STUDIES IN BOVINE MASTITIS.

I. Non-Hemolytic Streptococci in Inflammation of the Udder.

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INTRODUCTION.

Mastitis is one of the more important diseases affecting cows. Not only is it of serious nature economically, but within the past few years it has become of interest from the aspect of public health. Much of the recent work has tended to show that certain streptococci isolated from inflamed udders are closely related to if not identical with those found in certain epidemics of sore throat. Smith and Brown,[1] Davis and Capps,[2] and others believe, however, that these streptococci are of human origin and gain access to the udder through the teat canal.

Nocard and Mollereau[3] appear to have been the first to undertake a study of the etiology of bovine mastitis. They succeeded in isolating streptococci from milk obtained from the udders of ten cows suffering from mammitis. The streptococci are described as growing in long chains in bouillon and staining well with Gram's method. In addition they state that the streptococci fermented sugar media.

Lucet[4] examined milk from twenty-one animals affected with mastitis. From twelve he obtained non-gelatin-liquefying, Gram-negative bacilli; seven were infected with gelatin-liquefying, Gram-positive micrococci. The other two were suffering from streptococcic infection of the udder.

Guillebeau[5] found ten species of organisms associated with infections of the mammary gland. He grouped them as follows: three species of non-gelatin-

[1] Smith, Theobald, and Brown, J. H., *J. Med. Research*, 1914–15, xxxi, 455.

[2] Davis, D. J., and Capps, J. A., *J. Infect. Dis.*, 1914, xv, 135.

[3] Nocard and Mollereau, *Bull. et mém. Soc. centr. méd. vét.*, 1884, 188.

[4] Lucet, *Rec. méd. vét.*, 1889, vi, series 7, 423.

[5] Guillebeau, *Landswirtsch. Jahrb. Schweiz*, 1890, iv.

liquefying micrococci, one group of gelatin-liquefying cocci, two species of streptococci, three kinds of bacilli producing gas in sugar gelatin, and a single strain of rods liquefying gelatin. The results of his bacteriological findings in 85 cases may be given as follows:

Staphylococcus mastitidis	33 times.
Galactococcus versicolor	12 "
" *fulvus*	5 "
" *albus*	2 "
B. guillebeau a	20 "
" " b	once.
" " c	"
Streptococcus mastitidis sporadicæ	8 times.
" " *contagiosæ*	3 "

Jensen[6] subsequently identified *B. guillebeau* as *B. coli*. In addition he obtained *B. coli communis* and *B. lactis aerogenes* from severa cases of garget.

Steiger[7] undertook a detailed study of the disease and in all examined forty-five cases in cows and one in a goat. The summary of the bacteriological findings may be given as follows:

Staphylococcus mastitidis	6 cases.
Galactococci	10 "
Streptococci	10 "
B. coli	14 "
Mixed infections, *B. coli*, streptococci, and *B. necrophorous*	6 "

In addition, he had ample opportunities for clinical observation and was able to obtain considerable material for histological study. He discusses the possible modes of infection and considers that the usual mode is through the milk duct. It is admitted, however, that localization of a specific infection in the mammary gland may be caused by metastasis through the blood stream or lymph channels. The possibility of wound infection is also considered.

Unfortunately at the time of Steiger's investigation the differentiation of streptococci by their action upon carbohydrates and hemoglobin had not become a common practice. In describing both the streptococci and micrococci he employed grape and milk sugar. The streptococci were described as having a diameter of 1 micron. They grew in bouillon as diplococci or in short or long chains. Some grew diffusely throughout the medium, others left it clear. In agar they grew as small punctiform colonies. White mice were used to test the pathogenicity of the isolated streptococci. Some strains were highly virulent, others less so, and some produced apparently no ill effect.

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[6] Jensen, C. O., *Ergebn. allg. Path. u. path. Anat.*, 1897, iv, 830.

[7] Steiger, P., *Centr. Bakteriol., 1 te Abt., Orig.*, 1904, xxxv, 326, 467, 574.

Prior to Savage's[8] studies of microorganisms in mastitis, attempts to differentiate many of the organisms by more recent methods had not been made. Many believed that all streptococci isolated from inflamed mammæ were identical. Savage appears to have been the first to undertake a study of the action of bovine streptococci upon carbohydrate media. He observed many strains isolated from the milk of normal cows and from the milk of cows suffering from inflammation of the udder. Inoculations of white mice were resorted to in order to establish pathogenicity. Mention is not made of the action of any strains upon hemoglobin. Examinations of milk from thirty-one cows afflicted with mastitis were recorded as follows:

Due to streptococci	21 cases.
" " staphylococci	5 "
" " *B. coli*	1 case.
" " *B. tuberculosis*	1 "
Of doubtful origin	3 cases.

Savage did not consider the problem from its economic standpoint but from the relation of the disease to that of the public health. The more important points in the clinical data of the cases were available and his cytological studies were of considerable value.

Henderson[9] examined the secretions from fourteen cases of mastitis. Usually the examinations were made late in the course of the disease. Two were tuberculous, two were of the purulent type, and the remainder were described as parenchymatous. He believed that he was dealing with a mixed infection of streptococci and *B. coli* in twelve of the animals.

Zwick and Weichel[10] succeeded in isolating *B. lactis aerogenes* from nineteen out of twenty-one cases of acute mammitis. From two individuals they obtained *B. paratyphosus*. Inoculation of the mammary glands of goats with these organisms produced severe inflammations of that organ.

Gilruth and Macdonald[11] also reported an outbreak of acute contagious mastitis caused by *B. lactis aerogenes*. They believe that this organism is not usually pathogenic to cattle when inoculated into the blood stream but it may gain entrance into the milk duct and set up serious inflammation of the mammary glands of lactating cows. Recently Ward[12] reports the isolation of *B. pyogenes* from udder lesions. In reviewing the literature on *B. pyogenes* he cites one reference with regard to its etiological relation to a certain type of mammitis.

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[8] Savage, W. G., *Rep. Med. Off. Local 19Gov. Bd. 06–07*, xxxvi, 253; *Rep. Med. Off. Local Gov. Bd. 1907–08*, xxxvii, 359, 425; *Rep. Med. Off. Local Gov. Bd. 1908–09*, xxxviii, p. xxxiii.

[9] Henderson, J., *J. Compt. Path. and Therap.*, 1904, xvii, 24.

[10] Zwick and Weichel, *Arb. k. Gsndhtsamte.*, 1910, xxxiv, 391.

[11] Gilruth, J. A., and Macdonald, N., *Vet. J.*, 1911, lxvii, 217.

[12] Ward, A. R., *J. Bacteriol.*, 1917, ii, 619.

Kitt[13] reviews the literature and classifies the disease etiologically according to whether it is caused by members of the colon group, of the paratyphoid and *enteritidis* groups, or of the groups of staphylococci and streptococci. He states that it is possible to observe many forms such as catarrhal, parenchymatous, and purulent inflammation, abscess formation, sclerosis, and a general rapid necrosis of the mammary tissue. It is pointed out that one type may succeed another, according to the period of lactation and the general resistance of the animal. Like Steiger and others he believes that the teat canal offers a ready method of access into the gland. Mention is also made of infections through the blood and lymph streams. In this connection he refers to Guillebeau and Hess'[14] experiment in which they injected *B. coli* subcutaneously into goats, which was followed by a localization in the mammary gland.

Etiological Studies.

The following studies were undertaken to define more accurately the species of organisms responsible for disorders of the mammary glands of cows, and if possible to lighten the economic burden imposed upon dairying by these affections. In addition, a more complete description of the biological characters of bovine streptococci obtained from inflamed udders seemed desirable for the purpose of assisting those interested in the public health problem of milk-borne epidemics of tonsillitis.

Much of the material has been obtained from a large dairy herd. Mastitis was more or less endemic. During 1916 it was necessary to dispose of 65 cows because of chronic mammitis. In addition to the actual loss from chronic cases, many animals developed milder forms of the disease generally evidenced by flocculi in the milk and inflammation of the gland. Milk from such quarters was discarded and represented an absolute loss. It is interesting to note that during 1916 the number of animals disposed of because of chronic mammitis doubled the number reacting to tuberculin. It has also been possible to obtain clinical data and samples of milk from other sources.

The following routine procedure is used in obtaining milk from inflamed udders. Milk from the affected quarter is drawn directly into a sterile 6 ounce wide mouthed bottle, and except in winter it is

[13] Kitt, T., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1913, vi, 96.

[14] Guillebeau and Hess, cited from Kitt.[13]

iced at once. A note is made of the animal's general condition and the appearance of the involved quarter. The animal's herd number and its location in the various barns are recorded. The milk is usually plated within a few hours in dilutions of 1: 10, 1: 100, and 1: 2,000. Culture medium employed in all initial examinations consists of 1 cc. of defibrinated horse blood and 12 cc. of 2 per cent veal bouillon agar, to which is added the diluted milk, and the whole plated. The effect on hemoglobin is noted at the end of 24 and 48 hours. Readings are always made from deep colonies.

In counting the leukocytes and other cells in milk a modification of the Doane-Buckley[15] method has given satisfactory results. The original method may be described briefly as follows: 10 cc. of milk are poured into a graduated centrifuge tube and centrifuged rapidly for 10 minutes. The fat and supernatant liquid are drawn off down to the 1 cc. mark. A little saturated alcoholic solution of methylene blue is added and mixed. The counts are made with a Thoma-Zeiss blood counter. Campbell[16] modified the technique by washing the sediment several times with distilled water. He considers the stain unnecessary. Campbell's technique proved very satisfactory, although 0.9 per cent salt solution was substituted as a washing fluid. Stained films from the sediment of the centrifuged milk were prepared for microscopic study.

The examination of milk obtained from 81 animals suffering from various forms of mastitis has revealed the following bacterial associations:

With non-hemolytic streptococci	31
" hemolytic "	17
" mixed hemolytic and non-hemolytic streptococci	2
" micrococci	24
" *B. coli*	2
" pleomorphic Gram-positive rods	4
" *B. lactis aerogenes*	1
	81

[15] Doane, C. F., *Maryland Agric. Exp. Station, Bull. 102,* 1905.

[16] Campbell, H. C., *U. S. Dept. Agric., Bureau of Animal Industry, Bull. 117,* 1909.

Early in the investigation it became apparent that non-hemolytic streptococci were responsible for many udder infections, and it was decided to study several infections of this type. Among these the following cases represent typical spontaneous infections.

Case Records.

Cow 55.—Holstein cow, age about 6 years. Said to have had an attack of mastitis in Jan., 1917. Date of parturition unknown. Developed mastitis in the left hind quarter on May 20, 1917.

May 21. First examination of the milk from the affected quarter. The quarter was enlarged, very firm, hot, and painful when manipulated. The walls of the milk duct were thickened. Milk could only be expressed with great difficulty in a very fine stream. A slight rise in temperature was recorded (102.4°F.). The milk was watery and contained many irregular flattened, white flocculi.

Agar plates prepared from milk of the affected quarter revealed 3,600 non-hemolytic streptococci per cubic centimeter. Attempts to ascertain the number of cells failed because they clumped and formed an unbreakable viscid mass after centrifugation. Examination of films from the milk sediment revealed streptococci in chains up to nine cocci and great masses of polymorphonuclear leukocytes. The other quarters appeared normal.

May 23. The milk was less watery but contained many fine flocculi in suspension. It contained 16,950,000 cells per cubic centimeter and the plates revealed 47,000 streptococci per cubic centimeter.

May 25. The quarter was still firm but not feverish or painful. Milk was watery and contained very little fat; the flocculi were numerous. The animal's temperature was normal (101.4°F.).

Left hind quarter:
Cells 16,800,000 per cubic centimeter.
Colonies 5,000 " " " pure culture of non-hemolytic streptococci.

Right hind quarter:
Cells 185,000 per cubic centimeter.
Colonies 50 " " " no streptococci. The milk appeared normal.

Left fore quarter:
Cells 90,000 per cubic centimeter.
Colonies 510 " " " no streptococci.

Right fore quarter:
Cells 460,000 per cubic centimeter.
Colonies 2,100 " " " 95 per cent of the colonies were similar to those observed in plates from left hind quarter.
Films from sediment contained polymorphonuclear leukocytes and a few streptococci. The quarter appeared normal. The milk failed to show flakes.

Examinations were made at usually 3 day intervals, but as they failed to show marked differences the results of many will be omitted. It is interesting to note, however, that on May 28 the milk from the left hind quarter revealed 24,800,000 cells and 723,000 streptococci per cubic centimeter. The quarter had a tendency to become less firm from day to day and on June 8 the corded portion was confined to the lower half of the quadrant. The cells had fallen to 14,200,000 and the streptococci to 250 per cubic centimeter.

June 5. The blood serum of this cow in a dilution of 1:500 completely agglutinated cultures of streptococci from the affected quarter The highest agglutination recorded was on Aug. 1 when the streptococcic suspensions were partially clumped in a dilution of 1: 2,000.

June 8.
Left hind quarter:
Cells 5,300,000 per cubic centimeter.
Colonies 364,000 " " " pure culture of streptococci.
The quarter was still firm. The milk was less watery but still contained flocculi.
Right hind quarter:
Cells 110,000 per cubic centimeter.
Colonies 120 " " " no streptococci.
Left fore quarter:
Cells 110,000 per cubic centimeter.
Colonies 180 " " " no streptococci.
Right fore quarter:
Cells 1,620,000 per cubic centimeter.
Colonies 14,600 " " " pure culture of non-hemolytic streptococci.
The quarter failed to show inflammatory changes and the milk appeared normal.

This animal was under constant observation until July 12. The original swelling of the quarter had contracted into an irregular nodule about the milk cystern. From June 8 until July 12 the elimination of streptococci from the diseased quarter had been very irregular, once falling as low as 8,000 per cubic centimeter. A maximum cell count of 91,000,000 was recorded, although at one time the cells fell as low as 8,800,000 per cubic centimeter. The right fore quarter continued to harbor streptococci.

July 12.
Left hind quarter:
Cells 53,000,000 per cubic centimeter.
Colonies 160,000 " " " pure culture of streptococci.
Right hind quarter:
Cells 210,000 per cubic centimeter.
Colonies 460 " " " no streptococci.

Left fore quarter:
Cells 120,000 per cubic centimeter.
Colonies 340 " " " no streptococci.
Right fore quarter:
Cells 280,000 per cubic centimeter.
Colonies 16,600 " " " pure culture of streptococci.

It was possible to examine the milk from time to time throughout the period of lactation. The left hind quarter ultimately became smaller than the others. The secretion was greatly diminished. The milk continued to contain flocculi and streptococci could always be recovered from the agar plates. The right fore quarter harbored streptococci throughout the lactation period but never revealed abnormalities. The cow gave birth to a calf in December. Up to Feb. 1, 1918, signs of streptococcic mastitis failed to appear. The subject has been averaging 20 quarts of milk per day. Plates from the left hind and right fore quarter have not revealed the presence of streptococci, since the animal calved.

Cow 56.—Holstein heifer, lactating for the first time. The animal calved Dec. 19, 1916. Mastitis developed in the right hind quarter May 21, 1917.

May 23. The animal was slightly depressed and a temperature of 102°F. was recorded. The cow had refused all food; the rumen was impacted. The right hind quarter was tense, feverish, and tender, but not noticeably enlarged. The milk from this quarter was much decreased in amount and was extremely thick. The other quarters appeared normal but the milk flow had decreased.

The exudate from the right hind quarter contained 27,300,000 cells and 5,600 streptococci per cubic centimeter. The cells in the milk from the other quarters were well within normal limits and plates prepared from the milk failed to reveal streptococci.

This animal was under observation for 56 days. The quarter softened somewhat but failed to regain its normal appearance; the milk became less purulent but always contained flocculi composed of casein, fibrin, and leukocytes. The highest cell count was recorded on June 11, when 182,000,000 were noted. The plates on this day revealed 1,300,000 streptococci per cubic centimeter.

The other quarters did not become involved, and streptococci were not observed in the plates.

The cow was slaughtered on July 18 and the udder was obtained for further study. The right hind quarter was firm and a trifle smaller than the others. On section the larger milk ducts were practically filled with yellowish flocculent milk. The parenchymatous tissue was pinkish yellow in color and when freshly cut appeared dry and granular; within a short time milk began to exude from the cut surfaces. There was an increase in interlobular connective tissue. The other quarters appeared normal. Pieces of the involved quarter were fixed in Zenker's fluid. Sections for study were stained with methylene blue and eosin. Examination of these sections revealed considerable degeneration and necrosis of portions of the secreting epithelium. In certain lobules it appeared granular; the

nuclei were shrunken and often forced to one side of the cells. The lumen of such an acinus was usually occluded with milk containing many fat cells and polymorphonuclear leukocytes. The interacinar vessels were engorged with leukocytes and red blood cells. In other lobules the secreting epithelium had been blotted out; the acini appeared indistinct. All that remained of the original structure was the supporting framework, a few necrotic epithelial cells, and dense masses of leukocytes.

The epithelium of many of the small lactiferous ducts stained indistinctly. Much of it was degenerated and infiltrated with leukocytes. Leukocytes and fibrin comprised the contents of the lumen. Many of the larger ducts had suffered severely. Much of their lining epithelium had become necrotic or was badly degenerated. The degenerated portions stained poorly, the nuclei were shrunken, and leukocytes had invaded the epithelial cells. The subepithelial connective tissue contained large numbers of round cells Fibrin, necrotic leukocytes, milk, and microorganisms filled the lumen of the ducts.

Mammary glands of several other cows suffering from infection with non-hemolytic streptococci have been studied. In some the lesions are much more marked than in others. In Cow 66, slaughtered early in the course of the disease, some lobules consisted of purulent masses. In others the central acini were necrotic, while the peripheral had been invaded with dense masses of polymorphonuclear leukocytes. Widespread degenerative changes of the secreting epithelium had occurred. The interlobular connective tissue was congested and edematous.

In the left fore quarter of Cow 72 streptococci were found in the milk 13 days before gross changes in the quarter were observed. 75 days later the animal was slaughtered. On gross examination of this quarter much of the epithelial structures of the upper third of the gland had been replaced by connective tissue. The infiltration had extended downward into the center of the quarter in the form of fibrous strands constricting and blotting out many of the lobules.

Cow 141 had suffered from the same type of infection. The disease was chronic. The principal lesions were confined to the larger milk ducts.

Cow 70 affords an excellent example of a severe type of infection with non-hemolytic streptococci. Both hind quarters and the right fore quarter became so severely involved that the animal was killed. Lesions similar to those found in the sections of the gland of Cow 56 were observed.

The milk from affected quarters often varies in different individuals. Its character is influenced by the stage and severity of the infection. Usually at the onset it is more or less watery in appearance and contains many irregular white flakes of casein, fibrin, and cells. The reaction is slightly alkaline to litmus. It fails to coagulate when boiled. In chronic cases the exudate is usually yellow and less watery. The particles are larger, elongated, and have a tendency to coalesce on standing. The reaction is alkaline. Boiling usually produces a prompt coagulation.

It was possible in one instance to study an early infection. The animal had been under observation for 10 days before mastitis developed in the left fore quarter. The first count, on November 12, revealed 750,000 cells and 5,500 streptococci per cubic centimeter of milk. The quarter appeared normal and the milk was unchanged. 4 days later the streptococci had risen to 86,000 per cubic centimeter. Gross changes could not be detected in the quarter. Mastitis developed clinically on November 25. The count on November 27 revealed 19,000,000 cells and 1,640,000 streptococci per cubic centimeter.

Rühm[17] had noted a similar condition previously. In making routine bacteriological examinations from individual cows he was able to detect streptococci in considerable numbers before clinical signs of inflammation appeared.

Streptococci identical with those responsible for the more severe inflammations may gain access to udders and inflict little or no gross changes in the gland or its secretion. Cow 69 was suffering from an infection of the left fore quarter with *Bacillus lactis aerogenes*. The other quarters were not involved. The right hind quarter became invaded with streptococci. The cell count rose to 610,000 per cubic centimeter at the end of a week and 10,800 non-hemolytic streptococci were noted in a cubic centimeter of milk. 1 week later the cells had risen to 1,000,000. The streptococci fell to 7,300. At the end of 10 days the cell count remained stationary but the streptococci had disappeared. It was only after pouring a considerable quantity of this milk through a sieve having 100 meshes to the inch that flocculi could be detected. Clinical mastitis did not develop in this quarter during the succeeding 10 weeks.

[17] Rühm, G., *Woch. Thierheilk. u. Viehzucht*, 1908, lii, 125.

Morphological and Biological Characters of Non-Hemolytic Streptococci from Inflamed Udders.

In Table I the morphological and cultural characters of forty strains of non-hemolytic streptococci obtained from cases of mastitis are recorded. Fermented veal bouillon containing 1 per cent of the various carbohydrates and other substances was used to test the fermentative action of the strains. The initial reaction of the media varied between 0.6 and 0.8 per cent acid to phenolphthalein. Tubes 1.5 cm. in diameter, containing 13 cc. of media were used throughout. The column of liquid varied in height from 6.5 to 7 cm. Titrations were made after an incubation of 5 days at 38°C. The figures under each column denote the net production of acid.

The surface colonies on agar are usually round, delicate, slightly raised, almost transparent, and measure between 1 and 2 mm. in diameter. Occasionally one observes larger, flattened colonies. The deep colonies are tiny, ovoid, or biconvex in appearance. A few strains when grown in horse blood agar plates have produced a narrow, green-tinted zone about the deep colonies.

Morphologically the individuals appear as spherical or slightly elongated cocci. The chain formation varies over a considerable latitude. All stain by Gram's method.

Freshly isolated strains have not proved pathogenic for rabbits weighing between 1,500 and 2,000 gm. 1 cc. of a 24 hour bouillon culture injected intravenously failed to produce symptoms in most instances. Two animals revealed slight irregularities in temperature for a few days subsequent to inoculation but localizations failed to develop. Davis[18] isolated non-hemolytic streptococci from three cases of mastitis. He was able to produce joint localizations in rabbits only after the intravenous injection of the growth from two or more blood agar slant cultures.

From Table I it will be noted that the mastitis streptococci fall into two groups. The larger, composed of thirty-four strains, produces acidity in dextrose, lactose, saccharose, maltose, and salicin. The five individuals of the other group agree as to their general characters except that they fail to act upon salicin. The acid produc-

[18] Davis, D. J., *J. Infect. Dis.*, 1916, xix, 236.

TABLE I.

Morphological and Biological Characters of Non-Hemolytic Streptococci from Inflamed Udders.

Strain No.	Grouping.	Gram's stain.	Growth in bouillon.	Milk.	Production of acid in.							
					Dextrose.	Lactose.	Saccharose.	Maltose.	Raffinose.	Inulin.	Mannite.	Salicin.
					per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
C.51C.	L.C.*	+	Turbid.	Firmly coagulated.	4.8	4.0	3.7	4.0	0.0	0.0	0.1	3.9
C.51D.	"	+	"	" "	4.4	4.0	3.8	3.2	0.1	0.0	0.0	4.2
C.55L.H.Q.	M.C.	+	"	" "	4.2	3.7	3.6	3.9	0.2	0.1	0.0	3.3
C.55R.F.	L.C.	+	"	" "	4.5	4.0	3.9	3.8	0.2	0.2	0.0	3.0
C.56R.H.	M.C.	+	"	" "	4.6	3.7	3.9	4.1	0.0	0.0	0.0	3.5
C.65A.	L.C.	+	Clear.	" "	5.0	3.5	3.5	4.6	0.2	0.0	0.0	2.8
C.67D.	M.C.	+	Turbid.	" "	5.3	4.4	4.5	4.5	0.1	0.1	0.0	2.9
C.66	S.C.	+	"	" "	3.6	3.8	3.9	3.9	0.1	0.0	0.2	3.5
C.70R.H.	L.C.	+	Clear.	" "	3.8	4.0	3.7	3.1	0.0	0.2	0.1	2.6
C.70L.H.	"	+	"	Partially "	4.1	4.1	3.7	3.7	0.0	0.0	0.0	3.5
C.70R.F.	"	+	"	Firmly "	3.5	3.8	3.2	3.2	0.1	0.1	0.0	3.0
C.68R.H.	M.C.	+	Turbid.	" "	3.9	3.5	3.2	3.4	0.1	0.0	0.1	2.8
C.72B.	S.C.	+	Clear.	" "	4.2	3.5	3.1	3.8	0.0	0.1	0.1	3.1
C.141	"	+	"	Partially "	4.5	4.2	3.4	2.9	0.2	0.1	0.0	4.0
M.L.	M.C.	+	"	" "	2.2	2.7	2.5	2.1	0.0	0.0	0.1	2.0
M.S.	"	+	Turbid.	Firmly "	5.3	3.5	4.5	2.9	0.1	0.0	0.0	3.6
M.U.	"	+	Clear.	Partially "	3.4	3.6	3.5	3.5	0.0	0.1	0.0	3.0
M.X.	"	+	Turbid.	Firmly "	3.8	3.1	3.2	4.0	0.0	0.0	0.0	2.3
M.2	"	+	Clear.	Partially "	2.3	2.9	3.2	2.5	0.1	0.0	0.0	0.2
M.5	S.C.	+	"	" "	2.2	2.3	2.4	2.0	0.2	0.2	0.2	0.2
M.8	"	+	"	Firmly "	2.7	3.0	2.6	2.3	0.1	0.1	0.1	0.1
M.10	"	+	"	" "	6.5	4.2	3.7	4.7	0.1	0.0	0.0	0.5

M.11	L.C.	+	Turbid.	Firmly coagulated.	4.9	3.8	3.8	5.0	0.0	0.0	0.0	2.9
Abs.4	M.C.	+	"	" "	2.3	2.6	2.3	2.0	1.5	1.1	1.5	1.2
M.21	"	+	"	" "	4.2	3.4	3.0	3.3	0.1	0.0	0.1	0.0
M.22	S.C.	+	"	" "	4.4	4.5	4.1	4.0	0.1	0.0	0.0	2.4
M.25	M.C.	+	"	" "	3.3	2.9	2.4	3.0	0.1	0.1	0.1	1.5
M.34	"	+	Clear.	Coagulated on boiling.	3.0	3.3	3.3	3.5	0.0	0.0	0.0	0.0
M.35	S.C.	+	Turbid.	Firmly coagulated.	4.9	4.4	4.1	5.0	0.0	0.0	0.0	3.9
M.37	M.C.	+	"	Coagulated on boiling.	3.3	2.5	2.8	3.3	0.0	0.0	0.0	1.9
M.38	"	+	Clear.	Firmly coagulated.	4.5	3.8	3.7	4.1	0.1	0.0	0.1	3.1
M.40	S.C.	+	"	" "	5.6	3.8	4.3	4.9	0.1	0.1	0.0	3.1
M.42	L.C.	+	"	Partially "	3.9	3.7	3.3	3.1	0.0	0.0	0.1	2.6
M.45	M.C.	+	"	Firmly "	3.6	3.5	3.4	3.2	0.1	0.0	0.1	2.4
M.46	"	+	"	" "	3.9	3.5	3.4	3.5	0.1	0.1	0.1	2.6
M.58	"	+	"	" "	4.0	3.6	3.6	3.4	0.0	0.1	0.0	2.6
M.61	L.C.	+	"	" "	4.1	3.8	3.7	3.4	0.1	0.1	0.0	2.9
M.62	M.C.	+	Turbid.	" "	4.2	3.6	3.6	3.5	0.0	0.0	0.1	3.4
M.73	"	+	"	" "	4.2	3.9	3.9	4.0	0.0	0.0	0.0	3.7
M.75	"	+	"	" "	4.0	3.7	3.7	3.5	0.1	0.0	0.0	3.3

* The length of the chains in bouillon has been indicated as follows: S.C., chains of six or eight elements; M.C., threads of ten to twenty cocci; and L.C., chains of more than twenty.

tion of Strains M.5, M.8, and M.2 in dextrose, lactose, saccharose, and maltose bouillon is uniformly lower than that generally recorded. Strain Abs. 4 differs markedly from the others in that it ferments all the carbohydrates, although acid production is lower in raffinose, inulin, mannite, and salicin than in the others. This streptococcus was isolated from a subcutaneous abscess of the udder of a cow.

All streptococci grew well in bouillon, some left the medium clear, others produced a diffuse turbidity. A large majority firmly coagulated milk, others produced only a partial clotting after incubation for 5 days. In two instances the medium appeared unchanged when removed from the incubator, but coagulated promptly on boiling.

Since all strains failed to show major differences in their cultural characters, it seemed well to test their interagglutinability with a serum obtained from the injection of a single strain of streptococci. A nonlactating cow was chosen as an experimental animal. Before commencing the injections the serum of the animal was tested for agglutinins against three typical strains of streptococci. Agglutinations were not observed at dilutions of 1: 10. Immunization with killed cultures of Streptococcus C.55 was begun on October 15, 1917. The doses were increased gradually, and when it seemed that a resistance had been established, living cultures were inoculated. On December 12 the serum completely agglutinated the streptococci at a dilution of 1: 20,000.

The following method was employed in testing the agglutinating properties of each strain. The growth from 24 hour agar slant cultures was suspended in sterile 1 per cent solution of sodium chloride. Usually the suspensions were agitated with a platinum loop to break up the larger particles. All suspensions were diluted with the salt solution to a uniform density. To each cubic centimeter of this test fluid varying amounts of immune serum were added. Readings were made after incubation for 24 hours at 38°C. A tube containing only the suspension was incubated as a control.

Bovine streptococci usually produce homogeneous suspensions in a 1 per cent solution of sodium chloride and do not tend to precipitate spontaneously during the 24 hour incubation period.

All strains are agglutinated to a greater or less degree by the antiserum produced by immunization with a single species. The non-

TABLE II.

Agglutination Titer of Non-Hemolytic Streptococci Tested with a Serum Produced by the Immunization of a Cow with a Single Strain.

Strain No.	Dilutions.						
	1:100	1:500	1:1,000	1:2,000	1:5,000	1:10,000	1:20,000
C.55	+++*	+++	+++	+++	+++	+++	+++
C.55R.F.	+++	+++	+++	++	+	−	−
C.56R.H.	+++	+++	+++	+++	+	−	−
C.51C.	+++	+++	+++	+++	++	+	−
C.65A.	+++	+	+	−	−	−	−
C.67D.	+++	+++	+++	+++	+++	+	?
C.66	+++	+++	+++	+++	+++	++	+
C.70R.H.	+++	+++	+++	++	+	−	−
C.70L.H.	+++	+++	+++	++	++	−	−
C.70R.F.	+++	+++	+++	+++	+	?	−
C.72B.	+++	+++	+++	+++	+++	++	+
C.68R.H.	+++	+++	+++	+++	++	−	−
C.51D.	+++	+++	++	+	−	−	−
M.L.	+++	+++	+++	+++	++	−	−
M.S.	+++	+++	+++	+++	+++	+	−
M.U.	+++	+++	+++	++	+	+	−
M.2	+++	+++	+++	+++	+++	++	+
M.5	+++	+++	+++	+++	++	+	−
M.8	+++	+++	+++	+++	+++	+++	+++
M.10	+++	+++	++	+	−	−	−
M.11	+++	+++	+++	+++	+++	+++	+
Abs.4	+++	+++	+++	++	−	−	−
M.21	+++	+++	+++	+++	+	−	−
M.22	+++	+++	+++	+++	++	+	−
C.141	+++	+	−	−	−	−	−
M.25	+++	+++	++	−	−	−	−
M.34	+++	+++	+++	+++	+++	++	−
M.35	+++	+++	+++	+++	+++	++	+
M.37	+++	+++	+++	+	−	−	−
M.38	+++	+++	+++	+++	+++	+++	+++
M.40	+++	+++	+++	+++	+++	+++	+++
M.45	+++	+++	+++	+++	+++	+++	++
M.46	+++	+++	++	+	−	−	−
M.58	+++	+++	+++	++	+		−
M.61	+++	++	+	−	−	−	−
M.62	+++	++	+	−	−	−	−
M.73	+++	+++	+++	+++	+	−	−
M.75	+++	+++	+	+	−	−	−

* Clumping attended by complete clearing of the fluid has been recorded as +++. ++ indicates considerable agglutination without the entire clearing of the fluid. A moderate precipitation has been considered +. A negative reaction has been recorded as −.

salicin-fermenting strains—M.2, M.5, M.8, M.21, and M.34—were agglutinated as readily as those which attacked this substance (Table II). Streptococcus Abs. 4 which differed from all the others was likewise agglutinated at a serum dilution of 1: 2,000.

All the streptococci except Strains C.141, M.58, M.61, M.62, M.73, and M.75 were isolated from cases of mastitis occurring on one farm. In each case agglutination occurred at a minimum serum dilution of 1: 1,000. Of the six strains from other farms five agglutinated at 1: 1,000, the other (Strain C.141) was partially clumped at 1: 500. Cultures obtained from the same source as the immunizing strain were uniformly agglutinated at higher dilutions than those obtained elsewhere.

Five strains of hemolytic bovine streptococci isolated from inflamed udders were also tested. Each strain possessed many characters in common with the non-hemolytic types. Usually the only distinguishing difference was their action upon hemoglobin. In no instance were any of them agglutinated at dilutions as low as 1: 100.

Feeding of Mastitis Milk to a Pig.

Although freshly isolated strains of non-hemolytic streptococci failed to produce marked effects when inoculated into rabbits, it was considered necessary to test the effect of ingestion of large quantities of these organisms. A young pig weighing 99 pounds was chosen for the experiment. It was fed for 15 days with flocculent milk from Cows 55 and 56. The animal averaged about 2 quarts of purulent milk a day. The diet was augmented with a small amount of grain. Morning and evening temperatures were taken before the feeding was begun and during the experiment. The slight variations recorded were well within normal limits. The animal was under observation for 10 days after the milk feeding was discontinued but failed to show symptoms of any disorder. The pig gained 25 pounds during the experiment.

DISCUSSION.

Mastitis caused by infections with non-hemolytic streptococci is more prevalent than that caused by other classes of microorganisms. Data point to the extreme severity of these infections. Of the thir-

teen animals under observation for a considerable period but four have recovered. The others have either lost the function of the involved quarters or the disease has progressed to such an extent that they no longer remained profitable as milk producers.

It has been the custom of many investigators to consider the entrance of pathogenic microorganisms into the mammary gland in three ways: (1) metastasis from another disease focus within the body; (2) through wounds; and (3) through the teat canal. Localizations through the blood and lymph vessels occur in tuberculosis and actinomycosis as well as in some other maladies. Wound infection is probably responsible for gangrenous forms of mammitis. The probable mode of infections caused by non-hemolytic streptococci is through the teat canal. The disease is local, usually only one or two quarters are involved, and the general condition of the animal is not markedly affected. The elimination of streptococci several days before symptoms develop also points to entrance through the duct of the teat.

Injury has been considered by many investigators to be an important predisposing factor in udder inflammation. This has not been my experience. If injuries occurred they were of such a minor nature that they escaped detection. If injury plays a major part as a predisposing factor one would expect to find, in many cases, ulceration of the lining membranes of the large ducts and milk cystern and abscesses of the parenchyma. Such lesions have not been observed in the material examined.

It has been difficult to trace infection from one animal to another. On the farm where a large proportion of the material was obtained a "gang" system of milking has been adopted. The milkers are each assigned a cow to milk. The attendant washes his hands after milking each animal, and he is assigned another. In this way each man milks two or three cows irregularly spaced about each barn. This procedure renders the tracing of infections difficult. It seems reasonable to suppose that the extreme irregularity of the occurrence of infection throughout the herd may be explained by the transfer of the virus on the hands of the milkers. Clinical cases are constantly appearing. Cows revealing gross changes in one quarter and harboring streptococci in apparently normal quarters must be considered as dan-

gerous virus reservoirs. Incipient cases eliminate streptococci before symptoms develop. One animal suffered from an udder invasion with streptococci identical in every respect with those obtained from severe inflammations but never developed clinical mastitis. These conditions account at least for the spread of the virus. Contamination of the ends of the teats with feces and vaginal secretions may explain other possible sources of infection.

On one farm visited the incidence of udder inflammations approximated 10 per cent of the cows in one barn. It was customary to milk with a milking machine. The general sanitary conditions were excellent, except that the teat cups were not disinfected or sterilized between the milking of individual cows. The infection was probably spread by the contaminated milk cups.

Definite evidence is lacking to show whether the non-hemolytic streptococci isolated from inflamed mammæ are pathogenic for consumers of milk. Milk-borne epidemics of tonsillitis have been attributed to hemolytic streptococci and up to this time the non-hemolytic forms have not been incriminated, although it must be assumed that non-hemolytic streptococci from inflamed udders gain frequent access to the milk supply. The lack of virulence of these organisms when injected into rabbits and when milk containing enormous numbers of these streptococci was fed to a pig indicates their low pathogenicity for species other than bovines.

SUMMARY.

It seems clearly established that non-hemolytic streptococci are responsible for a considerable number of cases of bovine mastitis. Of the 81 animals examined, 31 were suffering from infections of this type. The lesions produced in invaded quarters varied from an involvement of only the lining epithelium of the large milk ducts to severe degeneration and necrosis of the secreting epithelium. In one instance a considerable portion of the glandular elements had been replaced with connective tissue.

The streptococci fall into two groups when their action on the various carbohydrates is considered. Thirty-four strains fermented dextrose, lactose, saccharose, maltose, and salicin; five others attacked

the first four sugars but failed to produce acid in salicin. All mastitis streptococci failed to act upon raffinose, inulin, or mannite. One species isolated from a mammary abscess produced acid in all the carbohydrates.

All the strains were agglutinated with an antiserum prepared from one typical strain. The agglutination titer varied over wide limits, although all the streptococci were agglutinated at a dilution of 1: 500. None of the strains inoculated proved pathogenic for rabbits. A pig fed on the milk from two typical cases of mastitis remained well.

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