# A STUDY OF STREPTOCOCCI ISOLATED FROM CERTAIN PRESUMABLY MILK-BORNE EPIDEMICS OF TONSILLITIS OCCURRING IN MASSACHUSETTS IN 1913 AND 1914.*

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## I.

There have occurred more or less extensive milk-borne epidemics of tonsillitis in the vicinity of Boston and elsewhere in the State of Massachusetts in 1911, 1913, and 1914. The prevalence of tonsillitis in epidemic proportions was reported from Chicago and Baltimore in 1912, and from Homer and Cortland in the State of New York in 1913. England has had many small epidemics traceable to milk as a vehicle, and it is now quite well established that milk is the chief, if not the only, disseminator of this type of disease on a large scale. This relatively new and unexpected manifestation of a well-known type of disease presents a problem, the solution of which is by no means ended by the proof that the disease is scattered by milk. The term "milk-borne" is made up of many subsidiary problems which must be cleared up unless all milk is to be efficiently pasteurized in the final container. Unless we know precisely the ways in which milk becomes infected, the raw product involves a menace not wholly eliminated even by very great care on the farm and in the dairy.

The disease disseminated by milk has been regarded by physicians as of a slightly different, more virulent type than the usual sporadic tonsillitis. The infection has shown more invasive tendencies with capacity to set up inflammation in distant organs and tissues. The more local complications are peritonsillar abscess, swelling and suppuration of cervical lymph nodes, middle ear disease, and rarely meningitis. Erysipelas, pleuritis and empyema, arthritis, nephritis and

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especially peritonitis have been observed. The fatalities of the epidemic disease have been regarded as much greater than in the sporadic cases. This is not generally true. It may be that the season is more or less responsible, for the summer outbreaks appear to be less virulent.

The agent of these complications has been quite definitely settled upon as a streptococcus. The same strain has also been found in the throat in certain cases, usually associated with other types of streptococci. It has been assumed that the strain associated with the secondary, metastatic lesions was also the agent of the primary throat lesion. In the material supplied this laboratory by the State Board of Health from certain smaller epidemics occurring in Massachusetts in 1913 and 1914, we have, therefore, focussed our attention upon the streptococci. Several theories have crystallized out of the investigations made thus far. They are:

1. The streptococci of the human epidemic are such as are commonly associated with inflammations of the udder, *i.e.*, they are organisms belonging to the cow which accidentally possess a relatively high degree of virulence for man.

2. They are human streptococci which have been shed into the milk. This serves as a medium for temporary multiplication and as a vehicle for their redistribution.

3. The heightened virulence of the streptococci causing these outbreaks needs explanation whether we regard them as essentially bovine or human. If bovine, they must represent a special strain rather uncommon, otherwise we should expect epidemics all the year around. If human, they may also represent some peculiar type or else the product of a temporarily increased virulence due to two coöperating factors, reduced resistance on the part of human beings in the late winter and early spring brought about by a combination of untoward living conditions, and the better opportunity for rapid passages from throat to throat — a process tending to raise virulence in many microörganisms.

4. There finally remains the important problem of how the milk is infected, provided the infection be essentially human. Our studies have led us to regard the infection as

human and to place the infection, in case of large, prolonged outbreaks, back into the udder itself whence the introduced streptococci may be shed in large numbers after multiplying in the udder ducts. Whether this udder infection has been demonstrated or not the reader must decide from the facts to be submitted.

Owing to the very large number of publications dealing with the recent epidemics, with diseases of the udder and the associated bacteria as well as with the bacteria encountered in normal udders, we shall briefly describe our own studies and then discuss our results in conjunction with such earlier researches of others as bear directly upon our findings.

## II.

The material to be studied was brought to the laboratory in the form of swabs from throats, peritoneal exudate, discharge from suppurating lymph nodes, or as cultures from such swabs on Löffler's blood serum. The milk samples were with few exceptions taken by a veterinary inspector directly from the udder. A small amount was milked into sterile, heavy-walled test-tubes and these closed with sterile rubber stoppers.

The streptococci studied were isolated on horse-blood-agar plates directly from the swabs, milk samples or Löffler's tubes, and were maintained on horse-blood agar.

The tests upon rabbits were made from a week to fourteen months after isolation. The cultures were kept, in the meantime, on agar plus several drops of defibrinated horse blood. The cultures from the earlier outbreaks had been kept on plain agar until the summer of 1913, when they also were grown with some horse blood.

OUTBREAK A (Norton, Mass.). — This occurred in an educational institution for young women. Fifty-five cases of sore throat developed May ninth to eleventh, 1913. On May twenty-first, swabs were taken during operation from peritoneal pus of two of the inmates, desperately ill, although neither had complained of sore throat at the time of the

epidemic. In addition to these, some five or six throat cultures came into our hands from three of which streptococci were isolated (Nos. 100, 103, 104). Samples of milk from the individual cows of the herd supplying the institution were received May twenty-third.

The culture S. H. from peritoneal pus, made with a swab obtained during the operation, was contaminated with a large spore-bearing bacillus of the subtilis type. The purified culture had the following characters: On horse-blood-agar plates, when colonies were well scattered, the deep ones after forty-eight hours were lenticular in outline, opaque, about one millimeter long. The clear hemolytic area was about 3.5 millimeters in diameter. The dimension given applies here as elsewhere to the diameter of the total laked area. That is, it includes the colony. A few surface colonies were present as very delicate, nearly translucent expansions up to eight millimeters in diameter. The clear area was about ten millimeters. The colonies on plain agar slants appeared as minute conical droplets, very translucent. The bouillon cultures showed, at first, only very faint clouding. Growth chiefly deposited on bottom and sides of tube. There was no absolute limpidity of culture fluid sometimes seen in streptococcus cultures. Later cultures became well clouded within twenty-four hours, but cleared up on standing. Milk cultures after six days in the incubator showed no changes even after boiling. That no acids are produced was shown by titration. This result agrees with the observation that lactose was not attacked. The streptococci appeared as long chains sometimes convoluted into clumps. The individual cocci were roundish; swollen forms were rare.

A swab from the peritoneal pus of S. T. received with that of S. H. was the starting point of pure cultures of streptococci which were studied side by side with those of S. H. and found identical with the latter. Their characteristics need not, therefore, be detailed again.

Two other patients from the same institution were admitted to the same hospital May 23, with symptoms of peritonitis. Both had had tonsillitis two weeks before, one

a severe, the other a mild attack. In both, the symptoms of abdominal infection disappeared in a short time without operative interference. Streptococci had been found by others in cultures from the throat of the first mentioned at the time of the tonsillitis. A culture from the throat of the other case showed a different type. This culture is designated as BL under Outbreak A.

From the throats of a certain number of associates of S. H. and S. T., swabs were obtained and the hemolytic streptococci isolated from three of these (Nos. 100, 103, and 104). These were studied side by side with the above two cultures. Of these, two (100 and 104) are identical with the cultures S. H. and S. T. (peritonitis). The third (103) differs in several particulars. It does not ferment mannite, whereas the above four strains do. A comparative study of the cultures at the beginning of the investigation showed that, in bouillon, No. 103 tended to settle out and leave the fluid clear. The fluid in case of Nos. 100 and 104 remained clouded. The long chains in the latter were, as a rule, free; in No. 103 they were more or less twisted and interwoven with one another. On blood agar the elements of the latter appeared a trifle smaller than parallel cultures of Nos. 100 and 104.

These may seem minor differences upon which to base distinctions, but they did exist and they proved that No. 103 was not identical with the streptococci which produced the disease, although it agreed with the others (S. H., S. T., 100, and 104) in not acting upon lactose (and milk) and thus differing with these from all streptococci of this same type of hemolysis subsequently isolated by us.

The action of the human and the bovine strains on fermentable carbohydrates is shown in Table I. The procedure consisted in adding from a sterile concentrated solution of the substance to be acted upon one per cent to sugar-free bouillon in test-tubes. After a week's stay at 36° C. the contents of the tubes were titrated with N/20 NaHO. The figures in the tables represent per cent of normal acid found. The italics indicate that the strain has produced acid.

The relation between the strains S. T., S. H., Nos. 100, 103, and 104 is further developed in Table II. The serum of a rabbit treated repeatedly with heated cultures of Streptococcus S. T. was used to determine agglutination relationships. Culture No. 103 contained clumps of cocci and abundant sediment in control as well as serum tubes, but there was no evidence of any specific clumping. The method used was briefly as follows:

TABLE I.

*Cultures from Outbreak A.*

| Designation of Culture. | | Dextrose. | Lactose. | Saccharose. | Maltose. | Raffinose. | Mannite. | Inulin. | Salicin. | Milk. |
|---|---|---|---|---|---|---|---|---|---|---|
| Type of Hemolysis. | | | | | | | | | | |
| Alpha. | Beta. | | | | | | | | | |
| BL. . . | . . . . . | 5.4 | 4.9 | 3.3 | 3.7 | 1.1 | 0.8 | 0.8 | 3.55 | Coag. |
| | S. H. (peritoneum) . . | 3.85 | 1.0 | 3.65 | 3.85 | 1.0 | 3.4 | 1.2 | 4.35 | Not coag. |
| | S. T. " . . | 3.95 | 1.0 | 3.85 | 4.0 | 1.15 | 3.2 | 1.15 | 3.0 | " " |
| | 100 (throat) . . . . . | 3.7 | 1.0 | 4.1 | 3.8 | 1.2 | 3.1 | 1.25 | 4.5 | " " |
| | 103 " . . . . . | 4.5 | 1.15 | 4.25 | 4.65 | 1.1 | 1.2 | 1.15 | 4.4 | " " |
| | 104 " . . . . . | 4.2 | 1.3 | 3.8 | 3.95 | 1.3 | 3.55 | 1.3 | 3.1 | " " |
| | 1 (cow) . . . . . | 5.65 | 4.4 | 4.65 | 4.75 | 1.15 | 0.95 | 1.15 | 1.4 | Coag. |
| | 8 " . . . . . | 6.0 | 4.85 | 4.8 | 5.05 | 1.1 | 1.0 | 1.15 | 1.45 | " |
| | 18 " . . . . . | 5.55 | 5.0 | 4.7 | 4.8 | 1.3 | 1.0 | 1.0 | 1.48 | " |
| | 25 " . . . . . | 6.0 | 5.0 | 4.75 | 5.0 | 1.1 | 1.1 | 1.1 | 1.15 | " |

TABLE II.

*Agglutinins in serum of rabbit immunized with streptococcus S. T.*

(Readings next day. Over night in refrigerator. The heavy line extends to agglutination limits.)

| Designation of Culture. | Serum Dilutions. | | | | | | | | | Control. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1/20 | 1/40 | 1/80 | 1/160 | 1/320 | 1/640 | 1/1280 | 1/2560 | 1/5120 | |
| S. T. . . . . | $\frac{+}{++}$ | ++ | ++ | + | + | + | + | $\frac{+}{-}$ | — — | — — |
| S. H. . . . . | $\frac{+}{++}$ | $\frac{+}{++}$ | ++ | + | + | + | + | + | $\frac{+}{-}$ | — — |
| 100 . . . . . | $\frac{+}{++}$ | $\frac{+}{++}$ | ++ | + | + | + | + | + | — — | — — |
| 103 . . . . . | C | ++<br>++ | ++<br>++ | ++<br>++ | ++<br>++ | ++<br>++ | ++<br>++ | ++<br>++ | ++<br>++ | ++<br>++ |
| 104 . . . . . | $\frac{+}{++}$ | $\frac{+}{++}$ | ++ | ++ | ++ | ++ | + | + | — — | — — |

After two and one-half hours at 36° C. all 1/20 tubes and control tube of 103 showed some agglutination.

In the above and following tables C signifies complete clumping and sedimentation; $\frac{++}{++}$ complete clumping but not complete subsidence; $\frac{+}{++}$ nearly complete clumping; ++ partial; + slight, and $\frac{+}{-}$ doubtful; — — indicates no clumping.

Twenty-four-hour bouillon cultures were shaken thoroughly in a mechanical shaker to break up clumps or chains. This fluid suspension was added to serum dilutions in small test-tubes and the mixtures placed at 36° C. for one and a half to two hours. After an inspection of the tubes they were placed at about 10° C. over night and the final reading taken next morning. In addition to the tests tabulated, a number were made with other immune sera, but the degree of clumping was uniformly too low and hence the results are omitted.

To determine any pathogenic action of the strains the rabbit was chosen in preference to the mouse. A uniform dose of one cubic centimeter of a bouillon culture twenty-four hours old was injected into an ear vein. It should be noted that this dose, which has been adhered to throughout our work, is relatively small as compared with the dosage

used by many other workers. In Table III. a synopsis of the inoculations of the strains obtained from Outbreak A is given. It will be noticed that all these strains except No. 103 had some pathogenic effect. Unfortunately, some of the rabbits used had a latent infection of snuffles (rabbit septicemia), which showed itself later as a necrotic pneumonia with suppurative pleuritis or as a more or less extensive suppurative infiltration of the subcutis. In one case the lymphatic apparatus of the intestine was involved. It will be noticed that the injection produced a relatively high temperature (104°–106° F.) for a variable number of days, ending in death or recovery. In those that died extensive suppurative lesions were found in the medulla of kidneys (S. T.), in peritoneum (No. 100). Suppurative joint affections involving the muscular and tendinous structures and the joints themselves were common (S. T., 100, 104). In one case (S. H.) the rabbit died of acute septicemia.

Samples of milk from twenty-five cows were received about ten days after the beginning of the outbreak. After standing in a refrigerator of low temperature over night, the sediment of every one was examined microscopically. Samples Nos. 1, 4, 6, 8, 9, 18, and 25 showed many leucocytes. Samples Nos. 7, 10, 11, 12, 17, 19, and 21 showed a few leucocytes. Nos. 13, 14, 15, 20, 22, 23, and 24 showed none. Bacteria were scarce in some samples, more abundant in others. A few streptococci were clearly seen in No. 6 only. It should be borne in mind that only about ten cubic centimeters of milk were submitted. The result of the examination might have been somewhat different if the sediment of fifty or one hundred cubic centimeters had been available.

Four guinea-pigs receiving .5 cubic centimeter sediment of milk into the peritoneal cavity from samples of Cow Nos. 1, 4, 6, and 8 showed no disease and increased in weight. No chronic lesions were found when they were autopsied after several weeks. These samples were chosen because of the abundant leucocytic content.

From samples of Nos. 1, 8, 18, and 25 streptococci were isolated apparently identical and with the following characters:

On horse-blood-agar plates the deep colonies are lens-shaped, opaque, frequently with minute daughter colonies attached, situated in a clear, hemolyzed, circular, sharply defined area from three to ten or twelve millimeters in diameter. In one case (No. 18) there was observed a clear inner circle three millimeters in diameter and a partly cleared outer zone, up to ten millimeters in total diameter. In other words, the hemolytic activity of these strains was very marked.

In bouillon after twenty-four hours a heavy deposit of long, loosely intertwined chains of fairly large roundish cocci appeared. The bouillon was clear. When shaken it became heavily turbid. On slanted agar the colonies tend to become confluent into patches with clear spaces between. The colonies resemble droplets of paraffine.

Three rabbits were inoculated with three hemolytic cow strains, respectively (Nos. 8, 18, 25), in precisely the same way as were the human strains from this outbreak. None of these rabbits showed either fever or any local reaction of any kind; none were made ill. The temperature fluctuations were within the normal. When we compare these results with those obtained after inoculation of rabbits with the hemolytic streptococci from the throats and peritoneal pus, we find a distinct demarcation between the human and bovine streptococci.

The absence of any agglutination relationship between one of the human strains and the bovine strain No. 18 is shown in Tables IV. and V. The cow culture showed slight clumping in the control, but the specific action of its serum was unmistakable.

TABLE III.

*Effect of hemolytic streptococci from throats and cow's milk in Outbreak A on rabbits following intravenous injection.*

| Designation of Strain. | Weight of Rabbit (grams). | Effect on Temperature. | Localizations. | Result. | Remarks. |
|---|---|---|---|---|---|
| S. H. β . . . | 2490 | High. | . . . . . | Dies in 3-4 days. Weight, 2185. | Septicemia. Cultures from various organs and urine positive. |
| S. T. β . . . . | 2720 | " | Right tarsus. Left knee joint. | Dies in 8 days. Weight, 2215. | Septicemia. Suppurative foci in kidneys. |
| 100 β . . . . . | 1610 | " | Ears swollen. Carpal and tarsal joints affected. | Chloroformed, after 10 days in dying condition. Weight, 1265. | Septicemia. Peritonitis complicated with necrotic pneumonia (snuffles). |
| 100 β . . . . . | 1480 | High for 8 days with relapses. | Left shoulder joint. Probably others transiently affected. | Chloroformed after 2 months. Weight, 1630. | Pure culture from pus of shoulder joint. |
| 103 β . . . . . | 2535 | None. | None. | No effect. Weight, 2835 after 40 days. | |
| 104 β . . . . | 2800 | High for 6 days. | Local lesion only. | Recovered. Weight, 2405 after 10 days. | Injection into abdomen. |
| 104 β . . . . | 2685 | High for 40 days. | Left carpus and right tarsus. | Chloroformed after 40 days. Weight, 1940. | Suppurative foci in mesentery and mesenteric lymph nodes; in Peyer's patch at ileo-cecal valve and in appendix. |
| BL α . . . . | 2660 | Slight (one day). | None. | No effect. | |
| 8 β (cow's milk) . . . | 2270 | None. | " | " " | Weight, 2300 on 13th day. |
| 18 β (cow's milk) . . . | 1620 | " | " | " " | Weight, 1790 on 21st day. |
| 25 β (cow's milk) . . . | 2315 | " | " | " " | Weight, 2415 on 25th day. |

TABLE IV.

*Agglutinins in serum of rabbit immunized with Streptococcus S. T.*

(1½ hours at 36° and over night in refrigerator.)

| Designation of Culture. | Serum Dilution. | | | | | | | | | Control. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1/20 | 1/40 | 1/80 | 1/160 | 1/320 | 1/640 | 1/1280 | 1/2560 | 1/5120 | |
| S. T. . . . . | + / ++ | + / ++ | ++ | ++ | ++ | ++ | + | + / − | − − | − − |
| L. F. (Outbreak B) . | + | + | + / − | + / − | + / − | + / − | + / − | − − | − − | − − |
| Cow 18 (Outbreak A). | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| No. 38 (Baltimore) . . | + | + / − | + / − | + / − | − − | − − | − − | − − | − − | − − |
| No. 40 (Chicago) . . . | + / − | − − | − − | − − | − − | − − | − − | − − | − − | − − |

TABLE V.

*Agglutinins in serum of rabbit immunized with streptococcus of Cow 18 (Outbreak A).*

| Designation of Cultures. | Serum Dilutions. | | | | | | | | | Control in Salt Solution. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1/20 | 1/40 | 1/80 | 1/160 | 1/320 | 1/640 | 1/1280 | 1/2560 | 1/5120 | |
| A. S. T. . . . . | + / − | + / − | − | − | − | − | − | − | − | − |
| B. L. F. . . . . | − | − | − | − | − | − | − | − | − | − |
| A. 18 (Cow) . . . | C | C | C | ++ / ++ | + / ++ | + / ++ | ++ | ++ | + | + |
| No. 38 (Baltimore) . | ++ | ++ | + | + / − | − | − | − | − | − | − |
| No. 40 (Chicago) . | ++ | + | + | + / − | − | − | − | − | − | − |

SUMMARY. — In this outbreak, certain hemolytic streptococci producing peritonitis were found identical with streptococci from the throats of associated tonsillitis cases. The streptococci were peculiar in that they acted upon mannite and not upon lactose and hence not upon milk. A nonpathogenic, hemolytic strain from the throat of one patient

also failed to act upon lactose and milk but differed from the rest in not fermenting mannite. The streptococci produced variable lesions in rabbits. Hemolytic streptococci, isolated from the milk of cows supplying the institution, differed from the human strains in acting upon lactose (and milk), in not acting upon salicin, in producing a somewhat higher acidity in bouillon containing sugars and in not causing any disturbance in inoculated rabbits. They also were not related to the human strains in their agglutinative affinities.

Since the samples of milk examined were drawn some days after the height of the tonsillitis, we cannot assume that the human strain was not in the milk as it came from the udders at the time of the epidemic. We feel, however, fairly certain from the results obtained that the hemolytic bovine streptococci, probably associated with slight mastitis or garget in the cows, were not the cause of the epidemic.

The hemolytic activity of this group of streptococci from human cases has remained unimpaired during the year.

OUTBREAK B (Canton, Mass.). — In a town not far from the one in which Outbreak A prevailed, a severe epidemic of tonsillitis occurred, also in May, 1913. That there was no connection between these may be accepted at the start, for the underlying streptococci as will be seen later were not identical. From this epidemic were received swabs and cultures from a number of patients and samples of milk from the cows of the dairy herd assumed to have been the source of the infection. In most of the cultures, two types of streptococci, with reference to hemolysis, were found and, as there were at hand no pure cultures from metastatic lesions to guide us, both types were taken into consideration. We give below a brief description of both types, since in recent publications about milk-borne epidemics the description of the streptococcus involved does not tally with ours. It should be borne in mind that we have used throughout horse-blood as a hemolytic indicator on agar plates, whereas other observers have used human and rabbit blood. We have, however, as will be seen later, drawn into our studies cultures kept in

this laboratory from the Boston epidemic of 1911 as well as cultures from the Baltimore and Chicago epidemics. To these we will return later.

Type *α*. As observed after forty-eight hours' incubation the change produced by streptococci of this type may be described as a somewhat greenish discoloration and partial hemolysis of the blood corpuscles immediately surrounding the colony forming a rather indefinitely bounded zone one to two millimeters in diameter and surrounded by a second narrow, clearer, not discolored, partially hemolyzed zone. The inner (discolored) zones were fairly constant in size and composition on all the plates. Under the microscope many of the corpuscles were seen to be present but obviously discolored, the discoloration differing a good deal in intensity for different strains of streptococci. The corpuscles remaining in the outer (clearer) zones were much fewer and never discolored. The outer zones were much more conspicuous on crowded plates than on plates containing only a few colonies. The deep colonies themselves were small (greater diameter, .5–1 millimeter) and bi-convex or complex (lobulated or asteroid), the formation of bi-convex or complex colonies being quite constant and characteristic for different strains. Generally strains isolated from bi-convex colonies produced in bouillon greater clouding and less sediment than those isolated from complex colonies and, under the microscope, the former cultures were seen to consist of shorter chains. The growth of all alpha strains on the agar slant took the form of small, round, convex, glistening, punctiform, discrete colonies. Bouillon cultures examined in the hanging drop after twenty-four hours' incubation consisted of chains of small round and more or less distinctly oval or elongated elements.

Type *β*. Streptococci of this type produced hemolyzed zones on horse-blood-agar plates radically different from those of the alpha type. The former produced sharply defined, clear, transparent, completely hemolyzed, colorless zones two to four millimeters in diameter. Under the microscope no corpuscles were seen to remain within the zones. The

colonies themselves were simple and bi-convex, never complex (greater diameter, .5–1 millimeter). The characteristics of growth in bouillon differed somewhat for different strains, from a well clouded suspension with a small amount of flocculent sediment to a perfectly clear bouillon containing an abundant fleecy sediment. As among strains of the alpha type, greater clouding of the bouillon was associated with the formation of shorter chains. On the agar slant, two types of growth were distinguishable. Some strains produced small, round, discrete, convex, glistening colonies. Others produced fairly large, generally dull, flat, confluent, amebiform colonies. Most of the strains pathogenic for rabbits produced colonies of the latter type on the agar slant. Bouillon cultures examined after twenty-four hours' incubation consisted of chains of round, flattened (transverse axis the greater), and rarely elongated elements. In size, the elements differed a good deal for different strains and were generally more refractive to light than those of the alpha type.

There was nothing particularly distinctive about the surface colonies of either type on blood agar plates. All were round, glistening, grayish, translucent, flatly convex colonies surrounded by hemolyzed zones corresponding to their respective types.

In the following table (Table VI.) the occurrence of the two types in the throat cultures is shown. From some patients swabs and cultures were obtained three different times. It will be seen that the markedly hemolytic type of streptococcus was encountered in nearly all of the cases.

At the same time, samples of milk from all eighteen cows of the suspected herd were examined microscopically, and of these four selected on account of the relatively large numbers of leucocytes. Cultures which caused hemolysis on blood agar plates were obtained from each cow (Nos. 2, 6, 15, and 18).

TABLE VI.

| Designation of Patient. | Source of Culture. | Type α (Feebly Hemolytic). | Type β (Markedly Hemolytic). |
|---|---|---|---|
| L.F. | Blood. | — | + |
| (2, 5, 8)* | Throat. | + | + |
| (1, 6, 7)* | " | + | + |
| (3, 4, 9)* | " | + | + |
| 10 | Gland. | + | + |
| 11 | Erysipelas. | + | Staphylococci. |
| 12 | Scarlet fever. | + | + |
| 13 | Ear. | Miscellaneous forms. | |
| 14 | Throat. | + | + |

* From every one of these three cases three different throat cultures were received with numbers as given in the table.

Of these various strains the one designated L. F. deserves individual mention. It was obtained after death from the blood of a patient in a large Boston hospital, who had come from Canton during the epidemic. There was no direct evidence that his death was due to milk infection. Nevertheless, the culture was included in our studies.

Among these various hemolytic human strains of β type from this outbreak there were slight differences in the form of the colonies on plain agar. Three fairly distinct types were made out in the earliest cultures:

Type a. Colonies appear conglomerate (hand lens) on slanted agar. (Culture Nos. 2, 6, 8.)

Type b. Colonies larger than preceding, flattish, round, thin, about 1.5–2 millimeters in diameter. (Culture Nos. 3, 9.)

Type c. Colonies more vigorous than either of preceding types. (Culture Nos. 10, 14.)

The significance of these slight variations is not understood. They may mean temporary differences due to varying amounts of viscid capsular substance, or they may

represent permanent differences belonging to races and varieties. Subsequent studies have shown that the cultures under Type a are distinguishable from the other types in several ways. Those of Type c agree closely with those of Type b in all characters except as given above.

Without going into details concerning the minute study of these strains from human and bovine sources, we may briefly state that the bovine cultures and the human cultures presented certain likenesses and differences with one exception. One of the bovine streptococci was wholly identical with culture No. 3 from a human throat. This culture came from a patient whose case ended fatally. These relationships and differences are brought out in two ways — first by inoculation of rabbits. In Table No. VII. are given briefly the results of animal tests with different streptococci. It will be seen that the culture of hemolytic streptococci from Cow No. 2 was virulent for rabbits, whereas the cultures from Cow 6 and Cow 18 were not. Two inoculations of the feebly hemolytic type from Cow 15 were negative as regards fever and other symptoms, but a joint lesion appeared in one animal quite late.

TABLE VII.

*Effect of hemolytic streptococci from Outbreak B on rabbits (intravenous injection).*

| Designation of Strain. | Weight of Rabbit (Grams.) | Effect on Temperature. | Localizations. | Result. | Remarks. |
|---|---|---|---|---|---|
| L. F. β . . . | 2035 | Very slight. | None. | No effect. | Weight in 7 days, 2090. |
| L. F. β . . . | 2045 | Moderate. | " | Slight. | Weight in 16 days, 1790. |
| 2 β . . . . . | 2020 | Slight, if any. | " | No effect. | Weight in 17 days, 2355. |
| 3 β . . . . . | 1760 | High. | Hemorrhagic inflammation of rectum. | Dies in 2½ days. | Streptococci in smears from various organs. |
| 3 β . . . . . | 1660 | High for 4 days. | Inoculated ear swollen, edematous, droops. | Recovered. | Weight after 27 days, 1780. |
| 6 β . . . . . | 1890 | None. | None. | No effect. | Weight after 12 days, 2020. |
| 10 β . . . . . | 1755 | High till death. | Small abscesses in psoas muscle. | Dies in 8 days. | Streptococci in various organs. |
| 10 β . . . . . | 1225 | High for 9 days. | Inoculated ear inflamed, swollen, droops. | Recovered. | Weight after 12 days, 1265. |
| 12 β . . . . . | 1575 | None. | None. | No effect. | |
| 14 β . . . . . | 1700 | Slight for 8 days. | Ear vein thrombosed. Suppurates. | Recovered. | |
| 7 α . . . . . | 1765 | Slight for 1 day. | None. | No effect. | |
| 10 α . . . . . | 1985 | None. | " | " " | |
| 2 β (Cow) . | 1400 | High. | " | Dies in 2½ days. | Septicemia. Streptococci isolated from blood, abundant on peritoneum. |
| 2 β " . | 1375 | High for more than a month. | Both ears inflamed, drooping. Abscess in right tarsal region (both ear veins tried when animal inoculated). | Chloroformed after 43 days. Weight, 1085. | Paralysis of both hind limbs on 30th day. No recognizable lesions of spinal column or cord. |
| 6 β " . | 1595 | None. | None. | No effect. | |
| 6 β " . | 1890 | " | " | " " | |
| 15 α " . | 2205 | " | Left knee joint and adjacent muscles. | Chloroformed after 3 weeks. Weight, 2075. | Streptococci in pure culture from joint. |
| 15 α " . | 1470 | " | None. | No effect. | |
| 18 β " . | 1580 | " | " | " " | |

The other relationships and differences are brought out in the fermentation tests given in Table VIII. Strains 2 $\beta$, 6 $\beta$, and 7 $\alpha$ ferment raffinose. The bovine strains 6 and 18 fail to act on salicin, whereas 2 and 15 do; 10 $\alpha$ fails to act on saccharose. All the bovine cultures yielded an acid concentration on titration, uniformly higher than the genuine human $\beta$ types if we except culture from Cow No. 2 which went with the human types. The cultures in milk are also instructive in this regard. The bovine strains produced a firm coagulum within three days. After seven days the milk cultures of the human strains either left the milk entirely fluid or else produced a thickening in the lower stratum only, but a few minutes exposure to boiling water caused firm coagulation in all. Here again the strain from Cow No. 2 went with the human strains.

In the work by Savage,[1] carbohydrates and alcohols are used to differentiate strains of streptococci. In many instances, lactose is acidified, but milk is unaffected. It is probable that he failed to boil his milk cultures. We have invariably found that when lactose is attacked, milk is not spared. The degree of acidity produced in certain groups is not, however, sufficiently great to cause spontaneous curdling, and it becomes necessary to place the tubes, for a few minutes, in boiling water.

TABLE VIII.

*Cultures from Outbreak B.*

| Designation of Culture. | | Dextrose. | Lactose. | Saccharose. | Maltose. | Raffinose. | Mannite. | Inulin. | Salicin. | Milk. |
|---|---|---|---|---|---|---|---|---|---|---|
| Type of Hemolysis. | | | | | | | | | | |
| Alpha. | Beta. | | | | | | | | | |
| | L. F. (Blood) . . | 4.3 | 3.85 | 3.4 | 3.9 | 0.8 | 0.75 | 0.85 | 3.25 | Coag. |
| | 2 (Throat) . . . . | 5.2 | 4.8 | 4.75 | 5.25 | 3.7 | 0.55 | 0.75 | 4.1 | " |
| | 3 " . . . . | 4.2 | 3.6 | 3.95 | 3.95 | 1.0 | 0.8 | 0.95 | 4.7 | " |
| | 6 " . . . . | 4.95 | 4.85 | 4.6 | 4.55 | 4.0 | 1.25 | 1.0 | 3.7 | " |
| 7 (Throat) . . . | . . . . . | 6.25 | 5.65 | 6.45 | 5.8 | 5.3 | 0.9 | 1.25 | 4.8 | " |
| | 9 (Throat) . . . . | 3.45 | 3.9 | 3.9 | 3.85 | 1.35 | 1.45 | 1.4 | — | " |
| 10 (Gland) . . . | . . . . . | 8.4 | 5.5 | 0.7 | 6.4 | 0.7 | 0.6 | 0.8 | 4.8 | " |
| | 10 (Gland) . . . | 3.9 | 3.3 | 3.95 | 3.75 | 1.25 | 0.95 | 1.15 | 4.8 | " |
| | 12 (Throat) . . | 5.1 | 4.35 | 4.55 | 4.6 | 1.15 | 1.1 | 1.0 | 1.5 | " |
| | 14 " . . . | 4.3 | 3.95 | 4.55 | 4.3 | 1.45 | 1.25 | 1.35 | 3.8 | " |
| | 2 (Cow) . . . . . | 4.0 | 3.65 | 3.6 | 3.75 | 1.05 | 0.85 | 0.9 | 4.8 | " |
| | 6 " . . . . | 5.6 | 5.0 | 4.9 | 5.2 | 1.05 | 1.15 | 1.3 | 1.1 | " |
| 15 (Cow) . . . . | . . . . . | 5.7 | 4.3 | 4.2 | 4.3 | 0.9 | 0.7 | 0.9 | 4.55 | " |
| | 15 (Cow) . . . . | 6.0 | 5.35 | 4.8 | 5.4 | 1.15 | 1.15 | 1.3 | 4.6 | " |
| | 18 " . . . . | 5.9 | 5.0 | 3.85 | 4.8 | 0.95 | 0.9 | 0.95 | 1.2 | " |

The following table (IX.) giving the results of agglutination tests with the serum of a rabbit treated with bovine streptococci of Outbreak A shows a close relationship existing among the streptococci from the four cows of this herd, and a somewhat less close relationship between the streptococci of this herd and that of Outbreak B. The strain from Cow No. 2 of this latter outbreak does not belong to the cow streptococci according to this test.

Cow No. 2 being the important link connecting the milk with the epidemic deserves some further attention. The udder of this cow had been injured, one quarter inflamed and giving a thick curdy product. The three remaining quarters were apparently not affected. The sample of milk from the inflamed quarter did not yield any hemolytic streptococci in cultures, but the mixed sample of the three other quarters

contained the human streptococcus as we are inclined to call it. It is of course not contended that this same strain may not have vegetated in the injured quarter, for the chances of missing bacteria in this kind of work are very numerous and we can only build on positive facts.

SUMMARY. — The strains included under the hemolytic ($\beta$) type of human streptococci of Outbreak B differ slightly among themselves as regards form of colonies and other cultural characters. There were probably not less than three varieties comprised in this type as shown by a comparison of cultural, fermentation and animal tests, and of hemolytic activities a year later (June, 1914). The hemolytic activity of cultures Nos. 3, 10, and 14 persisted during this period with little or no change. That of Nos. 2, 6, and 12 was much reduced. Similarly, the bovine strains of both Outbreaks A and B had lost much of their laking power, but that of Cow 2 of Outbreak B has persisted. The peculiar human streptococcus of Outbreak A (lactose not attacked) was not encountered in Outbreak B.

TABLE IX.

*Agglutinins in serum of rabbit immunized with streptococcus of Cow No. 18 (Outbreak A).*

| Designation of Strain. | Serum Dilutions. | | | | | | | | | Control. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1/20 | 1/40 | 1/80 | 1/160 | 1/320 | 1/640 | 1/1280 | 1/2560 | 1/5120 | |
| B. 3 β (human) . . . | + | − | − | − | − | − | − | − | − | − |
| B. Cow 2 β . . . . | + | − | − | − | − | − | − | − | − | − |
| B. " 6 β . . . . | ++ ++ | ++ ++ | + ++ | ++ | + | − | − | − | − | − |
| B. " 15 α . . . . | + ++ | + ++ | ++ | + | + − | − | − | − | − | − |
| B. " 15 β . . . . | + ++ | + ++ | ++ | + | + − | − | − | − | − | − |
| B. " 18 β . . . . | ++ ++ | ++ ++ | + ++ | + ++ | + ++ | + | + − | − | − | − |
| A. " 1 β . . . . | C | C | ++ ++ | ++ ++ | ++ ++ | + ++ | + | + | + | + − |
| A. " 8 β . . . . | C | C | C | ++ ++ | ++ | ++ | + | + | + | + |
| A. " 18 β . . . . | C | C | C | ++ ++ | + ++ | + ++ | + ++ | ++ | + | + |
| A. " 25 β . . . . | C | C | C | C | ++ ++ | + ++ | + ++ | + ++ | ++ | ++ |

From one cow of this Outbreak (No. 2) a streptococcus was isolated which corresponded precisely with strains from the throat of a fatal case, No. 3, and with No. 10 and No. 14 in cultural peculiarities, in agglutination and pathogenic characters. As far as studied, a second swab culture of No. 3 (No. 9) was identical with No. 3.

Besides material from these two outbreaks we have utilized material from a number of other groups of cases or outbreaks which were referred to milk as a source of the infection by epidemiological investigations. This work has added but little to our information, partly because the material submitted was inadequate, partly because other work prevented our utilizing it satisfactorily. We have, however, deemed it best to report upon and tabulate the results.

OUTBREAK C. — During the period covered by the epidemic in town B, cultures on Löffler's serum from the throats of a family affected with tonsillitis, but not in any way in contact with B, were received. This family had its own cow. The cultures were from the father and five children. From four of the latter, hemolytic streptococci were isolated resembling those of Outbreak B closely. From one throat a feebly hemolytic type was also isolated. This corresponded to the same type (*a*) of Outbreak B. The behavior of these types towards carbohydrates is shown on the following table (X.).

TABLE X.

*Cultures from Outbreak C.*

| Designation of Culture. | | Dextrose. | Lactose. | Saccharose. | Maltose. | Raffinose. | Mannite. | Inulin. | Salicin. | Milk. |
|---|---|---|---|---|---|---|---|---|---|---|
| Type of Hemolysis. | | | | | | | | | | |
| Alpha. | Beta. | | | | | | | | | |
| | 61 . . . . | 3.75 | 3.3 | 4.45 | 4.05 | 1.4 | 1.15 | 1.5 | 3.75 | Coag. (heated). |
| | 62 . . . . | 3.65 | 3.2 | 4.05 | 4.05 | 1.15 | 1.0 | 1.1 | 4.0 | " " |
| | 63 . . . . | 4.45 | 4.1 | 4.55 | 4.45 | 1.15 | 1.05 | 1.3 | 3.25 | " " |
| 64 . . . | . . . . . | 5.5 | 5.1 | 5.4 | 5.4 | 1.05 | 1.0 | 1.0 | 4.05 | Coag. |
| | 64 . . . . | 4.4 | 3.8 | 4.45 | 4.6 | 1.2 | 1.2 | 1.25 | 4.6 | Coag. (heated). |

They agree with certain strains of Outbreak B. Their hemolytic characters have persisted during the year and they possess a mild degree of pathogenic power as indicated in Table XI.

TABLE XI.

*Effect of hemolytic streptococci from Outbreak C on rabbits.*

| Designation of Strain. | Weight of Rabbit. | Effect on Temperature. | Localizations. | Result. | Remarks. |
|---|---|---|---|---|---|
| 64 β ..... | 2290 | Slight elevation for 10 days. | None. | Recovered; very little effect. | |
| 64 β ..... | 1285 | Moderate elevation for 2 weeks. | Right knee. | Chloroformed after 120 days. | Cultures from knee-joint negative. |
| 64 β ..... | 2140 | ..... | ..... | Dies in 1½ days. | Death probably due to snuffles infection (acute attack). |
| 64 α ..... | 2095 | None. | None. | No effect. | |

OUTBREAK D. — During the month of March of the present year (1914) cultures from a localized outbreak of tonsillitis in the towns of Wakefield and Stoneham were received. A brief account of the epidemiology has been published by F. L. Morse.[17] These cultures were obtained from the throats of individuals connected with a dairy from which the infection was supposed to have emanated and from patients outside. Samples of milk were not examined. From Table XII. it will be seen that both hemolytic types of streptococci were isolated from most of the swabs or cultures submitted. The fermentation reactions indicate at least three varieties of throat streptococci.

TABLE XII.

*Cultures from Outbreak D.*

| Designation of Culture. Type of Hemolysis. Alpha. | Beta. | Dextrose. | Lactose. | Saccharose. | Maltose. | Raffinose. | Mannite. | Inulin. | Salicin. | Milk. |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 . . . . | . . . . . | 5.6 | 5.3 | 5.15 | 5.2 | 1.15 | 1.0 | 0.9 | 4.25 | Coag. |
| | 1 . . . . | 3.4 | 3.7 | 3.3 | 3.5 | 1.7 | 2.0 | 1.85 | 3.05 | Coag. (heated). |
| 2 . . . . | . . . . . | 6.7 | 5.65 | 5.7 | 5.6 | 4.9 | 1.25 | 2.4 | 4.15 | Coag. |
| | 2 . . . . | 3.5 | 3.5 | 3.9 | 3.9 | 1.75 | 1.65 | 1.65 | 3.5 | Coag. (heated). |
| | 3 . . . . | 3.85 | 3.8 | 3.45 | 3.45 | 1.8 | 1.8 | 1.7 | 3.35 | " " |
| 4 . . . . | . . . . . | 3.0 | 3.35 | 2.9 | 2.95 | 1.7 | 1.55 | 1.8 | 1.25 | Coag. |
| | 4 . . . . | 3.35 | 3.45 | 3.25 | 3.3 | 1.05 | 0.95 | 1.15 | 4.25 | Coag. (heated). |
| AD. 4 . | . . . . . | 5.1 | 4.8 | 5.25 | 5.6 | 5.0 | 1.45 | 1.8 | 1.3 | Coag. |
| | AD. 4 . | 3.1 | 3.0 | 3.25 | 3.25 | 2.0 | 2.0 | 2.0 | 4.4 | Coag. (heated). |
| AD. 5 . | . . . . . | 5.45 | 5.15 | 5.2 | 5.2 | 5.2 | 0.85 | 1.2 | 3.9 | Coag. |
| AD. 8 . | . . . . . | 3.55 | 3.95 | 4.05 | 3.7 | 1.45 | 1.4 | 1.3 | 1.35 | " |

Only four strains were tested on rabbits (Table XIII.). Of two strains from the throat of the same patient supposed to have been the source of the milk infection, the one exhibiting the $\beta$ type of hemolysis was pathogenic, the other type was not. Two strains of the $\beta$ type from two patients at large were also pathogenic but more feebly so. These three pathogenic strains may be classed together under a definite formula of fermentation as shown in Table XII. The fourth, non-pathogenic, culture differs from these in acting upon raffinose, not upon salicin, and in producing a greater amount of acid.

TABLE XIII.

*Effect of streptococci from Outbreak D (Wakefield) on rabbits.*

| Designation of Culture. | Weight of Rabbit. | Effect on Temperature. | Localizations. | Result. | Remarks. |
|---|---|---|---|---|---|
| AD. 4 *α* .... | 1765 | None. | None. | Negative. | |
| AD. 4 *β* .... | 1855 | Moderately high until killed. | Both tarsal and right carpal joint. | Chloroformed after 23 days. Weight, 1255. | Culture from right tarsal joint positive. |
| 1 *β* ..... | 2200 | Slight elevation for 2 weeks. | Right knee joint. | Chloroformed after 23 days. Weight, 1950. | Culture from knee joint positive. |
| 2 *β* ..... | 1570 | High for 2 weeks. | None. | Recovery. | |

OUTBREAK E. — Material from a localized outbreak in a neighboring State was received during March, 1914. Four cultures from throats were isolated, the fermentation tests made and one strain tested upon rabbits. Nothing differing materially from earlier results was obtained, if we except the fermentation of mannite by the culture which was pathogenic to a rabbit. The inoculations are given in Table XVII., the sugar tests in Table XIV. A sample of milk supposed to have caused the outbreak was found to contain only staphylococci.

TABLE XIV.

*Cultures from Outbreak E.*

| Designation of Culture. | | Dextrose. | Lactose. | Saccharose. | Maltose. | Raffinose. | Mannite. | Inulin. | Salicin. | Milk. |
|---|---|---|---|---|---|---|---|---|---|---|
| Type of Hemolysis. | | | | | | | | | | |
| Alpha. | Beta. | | | | | | | | | |
| 4 ..... | ..... | 6.2 | 5.25 | 5.15 | 5.35 | 1.4 | 1.4 | 1.35 | 1.45 | Coag. |
| | 4 ..... | 3.4 | 3.4 | 3.2 | 3.5 | 1.5 | 2.9 | 1.3 | 3.55 | " |
| 2 ..... | ..... | 6.1 | 5.3 | 5.35 | 5.7 | 1.55 | 1.4 | 1.3 | 4.25 | " |
| 5 ..... | ..... | 4.05 | 4.1 | 4.6 | 4.2 | 1.75 | 1.1 | 3.4 | 3.75 | " |

OUTBREAK F (Westfield). — This outbreak occurred during May, 1914. From the material presented five strains were isolated, two corresponding to the $\alpha$ and three to the $\beta$ type of hemolysis. Two of the $\beta$ strains were tested on rabbits with positive results (Table XVII.). The sugar reactions (Table XV.) agree with the pathogenic $\beta$ type as hitherto described. Some suspected separator slime was examined at the same time but streptococci were not isolated.

TABLE XV.
*Cultures from Outbreak F.*

| Designation of Culture. | | Dextrose. | Lactose. | Saccharose. | Maltose. | Raffinose. | Mannite. | Inulin. | Salicin. | Milk. |
|---|---|---|---|---|---|---|---|---|---|---|
| Type of Hemolysis. | | | | | | | | | | |
| Alpha. | Beta. | | | | | | | | | |
| | 1 . . . . . | 3.9 | 3.35 | 3.65 | 3.35 | 2.15 | 1.4 | 1.6 | 3.4 | Coag. |
| | 2 . . . . . | 3.7 | 3.4 | 3.4 | 3.35 | 1.35 | 1.4 | 1.4 | 3.3 | " |
| | 5 . . . . . | 3.7 | 3.5 | 3.7 | 3.5 | 1.55 | 1.7 | 1.65 | 3.6 | " |
| 2 . . . . . | . . . . . | 5.25 | 5.3 | 5.55 | 5.65 | 5.15 | 1.15 | 1.1 | 1.0 | " |
| 3 . . . . . | . . . . . | 4.3 | 3.6 | 3.3 | 3.6 | 1.1 | 1.0 | 1.0 | 3.35 | " |

OUTBREAK G (Winthrop). — This epidemic, the last from which material was received, occurred in July, 1914. Only one throat swab and culture were received from which a variety of bacteria but no streptococci were isolated. Fifteen samples of milk from individual cows of the herd which was designated by epidemiological data as the source of the infection were obtained. These were drawn directly into sterile test-tubes. Microscopic examination of smears from sediments of about ten cubic centimeters of milk kept in a refrigerator over night showed great variation in the number of leucocytes present. Some were nearly free from cellular elements, others contained from ten to four hundred per field of a two millimeter objective. One sample contained immense numbers.

Owing to the large number of spore-forming bacilli in the samples, streptococci were isolated from only two samples,

although they were present in at least one other sample. Inoculation into rabbits of milk and of mixed cultures failed to yield pure cultures.

One sample of mixed milk from this herd was also examined and a hemolytic streptococcus of β type isolated. The three strains of hemolytic streptococci tested were of the β type. The fermentation reactions are given in Table XVI., the inoculations into rabbits on Table XVII. The two strains from individual cows are not identical. One ferments salicin, the other not. Differences were also noted in the size of the cocci and in the bouillon growth. They both acted alike on horse blood agar. The strain from the mixed milk resembles one of the preceding culturally. The test upon rabbits, however, differentiates it, for it was the only pathogenic strain of the three.

TABLE XVI.

*Cultures from Outbreak G.*

| Designation of Culture. | | Dextrose. | Lactose. | Saccharose. | Maltose. | Raffinose. | Mannite. | Inulin. | Salicin. | Milk. |
|---|---|---|---|---|---|---|---|---|---|---|
| Type of Hemolysis. | | | | | | | | | | |
| Alpha. | Beta. | | | | | | | | | |
| | 1 (mixed milk) . | 6.6 | 5.3 | 5.45 | 4.7 | 1.3 | 1.2 | 0.87 | 4.4 | Coag. |
| | 2 (Cow) . . . . . | 6.0 | 5.33 | 5.15 | 4.6 | 1.2 | 1.0 | 0.9 | 4.3 | " |
| | 3 " . . . . . | 5.95 | 5.1 | 5.2 | 4.4 | 1.4 | 1.0 | 1.1 | 1.23 | " |

Whether the pathogenic strain G–1 from mixed milk was a human type and the cause of the outbreak remains undecided since no material from throats containing streptococci was accessible. The culture resembles the throat types in all respects. The acid production is, however, somewhat higher than that of the β types of earlier outbreaks here reported.

TABLE XVII.

| Designation of Culture. | Weight of Rabbit. | Effect on Temperature. | Localizations. | Result. | Remarks. |
|---|---|---|---|---|---|
| E. 4 β..... | 2140 | High. | Left elbow, right knee. | Chloroformed in 16 days. Weight, 1325. | Blood cultures negative. Cultures from knee positive. |
| F. 1 β..... | 2015 | " | Left hip joint, right knee joint. | Chloroformed after 57 days. Weight, 1375. | Pure culture from hip joint. |
| F. 5 β..... | 1990 | " | Both tarsal and carpal joints. | Chloroformed after 21 days. Weight, 1410. | Pure culture from left tarsus. None from other joints. |
| G. 1 (mixed milk). | 1890 | Above normal throughout. | Both wrists and heels and right elbow. | Chloroformed after 29 days. Weight, 1360. | Streptococci recovered in pure culture from left wrist and right heel. |
| | 1850 | High. | Right elbow, left carpus. | Chloroformed after 35 days. Weight, 1690. | Cultures negative. |
| G. 2 (Cow) | 1825 | None. | None. | No effect. | |
| | 2465 | " | " | " " | |
| G. 3 " | 1950 | " | " | " " | |
| | 2450 | " | " | " " | |

Cultures from earlier epidemics. — In addition to the cultures from the various localized epidemics of 1913 and 1914, there were studied comparatively with these, four cultures of streptococci from the Boston epidemic of 1911, two from the Chicago epidemic of 1912, and two from the Baltimore outbreak of the same year. The four cultures from the Boston epidemic had originally been isolated by different persons and brought to one of us. They were studied at that time and maintained in the collection of this Department until the present.

No. 32 was from the spleen of a fatal case of tonsillitis; No. 33 from a case followed by adenitis; No. 34 from a case followed by empyema, and No. 35 from a case followed by

erysipelas. At that time all were hemolytic in both horse blood and rabbit blood agar.

Strains Nos. 29, 30, and 31 were obtained from Dr. D. J. Davis of Chicago; Nos. 38 to 41 inclusive, through the Rockefeller Institute. The numbers are ours.

Cultures of the Boston epidemic were sent to Dr. Davis in 1912 and he [16] states that the four strains obtained from this laboratory "appear to be in every respect identical with the Chicago streptococci."

The reactions of these cultures towards carbohydrates is given in Table XVIII. It will be seen that they all agree among themselves and with the $\beta$ strains of the more recent local outbreaks B, C, and D, but not with those of A. This latter group of streptococci stands apart from all the rest.

TABLE XVIII.

*Miscellaneous cultures.*

| Designation of Culture. | | Dextrose. | Lactose. | Saccharose. | Maltose. | Raffinose. | Mannite. | Inulin. | Salicin. | Milk. |
|---|---|---|---|---|---|---|---|---|---|---|
| Type of Hemolysis. | | | | | | | | | | |
| Alpha. | Beta. | | | | | | | | | |
| | 29b (Chicago epidemic), | 4.0 | 3.5 | 3.9 | 3.9 | 1.25 | 1.1 | 1.3 | 3.7 | Coag. (heated). |
| | 30 (Chicago epidemic), | 4.35 | 3.55 | 4.3 | 3.9 | 1.25 | 1.3 | 1.3 | 3.8 | Coag. (heated). |
| | 31 (Chicago epidemic), | 4.15 | 3.6 | 4.0 | 3.55 | 1.35 | 1.3 | 1.3 | 3.65 | Coag. (heated). |
| | 32 (Boston epidemic), | 3.85 | 3.55 | 3.55 | 3.6 | 0.9 | 0.95 | 0.75 | 4.7 | Coag. (heated). |
| | 33 (Boston epidemic), | 4.35 | 3.5 | 3.9 | 4.0 | 1.4 | 1.35 | 1.35 | 3.45 | Coag. |
| | 34 (Boston epidemic), | 4.25 | 3.95 | 4.15 | 4.0 | 0.95 | 0.8 | 0.85 | 3.5 | Coag. (heated). |
| | 35 (Boston epidemic), | 4.85 | 3.65 | 4.45 | 4.0 | 0.95 | 1.15 | 0.7 | 3.8 | Coag. (heated). |
| | 38 (Baltimore epidemic), | 4.2 | 3.65 | 3.95 | 3.45 | 0.8 | 0.8 | 0.85 | 4.8 | Coag. (heated). |
| | 39 (Baltimore epidemic, milk), | 4.6 | 3.55 | 4.55 | 4.55 | 0.7 | 0.7 | 0.8 | 4.7 | Coag. (heated). |
| | 40 (Chicago epidemic), | 3.65 | 3.15 | 3.65 | 3.35 | 0.8 | 0.7 | 0.8 | 4.9 | Coag. |
| | 41 (Chicago epidemic), | 4.05 | 3.25 | 3.65 | 3.85 | 1.4 | 1.15 | 1.25 | 3.65 | Coag. (heated). |
| 42 (laboratory stock, tonsillitis) . . . . | . . . . . | 6.5 | 5.7 | 5.8 | 5.95 | 5.15 | 1.0 | 1.55 | 4.25 | Coag. |

In 1914 one of the Boston strains together with one from the Chicago cases and two from the Baltimore group (throat and milk) were tested on rabbits by intravenous injection. All were pathogenic, though in varying degrees.

TABLE XIX.

| Designation of Culture. | Weight of Rabbit (grams). | Effect on Temperature. | Localizations. | Result. | Remarks. |
|---|---|---|---|---|---|
| No. 32 (Boston epidemic) .. | 2480 | Slight elevation one day. | None. | No effect. | |
| No. 32 ..... | 1360 | Elevated temperature for a month. | Left knee and right carpal joint. | Chloroformed after 2 months. Weight, 1080. | Suppurative lesions of thigh and right wrist. |
| No. 38 (Baltimore)... | 2510 | High till death. | ..... | Dies in 9 days. Weight, 2080. | Abscesses in kidneys, in follicles of appendix, heart muscle. Cultures from various organs positive. |
| No. 39 (Baltimore epidemic, milk) .... | 1660 | Very slight. | ..... | Dies in 9 days. Weight, 1620. | Transudates in pleural and pericardial sacs. Minute vegetation on mitral valve. Culture from heart's blood positive. |
| No. 40 (Chicago) .... | 1910 | Elevated temperature for 10 days. | Both knee joints. | Chloroformed in 10 days (dying). Weight, 1465. | Pure culture from heart's blood. Suppurative foci around both knee joints and in joints themselves. |

III.

Our studies extending over more than a year and a half have shown that cultures from throats affected with tonsillitis contained at least two types of streptococci well differentiated on horse-blood-agar plates. Our attention was largely restricted to one of these types, a streptococcus producing around the colony a clear zone from three to four millimeters in diameter. This type corresponded with the hemolytic strains of earlier milk-borne epidemics of tonsillitis (Boston, Chicago, and Baltimore). Within each of these groups a close analysis of morphological characters did not bring out differences beyond slight variations in size of the cocci, but on culture media differences were evident.

Almost every culture medium used enabled us to group these cultures according to certain slight, minor, either variable or fairly constant differential characters. Thus, in bouillon the degree of cloudiness and sedimentation varied. On plain agar and horse-blood agar the form of colonies varied slightly. Some strains produced small, discrete, conical colonies, others flat, disc-like. In some strains they ran together into a smooth, patchy growth; in others, they remained discrete. Some of these characters were inconstant, notably the sedimentation or clouding of bouillon. In a few cases the hemolytic activity disappeared in part.

It is not possible to give any definite value to these minor cultural differences. They probably depend, to a certain degree, on the quantitative variations of cohesive substances secreted by the cocci. Slight variations of this function might yield a great variety of appearances in bouillon and of the colony aggregation on solid media. It is often surprising how tenacious such minor differences are and how valuable they become in tracing certain strains. The strains often become individualized thereby. Only close study of such strains by repeated testing of their cultural peculiarities will enable us to evaluate their relative fixity.

TABLE XX.

| | Designation of Culture. | | Dextrose. | Maltose. | Saccharose. | Lactose. | Raffinose. | Mannite. | Inulin. | Salicin. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Type of Hemolysis. | | | | | | | | | |
| | Alpha. | Beta. | | | | | | | | |
| I. . | B-7, D-AD5, D-2, X-4a . | B-2, B-6 . . . . . | + | + | + | + | + | − | − | + |
| II. . | A-BL, B-15 (cow), C-64, D-1, E-2, F-3 . . . | B-LF, B-3, B-9, B-10, B-14, B-2 (cow), B-15 (cow), C-61, C-62, C-63, C-64, D-1, D-2, D-3, D-4, D-AD4, E-4, F-1, F-2, F-5, G-1 (milk), G-2 (cow), X-29b, X-30, X-31, X-32, X-33, X-34, X-35, X-38, X-39, X-40, X-41 . . . . . | + | + | + | + | − | − | − | + |
| III. . | . . . . . | A-SH, A-ST, A-100, A-104. | + | + | + | − | − | + | − | + |
| IV. . | . . . . . | A-103 . . . . . | + | + | + | − | − | − | − | + |
| V. . | B-10 . . . . . | . . . . . | + | + | − | + | − | − | − | + |
| VI. . | D-AD4, F-2 . | . . . . . | + | + | + | + | + | − | − | − |
| VII. . | D-4, D-AD8, E-4 . . . | A-1 (cow), A-8 (cow), A-18 (cow), A-25 (cow), B-12, B-6 (cow), B-18 (cow), G-3 (cow) . . . . . | + | + | + | + | − | − | − | − |
| VIII. . | E-5 . . . . . | . . . . . | + | + | + | + | − | − | + | + |

Milk was coagulated in every case where a fermentation of lactose is indicated.

In thus encountering minor differences, we have only repeated the experience of others, and gained the impression that the streptococci obtainable from human throats may be separated into a large number of races or varieties. To identify any particular strain, it therefore becomes necessary to use all the means of differentiation placed at our disposal. The existence of such a large number of races might readily lead to the inference that their characters are not fixed, but changeable under unknown conditions. Such changes may affect the fermentative capacities, hemolytic functions, and virulence independently.

When we, however, observe these races over a long period of time we are struck by the persistence of apparently minor cultural and other characters under laboratory conditions. In our hands none of the strains studied have lost or gained

any capacity to ferment certain sugars or alcohols. In Table XX. the various cultures studied are grouped according to their behavior towards dextrose, maltose, lactose, saccharose, raffinose, mannite, inulin, and salicin. No generalizations are permitted by this grouping. Tentatively, we may point out that the second group includes all of the pathogenic forms, except those of the third section, which comprises the pathogenic type from Outbreak A. The fourth section represents a non-pathogenic type, possibly a variant of the preceding group. The seventh group contains all of the milk streptococci which do not attack salicin. None of these produced any effect on rabbits.

The hemolytic activity of the $\beta$ types has remained fairly constant. In no case has it disappeared. Strain B–15 from a cow forms an apparent exception, but this strain has in the course of our studies split up into a series of forms differing in their laking capacity, some being non-hemolytic at present.

The behavior of all strains towards rabbits so far as tested enables us to classify them into a pathogenic and a non-pathogenic group. To the latter belong all the cow cultures except one $\beta$ type from Outbreak B, one from Outbreak G, and all $\alpha$ types with the possible exception of a milk culture from Outbreak B, 15 $\alpha$. This strain produced a knee-joint lesion in one rabbit without febrile reaction. Towards a second it was non-pathogenic.

The pathogenic group includes strains from all outbreaks. These strains produce, after intravenous injection in rabbits of a definite uniform dose, a febrile reaction, lasting from one to four or more weeks. Following this or accompanying it are localizations affecting hip, shoulder, knee, wrist, and foot. The tissues around the joint are as a rule involved, although in some cases the joint surfaces and the bones, notably the head of the femur, are eroded. In the more acute cases, foci occur in kidneys and heart. In a few instances, only the febrile reaction without localizations was present. In one case the heart valves were affected. It suggested itself that perhaps the handling of the animals during the taking

of the temperature may have favored the localizations. They did not occur among the afebrile cases, however, excepting in the rabbit injected with the *a* strain from Cow 15.

Taking all the facts into consideration it would seem that in searching for the particular streptococcus of an epidemic we must search for an individual strain rather than a type, for this may and does vary from outbreak to outbreak. Thus, the pathogenic non-lactose-mannite-fermenting strain of Outbreak A was not encountered subsequently. Yet the proof that it caused the epidemic is best supported by the data. The hemolytic streptococci from samples of milk of Outbreaks A and B belong to the $\beta$ type of hemolysis. With exception (2 $\beta$) noted above, they differed from the human types in (a) no appreciable virulence for rabbits, (b) somewhat higher acid production in fermentable media including a more active curdling of milk, (c) more vigorous hemolysis at the start, and (d) no action on salicin.

Perhaps the most significant result of these studies is the isolation from the milk of Cow 2 in Outbreak B a streptococcus closely agreeing with certain human strains and in so far differing from the bovine strains usually encountered. This strain agreed most closely in all minor particulars with a strain from the throat of a fatal case, No. 3. This strain was obtained from two swab-cultures of the same case made on different days (3 and 9). It is also identical with Cultures No. 10 and No. 14 of the same epidemic. It may appear that the significance of this fact is exaggerated, but it must be borne in mind that one positive determination is worth more than many negative ones where the chances for overlooking the object sought for are so numerous. The same may be said concerning the strain obtained from mixed milk of Outbreak G. We have here, however, no human strain from the same outbreak with which to compare and identify it.

It has been customary in casting about for a sufficient reason for the explosive appearance of milk-borne epidemics of tonsillitis to postulate the infection of the milk during the milking or later. We wish now to add one other possibility

as perhaps explaining better than the above assumption the appearance and persistence of certain epidemics. This is the infection of the udder ducts with human streptococci. Before taking up this phase of the subject it is of importance to consider mastitis or garget as a possible source of human disease.

It is now well-known that inflammation of the udder is always associated with certain bacteria, such as streptococci, staphylococci, B. coli, and paratyphoid bacilli. As far back as 1875, Franck[2] took the position that mastitis was not due, as then generally held, to exposure, injury, retention of milk, and certain general disturbances of health, but to specific bacteria. He produced severe mastitis by injecting putrescent fluids into the duct and by transferring the milk from an infected to a normal udder. It is quite evident at present that bacteria are not exclusively responsible for all forms of inflammation, but that certain predisposing conditions of the udder and the cow have much to do with giving the bacteria the opportunity to multiply in the udder and start the inflammation. It is, on the other hand, conceivable that a given bacterium may be endowed with such a high degree of virulence that predisposing conditions are not needed to give it a start. Such highly infectious organisms have been described as the cause of epizootics of mastitis.[7] The number of bacteria introduced is also of importance. The introduction into the milk duct of very few bacteria may not lead to any disturbance, whereas a large dose may do so. Kitt,[3] in 1886, found that certain strains of colon bacilli could produce mastitis. By simply smearing the bacteria on the opening of the duct he was able to produce inflammation. Bang succeeded by introducing bacteria on the end of a glass rod.

Bang,[4] in 1889, was the first to point out that different species of bacteria may produce different types of inflammation. Thus, a streptococcus from a case of mastitis in a cow produced after injection into a milk duct of another cow a slowly progressive, chronic catarrh leading to complete atrophy of the glandular tissue. Injection of the streptococcus of strangles in horses produced a severe, acute,

purulent inflammation ending in atrophy. Staphylococci produced acute inflammations both rapid and slow leading eventually to recovery. On the other hand, unlike bacteria may produce inflammations resembling each other closely.

Jensen,[5] in 1896, demonstrated that B. coli isolated from the normal intestine may produce a severe but short attack of mastitis when injected into a milk duct. He found it as the cause of several cases of parenchymatous inflammation. The same organism had been encountered previously by Kitt and by Guillebeau,[6] but given other names.

The experiments made by the foregoing and others show that a large number of species of bacteria may produce catarrhal or parenchymatous inflammation of the udder ending in complete restitution of the gland or in atrophy. Even the common spore-bearing bacilli (B. mesentericus vulgatus) have caused inflammation when injected experimentally.

Very few experiments on the persistence of infectious organisms in the milk after spontaneous and induced inflammation of the udder have thus far been reported. G. Fauss working under Zwick found that in infections due to the colon bacillus, the bacteria disappear in twelve to thirty days. Zschokke[8] found in streptococcus mastitis, induced in a goat, the injected organisms after ten weeks. In a cow they were present after six months.

The relation of mastitis to the public health depends on the susceptibility of the human subject to the bacteria found in inflamed udders. Because they happen to start or maintain disease of the cow's udder does not necessarily imply their capacity to multiply in the human body. Due caution, however, suggests that such milk should not be used even if it can be shown that the infected milk is harmless in certain cases.

W. G. Savage[1] made a series of experiments in England in 1906 to 1909, upon goats which help to throw some light on our own findings. He infected the udder by transferring a little of the growth of certain bacteria from solid

media with a blunt platinum needle, inserted into the milk duct. His results were as follows:

Three cultures of streptococci from garget in cows produced inflammation of the udder of goats.

Of two streptococcus cultures isolated from sore teats one caused inflammation, the other not.

One streptococcus from cow dung proved harmless.

Of human streptococci, four from cases of tonsillitis, two from scarlet fever, one from a healthy throat and four from other human disease, all failed to produce mastitis in the goat.

One streptococcus culture from Ludwig's angina and one from acute epiphysitis produced slight mastitis.

The special point of interest in these experiments lies in the fact that in some instances the streptococci could be detected in the milk some time after the infection. In the case of an erysipelas streptococcus it was found in the milk four and fifteen days after inoculation. One human strain was found in the milk twenty-six days after inoculation. In the case in which a strain from the spleen of a boy who died of acute lymphadenitis was inoculated, streptococci were detected for a long time after, although the gland was not affected. The streptococci obtained after seven days failed to act on salicin. Those obtained after one hundred and two, one hundred and ten, one hundred and twenty-two, one hundred and thirty, and one hundred and ninety-five days did act on salicin. Finally those isolated after two hundred and two hundred and nineteen days were again like the original strain. It is probable that there were two strains present, one fermenting salicin, the other not, one appearing at a time in his cultures.

Similarly, pure cultures of bacilli of human tuberculosis have been injected into the udder of cows without producing any recognizable lesions and leading to a prolonged infection of the milk with human tubercle bacilli. In order to test the action of mastitis streptococci on man, Savage inoculated his own throat with an abundance of streptococci from two cases of mastitis in cows. Neither inoculation was followed

by symptoms of any kind and the inoculated streptococci disappeared very rapidly.

After the completion of this manuscript in the summer of 1914, a paper by D. J. Davis and J. A. Capps appeared (Journ. Infect. Diseases, xv, 1914, 135) bearing directly upon the problem of udder infection. The authors attacked the question experimentally by infecting the udder of a healthy cow in active lactation with human streptococci. Smearing the teats with cultures of hemolytic streptococci and with exudate from a severe case of tonsillitis was negative. The udder remained normal and the milk free from the streptococci. When a slight abrasion of the skin near the meatus of one of the teats was made, and the wound infected with streptococci, the latter together with leucocytes appeared in the milk and were still present after four weeks.

In a third experiment, a relatively large dose of streptococci was injected directly into the canal, probably into the cistern. This was followed by evidences of slight inflammation, and large numbers of leucocytes and streptococci, which persisted for several weeks. The streptococci were not modified by their sojourn in the udder ducts. Rabbits, guinea-pigs, and a monkey were fed with the milk. Only the guinea-pigs responded with swelling and hemorrhage of the joints. We are inclined to think that the guinea-pigs were suffering from scurvy (Journ. Med. Research, xxiv, 1913, 317).

These experiments are of interest in that they harmonize with the various statements bearing on udder infection made in this paper. These data permit us to draw tentatively the following inferences:

1. The streptococci of cow mastitis or garget are different from the streptococci of human tonsillitis.

2. The virulent streptococci of man do not cause any appreciable inflammation of the cow's udder.

3. The mastitis streptococci do not cause throat affections in man.

4. The udder of a cow inoculated with virulent human streptococci may permit certain strains to multiply in the milk ducts. These are shed into the milk for some time after.

The theory that milk-borne epidemics of tonsillitis are due to human streptococci is strongly supported by the seasonal occurrence of such outbreaks during the greatest prevalence

of sore throats in the transition period between winter and spring. Mastitis is not a seasonal disease but depends rather upon injuries to the udder, the birth of a calf and exposure. Mastitis and milk-borne epidemics may, however, be made to coincide in time if the udder be manipulated and the udder ducts infected by a human being suffering with tonsillitis. Under such conditions two types of streptococci may appear in the milk simultaneously, the bovine and the human type. These, being so much alike as regards hemolytic properties, might be regarded as identical by the one studying the milk.

The grafting of human streptococci upon the udder, their multiplication under ideal conditions in the warm, fresh residual milk and their discharge twice daily into the milk at milking time, offers a much better explanation of the continuance of milk-borne epidemics over a number of days than any single infection of the milk through coughing, sneezing, spitting, and tasting. Such accidents may indeed infect the milk, but the infection could hardly last over a day and might be diluted to ineffectiveness in a very large supply. Such accidents would be more potent in a very small dairy.

The time of infection of the milk is of importance. Milk infected during milking, especially with organisms in an active state of multiplication would be far more dangerous than if the infection occurred after the milk had been chilled to a low temperature. The throat streptococci passing into the milk at milking time would not have such a good opportunity as those already adapted to the new medium in the milk ducts of the cow. They could, however, do much damage by entering the still warm milk as early as possible. We may illustrate our point by reference to the Boston epidemic of 1911.

C.-E. A. Winslow[9] made an extensive survey of the territory covered by this epidemic. The data collected with reference to the distribution of cases of tonsillitis and the milk supply pointed definitely to the milk. This was probably infected from human cases and not traceable to any

spontaneous udder disease. Winslow showed that the incidence of the infection covered at least an entire week. During this week (May 9–16) most of the cases occurred. How can we explain such a prolonged infection of the milk? It is scarcely conceivable that the accidental infection of the milk by a carrier could have been repeated so often, or that such accidental infection could have led to such widespread disease unless such infected milk had been allowed to stand in a warm place many hours in order to promote the multiplication of such streptococci so that they might leaven a large mass. This could hardly have occurred daily for a week or longer. The infection could not have gained headway after the milk had been chilled in the usual manner. If the milk had been infected at milking time, the repeated use of an uncleansed, undrained milking pail kept in a warm place or during unusually warm weather might have furnished enough streptococci each day to leaven a large amount of milk. It is scarcely conceivable that such a procedure could have taken place. Before accepting such a strained hypothesis, we should look elsewhere for an explanation more in accord with biological processes. This is furnished by the infection of the udder ducts with streptococci from human cases.

The relative rarity of such epidemics in spite of the many opportunities to infect the milk supply offered from day to day indicates that the infection of the milk ducts does not occur readily, nor with every strain of streptococci. It is not improbable that a certain degree of inflammation must exist to give the invading bacteria an opportunity to get a foothold. This requirement may account for the coincidence in time of milk-borne epidemics with cases of garget.

The way in which the udder may become infected is indicated by the experiments of Franck, Kitt, Guillebeau, Hess, and Savage The bacteria are probably introduced by some one manipulating the udder in the endeavor to draw milk from a swollen quarter. Milking tubes are frequently introduced to favor the discharge of milk. Other objects may be

used by the ignorant to open a supposedly plugged duct. To lubricate such a tube or other object with saliva is conceivable. D. J. Davis[10] in a study of epidemics of sore throat in Chicago in 1912 makes the following significant statement: "One farmer who was obliged to insert a tube into the teat, following mastitis, in order to empty one quarter of the udder, admitted that he put the tube regularly into his mouth to blow out the contents and never cleaned the tube otherwise. This same farmer admitted, too, that he had had sore throat for several days but paid little attention to it and continued to milk as usual."

Guillebeau[11] calls attention to the fact that severe cases of mastitis are produced by introducing pigeon feathers and other pointed bodies as catheters.

In the Baltimore epidemic, the epidemiological data brought together by Frost[12] show that the cases occurred in largest number February twentieth to twenty-ninth and March first to tenth and were traceable to a single dairy. The operation of this dairy was such as to convince Frost that the infection occurred prior to an inadequate pasteurization and that the source was to be looked for in one or more cows with udder infection. His reasoning is very much like ours, as the following quotation shows:

"It seems hardly probable that an infected person of reasonably cleanly habits handling the milk on one of the tributary farms could have introduced into it a number of organisms sufficient, when distributed throughout the whole supply and decreased by pasteurization, to have caused such a massive infection. Then, too, from the prolonged course of the epidemic among the consumers of this milk, it would appear to have been pretty constantly infected for a period of three to six weeks; and such continuous infection would hardly be expected from a human source.

"Supposing the infection to have originated from one or more cows with udder infection, it is very evident that the number of bacteria likely to be discharged into the milk would in all probability be many times more than the number that could probably be introduced accidentally from a human case. Also, if the infection came from cows with diseased udders it is readily understood that it would be more probably a constant, long-continued infection."

Frost's reasoning readily applies to the infection of the udder with human streptococci and their multiplication and persistence therein for a time.

In the same publication Stokes and Wachtel[13] report upon bacteriological studies of the milk supply of the suspected dairy. They recovered slightly virulent pneumococci which we now know are not infrequently associated with various septic diseases of calves. From the raw milk shipped to the dairy in question, an organism of the Streptococcus epidemicus type was isolated.

"This was recovered from the mixed milk of one shipper as it arrived at the railroad depot. Further investigations showed the presence in this shipper's herd of one cow whose milk contained inordinate numbers of pus cells. We were unable to obtain either pneumococci or streptococci from this milk, as the cultures were overgrown by B. coli.

"The examination of the herds on suspected dairy farms disclosed the presence of several gargety cows. These had not been milked for some time previous to the investigation. The cow whose milk contained one hundred pus cells to the field gave no physical evidence of mastitis, and was being milked at the time. Although a careful inquiry was made, no history of cases of septic sore throat on the farms could be elicited."

An outbreak of tonsillitis in the towns of Cortland and Homer in New York which occurred at the end of April, 1913 was reported by North, Avery and White.[14] Epidemiological evidence pointed strongly to a single herd of twenty-eight cows among which there were two cases of mastitis. Streptococci were isolated "from the throats of four patients apparently identical with strains of streptococci obtained from the milk slime of the two cows suffering from garget. Cultures from the throats of eight other patients contained streptococci of this same type but differed by slight variations only, in their carbohydrate fermentations."

The authors identify these with streptococci isolated previously from three cows of another herd known to be suffering from garget and from the milk slime of one cow supposed to be normal, "but which gave an abnormal amount of slime."

The authors group the streptococci found into four classes according to their behavior towards raffinose and salicin and refer the special pathogenic streptococci to Group D, which acts upon neither body. In our summary (Table XX.) the non-pathogenic cow streptococci are put into this same class. The authors do not draw any distinctions between the hemolytic bovine streptococcus and the human type resembling it very closely. They do not hesitate to consider the two cases of mastitis as the source of the epidemic. We cannot follow them in their conclusions. The particular streptococcus causing the epidemic, if such there was, is not sufficiently defined to be picked out of the material submitted. Was it the bovine hemolytic strain or a well-defined human strain to which they refer the epidemic? This streptococcus must necessarily have the same characteristics whether coming from the udder or from the human throat, at least within the brief space of time covered by the epidemic. It is not improbable that the true agent disappears rapidly from the throat and is replaced by other streptococci. In our plates, the hemolytic ($\beta$) type was present in small numbers among the many colonies of the other ($\alpha$) type and might be easily overlooked. Rosenow is even inclined to reject the blood-agar plate and use rabbits to bring out the hemolytic type which otherwise might fail to appear.

The relation of the hemolytic streptococci from the cows of Outbreaks A and B (excepting of course the presumably human strain from Cow 2) to the human strains remains to be determined. The eventual transformation of the inoculated human strains into the innocuous cow strains in the udder is a possibility to be reckoned with, but of no immediate consequence in tracing the source of epidemics.

E. C. Rosenow[15] has reported transformation of Streptococcus pyogenes into the streptococci of epidemic sore throat in unheated, sterile cow's milk. Our results do not assume any transformations but simply suggest multiplication in the udder. Both views may be harmonized by postulating for the streptococcus from man, a temporarily heightened virulence in the new medium with a gradual transformation

into the mastitis type which is to all appearances harmless to man.

A critical study of the literature which has grown up about the Baltimore and Chicago outbreaks does not give one the impression that the etiology of milk epidemics is precisely the same for the two cities. It may not have been the same for all the cases in the same city. Given a number of distinct strains of streptococci, capable of producing tonsillitis, and the opportunity for infecting the udders of cows and the milk after milking with such strains, there is no good reason to deny that several strains may be encountered, even in the milk supply of the same large city. Our own experience shows that even nearby towns may have epidemics almost contemporaneously due to easily distinguishable streptococci.

D. J. Davis in his bacteriological studies of the streptococci involved in the Chicago outbreak isolated a strain from a typical case of mastitis in a cow in a region whence came the suspected milk "which was pathogenic to animals, became encapsulated on animal passage and agreed in all essential respects to (with) the human streptococcus. A coccus identical in morphology, in culture, and in pathogenicity was obtained from a human case of tonsillitis and arthritis on the same farm." Davis further states: "The fact should be emphasized that streptococci which cause mastitis in cows may be pathogenic for animals and virulent for man."

In endeavoring to place the cause of epidemic, milk-borne tonsillitis in the infection of the milk ducts of the cow with streptococci belonging to man as well as in the subsequent miscellaneous infection which may take place from the time the milk enters the milk pail until it is finally consumed, we desire to call attention to the necessity of examining still further small sharply localized outbreaks for further proof. This is not an easy matter for it requires prompt notification, prompt epidemiological study to single out the herd, the immediate collection of throat cultures and samples of milk from every cow of such herd by a trained and trusted inspector, and lastly, a laboratory in which everything else can be pushed aside for the time being so that the various

samples can be analyzed as soon as possible. With this should be associated the experimental method which shall determine what strains are capable of multiplying in the udder and how long they may remain there before they disappear or lose their virulence.

We have no intention in suggesting udder infection of minimizing the dangers of milk infection in any stage. The earlier the infection, the greater the circle of its vicious influence. Multiplication in the udder ducts, under the best influences of warmth and freshness of the fluid and discharge twice daily, would furnish the most potent conditions for an intensive infection of a large supply over an indefinite period. Infection during milking is the next most dangerous procedure. When the milk has been chilled to 50° F. or below, streptococci cannot multiply, and infection at this stage could only affect a few persons. Infection during and after milking can hardly take place in sufficient degree to produce more than one batch of infected fluid unless the conditions surrounding the cleansing of utensils and the temperature of the milk are so unsanitary as to justify the peremptory shutting up of the dairy.

The still very obscure phenomenon of the transmutability and variability of bacteria with special reference to the group of streptococci needs thorough study. The many variations in morphological details, in behavior towards culture media, towards carbohydrates and other fermentable substances, and towards experimental animals indicate that the group of hemolytic streptococci is not only made up of several fixed species or races but that each has characters variable within certain limits. These will not as a rule interfere with etiological researches, provided uniform and minutely analytical methods are employed and the study not too long delayed. We believe that in the culture tube the only changes which take place are gradual quantitative modifications of existing characters. Changes signifying complete loss or the gain of well-defined functions such as the production of acid from certain carbohydrates do not appear to take place in our culture media, and it is quite probable that when they do

take place they occur under the immediate influence of life-processes — such as go on in the udder for instance. This problem can only be solved through prolonged experimentation. In our own repeated tests of the various races of streptococci extending over a period of fourteen months, we have observed some loss of hemolytic activity in a certain group and perhaps some decline of pathogenic power, but no loss of identity when all characters were considered.

We have thus far referred only to epidemics of tonsillitis due to infected milk. The many isolated and sporadic cases may at least in part be due to the same causes. This possibility places an obligation upon the physician and health officer confronted with the sudden unprovoked appearance of isolated and sporadic family outbreaks where there is no clear evidence of contact infection, to see if in such cases the milk or cream or even butter may not be at fault. Infection of the milk during milking and later accompanied with insufficient cleansing of utensils and containers or neglect to keep the milk cold may have been in many cases the source of isolated cases. Not only sporadic throat affections but also appendicitis associated with streptococci may now and then be due to infected milk.

## CONCLUSIONS.

The foregoing studies, though incomplete in many details, taken together with earlier quoted investigations warrant us, nevertheless, in formulating certain conclusions:

I. The streptococci causing epidemics of tonsillitis are not necessarily the same in different epidemics either in the same or different localities.

II. The success likely to attend the tracing of such epidemics to their source will depend upon a minute, detailed study of individual strains of streptococci and the discovery of certain minor distinguishing characteristics as guides.

III. The streptococci which have been the agents of recent outbreaks are all alike in that the colonies produce, immediately around them, a clear hemolyzed area on blood

agar plates (horse blood). They differ from the common throat coccus, our *a* type, in which the colony has a partly discolored and hemolyzed mantle between it and an outer narrow clearer zone.

IV. Spontaneous changes in cultural characters do not proceed rapidly enough if they go on at all, to interfere with current bacteriological methods. Tendencies towards slow changes may be used as further valuable distinguishing characters.

V. In Outbreak B, a streptococcus isolated from a specially drawn sample of milk from a cow in the suspected herd proved different from hemolytic udder streptococci from the same and another herd (Outbreak A), and identical in every respect with streptococci from the throats of three human cases, one of them fatal. From Outbreak G a strain not distinguishable from human pathogenic strains was isolated from mixed milk.

VI. The relation of different races of pathogenic human streptococci to the udder of the cow as regards capacity for establishing themselves, multiplying, persisting and becoming modified therein, can be defined only after carefully controlled experimental investigations on cows in various stages of normal lactation at different seasons of the year, and in those affected with various kinds of mammitis.

VII. There is at present no satisfactory evidence that bovine streptococci associated with mastitis or garget are the agents of tonsillitis in man. Whenever cases of garget are suspected as sources of infection in man, both human and bovine types should be looked for.

VIII. The manipulation of the udders of cows the milk of which is destined for public use should be controlled to the extent of forbidding the introduction into the milk ducts of any foreign object not properly sterilized.

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