the sugars, including raffinose, and their inability to utilize the alcohols.

The mouth cultures fermented dextrose, saccharose, lactose, mannite, and freqently raffinose, but were almost without effect on starch and glycerin.

The udder cultures were characterized by the general lack of fermentative ability, which was limited almost entirely to dextrose, saccharose, and lactose, with a comparatively small number utilizing mannite, glycerin, and gelatin.

When the udder cultures were divided on the basis of gelatin liquefaction, two groups were obtained. The fermentative activities of one

of these, which are similar to those of *Streptococcus pyogenes*, were limited to dextrose, saccharose, and lactose, with an occasional culture fermenting mannite, starch, or inulin. The second group fermented the three simple sugars, mannite, and usually glycerin and liquefied gelatin.

When the milk cultures were considered individually, it was found that with the exception of two which clearly came from feces they could be included in one or the other of the two groups into which the udder cultures were divided.

Of the 41 nonliquefying udder cultures 24 gave identical reactions. The remaining cultures differed from the type in one or two characters only.