DAIRY INFECTION WITH STREPTOCOCCUS EPIDEMICUS.

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Plate 9.

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The milk-borne streptococcus epidemic about to be reported is of interest not because of any unusual features or because of any intensity of the epidemic, but because of the definiteness of the findings and the promptness with which they were obtained. There were also certain unusual clinical features.

The dairy involved was one producing a high grade of raw milk for sale in Boston. In February, 1917, there was prevalent, especially among children, a malady which was usually diagnosed as epidemic adenitis, more rarely as sore throat, pharyngitis, or tonsillitis. The initial symptom was sore throat of moderate severity, rarely such as to be diagnosed as septic sore throat, a reportable disease in Massachusetts. A few patients experienced a peculiarly nauseating nasopharyngitis. A few cases of otitis media occurred, but the most constant development was inflammation of the submaxillary and cervical lymph nodes. Recovery was sometimes quite slow and characterized by recurrent periods of high temperature.

At the time the authors were consulted by the dairy several pediatricians had noticed that among their patients an undue proportion were consumers of this milk. On the other hand, an infants' hospital supplied with milk by this dairy had reported no cases of the disease.

On the afternoon of February 23, a conference was called by the officials of the dairy. Physicians whose patients were using this milk were invited to attend this conference and also the authors. Although there was no conclusive evidence that the milk was at fault, a complete investigation was undertaken at once. The following measures were adopted. (1) At the conference physicians were sup-

plied with sterile swabs for the collection of pathologic material from patients. (2) Arrangements were made to obtain samples of milk from individual cows of the dairy herd beginning the next morning. (3) Swabs from the throats of employees of the dairy were to be examined as soon as the milk had been studied. (4) All the milk of the dairy was to be pasteurized in the final bottles for the time being.

Apparently the epidemic among consumers of the milk ended immediately. The initial symptoms of the last case reported were noticed on February 24.

Since in a paper by Dr. Theobald Smith and the author (1) the milk-borne epidemics described were designated A to G inclusive, this one and the dairy involved will be referred to as Epidemic H and Dairy H.

The Human Material.

Swabs received were soaked for 15 minutes in about 1 cc. of sterile salt solution which was pipetted directly into the tube in which the swab was received. The salt solution was then diluted, added to fluid blood agar (standard meat infusion agar, 12 cc., plus defibrinated horse blood, $\frac{2}{3}$ to 1 cc., at 45°C.), and immediately poured into a Petri dish. After the moisture condensing inside the dish had been allowed to evaporate the plate was incubated at 37°C. in the inverted position.

Fifteen swabs were received from patients using milk of Dairy H. Of these, twelve were from throats or tonsils and three from ears. In the blood agar plates of twelve of these swabs, including those from ears, was found the organism which we have learned to recognize as *Streptococcus epidemicus* Davis.[1] The appearance of this organism in the blood agar plate is such as to make it readily recognizable (2). It produces the beta type of hemolysis. The surface colonies are large and watery, round when isolated but confluent and amebiform in the streak. Organisms in the fresh surface colonies are encapsulated. This streptococcus was present in large numbers on most of

[1] The name *Streptococcus epidemicus* is used tentatively. The organism is so nearly like *Streptococcus pyogenes* that it may be regarded as a well defined variety of the latter rather than as a true species, and if so should be called *Streptococcus pyogenes* (var. *epidemicus*).

the swabs; in pure culture on three of them, two from throats and one from an ear, and in almost pure culture on several others. It was not found on three of the swabs from patients using Dairy H milk. One of these was a convalescent patient and the material from the other two was not properly diluted and the plates were poor. These should have been done over again, but in view of the evidence already at hand and because of the great quantity of material from cows waiting for study at the time, this was not done.

Swabs were studied from four adults in families where children had the infection. The children in these families used milk from Dairy H but the adults did not use it regularly. A diagnosis of epidemic adenitis was not made in the case of the adults but they complained of coughs, colds, or sore throat. In none of them was *Streptococcus epidemicus* found.

Nine swabs were studied from patients not using milk from Dairy H. Three of these were from cases with a clinical diagnosis of epidemic adenitis. The others were diagnosed as sore throat or tonsillitis. From none was *Streptococcus epidemicus* isolated. Other pathogenic streptococci were recovered from some of them.

Most of the patients from whom *Streptococcus epidemicus* was isolated had developed initial symptoms on February 13 to 18. Most of the swabs were taken on February 24, 25, and 26. The last case reported developed initial symptoms on February 24, and the swab was taken on March 2.

The Dairy Herd.

All the milk sold by Dairy H was produced by its own herd, about 112 cows being milked at this time. The milk was produced and handled under exceptionally good conditions. Precautions were taken to exclude the milk from cows with garget. The herdsman drew the fore-milk from each teat onto a fine wire screen in order to detect the presence of flocculi or viscidity. The so called gang system of milking was in use, each man milking the next cow in line at the head of the gang of milkers as he finished milking the last one. The milk was quickly cooled and bottled at the farm.

The herd was housed in three barns, A, B, and C. On the morning of February 24 samples of milk were taken from twenty cows in Barn A. A stream of milk from each of the four quarters of every cow was milked directly into a sterile cream bottle. The samples were packed in ice and taken directly to the laboratory where they were centrifuged and the sediment was plated out in horse blood agar. A stained film of the sediment was also made. In making the blood agar plates it was found useful to employ both deep and surface streak inoculation of the same plate. The tube of blood agar was first inoculated in fluid condition, then after the plate had been poured and hardened it was streaked in three or four places. This gave an opportunity to observe both deep colonies and surface streaks on the same plate.

It happened that one of the first samples yielded in pure culture large numbers of *Streptococcus epidemicus* resembling in every way the organisms isolated from the patients (Fig. 1). The stained film of the milk sediment revealed large numbers of leucocytes and many short chains and pairs of round or flattened cocci. The film alone would have attracted suspicion but could not have been relied upon for a diagnosis. The milk of this cow (No. 108) had not yet roused the suspicion of the herdsman or milkers and was being used. As soon as this culture was discovered the cow was isolated on February 25, and on the morning of February 26 samples of milk were taken from each quarter of the udder. By this time the sample from the left fore quarter was noticeably thick and yellow, while samples from the other three quarters were normal in appearance. Cultures showed the left fore quarter only to be infected.

By February 26 samples of milk from all the cows of the herd had been similarly cultured and examined. None revealed any streptococci resembling those from the patients and Cow 108. Among these cows, however, were three known to have garget in one quarter of the udder. The milk of this quarter was regularly discarded. We studied samples of milk from these quarters culturally and found streptococci which produced more or less hemolysis in the blood agar plate but did not closely resemble the streptococci isolated from the patients and Cow 108.

The location of Barn A and the method of handling the milk at the farm offer a suggestion as to why the epidemic was not more widespread among the patrons of the dairy. They also serve to explain why there was not a single case at an infants' hospital where the milk was used. At the barns the milk was poured from the milking pails into large sterile cans and carried by wagon to the milk house where it was poured from the cans into the mixing tank. From the mixing tank it ran through the cooler and was bottled, the bottling going on continuously while fresh milk was being poured into the mixing tank. The apparatus was properly sterilized before each milking and the whole process carried on under very good conditions. There might be in the mixing tank the milk from not more than ten or fifteen cows at any one time. Barns B and C were within about 200 yards of the milk house and milk from these barns was therefore the first to enter the mixing tank. Milk from Barn A which was a quarter mile or more from the milk house was likely to enter the mixing tank toward the latter part of the milking. Hence, the larger part of the milk escaped contamination by the milk of Cow 108 which was in Barn A.

The Dairy Employees.

The theory advanced by Smith and Brown (1) that epidemic milk-borne tonsillitis is due not to the streptococci of ordinary bovine mastitis but to streptococci of human origin inoculated into the milk ducts of cows during milking has now received considerable support from the work of other authors.

In view of the recent remarks of Keegan (3) it seems necessary again to emphasize the fact that infection of the cow's udder by *Streptococcus epidemicus* may persist for some time without gross evidence of mastitis. That such an infection is not common is shown by the work of Jones (4) who studied over 80 cases of mastitis in cows without finding *Streptococcus epidemicus* except possibly in one instance. These facts make the finding of this organism in the udder or milk supply in association with an epidemic all the more significant. It was not the intention of Smith and Brown to deny the possibility of contact infection.

The theory of Smith and Brown offers a plausible explanation of the relative rarity of such milk-borne epidemics notwithstanding the wide prevalence of bovine mastitis, or garget, and its occurrence in

TABLE I.

Infected Individuals at the Dairy Farm.

Individual.	Employment and habitat.	Cultural history.	Clinical history.
W	Driver of automobile truck and miscellaneous work. Lived at farm boarding house.	*S. epidemicus* + Feb. 26, Mar. 20, Apr. 2, 9. Never present in large numbers. Negative cultures at intervals between above dates.	One tonsil large but no clinical symptoms at any time.
C	Milker. Lived at farm boarding house.	*S. epidemicus* + Feb. 26, 28, Mar. 1, 2, 5, 8, 10, 12, 13, 14. Often in considerable numbers. Negative cultures Mar. 15, 16, 17, 20, and thereafter.	Quinsy sore throat shortly before Feb. 26. No clinical symptoms after Feb. 26. Mar. 12 to 17. Daily irrigation of crypts of tonsils with hydrogen peroxide.
S	Milker and feed mixer. Lived at home. Father of K.	*S. epidemicus* + Mar. 2, 4, 5, 6, 8, 10, 12, 13, 14, 16. Often present in fair numbers. Negative cultures Mar. 17, 20, Apr. 2, and thereafter.	Large tonsils with large crypts but no clinical symptoms before Mar. 15. Mar. 12 to 17. Daily irrigation of crypts of tonsils with hydrogen peroxide. Mar. 15. Complained of rheumatism. Apr. 2. Confined to home for several days with rheumatism of back.
M	Milker. Lived at farm boarding house.	*S. epidemicus* + Mar. 2, 5, 6, Apr. 23, 30, May 14, 29, June 5, 11. At first present in considerable numbers; later very few. Negative cultures Feb. 26, 28, Mar. 3, 8, 10, 20, Apr. 2, May 7, 25.	No throat symptoms. Mar. 10. Complained of rheumatism. " 20. Confined to bed. Temperature. Left knee swollen and painful. Sent to hospital. Apr. 2. Back from hospital. Re-

			ports involvement of both knees, left elbow, and both hands. Able to resume work with some discomfort. Apr. 27. Began use of autogenous vaccine of *S. epidemicus*. Gradual improvement.
D	Milker. Lived at farm boarding house.	*S. epidemicus* + Mar. 8, May 14. Present in small numbers. Negative cultures Mar. 3, 5, 14, 20, Apr. 16.	Left tonsil enlarged. Mar. 15. Complained of rheumatism. " 20 to Apr. 16. Unable to work at times. Puffy swelling of wrist and ankle. Apr. 24. Part of left tonsil cut off by doctor.
K	Milker. Lived at home. Son of S.	*S. epidemicus* + Mar. 20, 23, 26. Present in fair numbers. Negative cultures repeatedly before and after above dates.	Throat apparently normal. No clinical symptoms at any time.
J	Milker. Lived at home.	*S. epidemicus* + Mar. 23, Apr. 2. Present in small numbers. Negative cultures Mar. 26 and after Apr. 2. No diphtheria bacilli found.	Had been quarantined at home with "diphtheria" for several weeks previous to Mar. 23. No swabs examined during that time. No subsequent clinical symptoms.
Miss V.	Father and mother employed on farm. Lived at home.	*S. epidemicus* + Apr. 30. Present in moderate numbers.	Said to have had several attacks of quinsy sore throat during past few months. The swab was taken just previous to removal of tonsils on Apr. 30. No other swabs taken.

practically all dairy herds of any size. With this theory in mind our next effort was to determine the source of the infection of Cow 108. Unfortunately two of the milkers who had attended this cow at the time the epidemic started had left the employ of the dairy and it was impossible to obtain material or clinical data from them. Under the system of milking in use it was impossible to connect the act of milking any given cow with any particular milker.

On February 26 swabs were taken from the throats of fifteen employees, mostly milkers, at the dairy farm. All these men reported themselves in good health. One milker, C, had recently recovered from quinsy sore throat, but this man was said not to have worked in Barn A where Cow 108 was kept. *Streptococcus epidemicus* was found on swabs from C and another man, W, who drove the milk truck and did other odd jobs about the farm but did no milking. Both C and W lived at the farm dormitory and boarding house. Cultures from the other men were negative on this date. Swabs were taken and cultured from as many of the men as possible every few days. In Table I is given a tabular summary of the study of infected employees listed in the order of the discovery of the infection. Special attention is called to J, a milker, living at home, who had just returned to work after being under treatment for diphtheria for several weeks. Two women were employed at the farm boarding house. Swabs from both of these were examined repeatedly with negative results. One, Mrs. H., reported that she had "lost her voice several weeks ago as a result of laryngitis," but appeared well at the time of this investigation. The other, Mrs. V., had no clinical history. Her husband also was employed on the farm but gave no clinical history, and no positive cultures were obtained from him. On April 30, however, it was learned that a young daughter of Mr. and Mrs. V. was to be taken to Boston to have her tonsils removed. It was learned that this child had suffered from several attacks of quinsy sore throat during the past few months. She did not live on the farm but a swab from her throat was obtained previous to operation. Moderate numbers of *Streptococcus epidemicus* were found. It was also learned from K on March 26 that he had a young sister who "came near having pneumonia lately" and another sister who had frequent "earache due to adenoids." Swabs from these people could not be obtained.

Altogether swabs were studied, in most cases at regular intervals of a few days, from twenty-six persons connected with the dairy. From eight of these *Streptococcus epidemicus* was isolated at one time or another. At least two harbored the organism when the first swabs were taken on February 26.

The incidence of rheumatism among the infected men is noteworthy. There was no complaint of rheumatism among the non-infected employees.

From the data it seems impossible to connect any of these individuals with the introduction of the infection into the dairy and the infection of Cow 108. There are too many possibilities. It may be that C, or J, some member of the family of S, or Miss V. was the source of infection, or it may have been one of the men who left the dairy before the investigation was started. On the other hand, it seems likely that some of these and perhaps other persons were infected by using the milk of Cow 108, since all the employees and their families used the milk of the dairy. It is also possible that there was a good deal of contact infection, and there seems no other way to account for the infection of K, D, and possibly S, M, and J after Cow 108 had been removed. It is also possible that J's disease was a pseudodiphtheria due to *Streptococcus epidemicus*.

After Cow 108 had been eliminated from the herd frequent examinations of the milk and of swabs from the men's throats were made. All infected men were kept from milking or handling the milk in any way.

Data Bearing on the Mastitis of Cow 108.

Cow 108 gave birth to a calf in November, 1916. She was apparently normal and gave a good quantity of milk from each quarter of the udder up to the time of this investigation on February 24, 1917. On this date, as related above, *Streptococcus epidemicus* was found in the mixed milk from the four quarters of the udder. A photograph of the blood agar plate made at this time is shown in Fig. 1. Samples of milk from individual quarters of the udder showed infection in only the left fore quarter. On February 26 the milk from this quarter was noticeably thick and yellow. *Streptococcus epidemicus* was present in pure culture. Samples of milk from individual quarters were

again studied on March 11. By this time the left fore quarter was manifestly shrunken, the milk from it diminishing in amount and being quite thick and yellow. The culture was as before. Milk from

TABLE II.

Infected Cow 108.

Feb. 24. Mixed sample of milk from all four quarters. Gross appearance normal. Large numbers of *Streptococcus epidemicus* per cc. of milk. Cow isolated.

Date.	Milk from left fore quarter.		Milk from left hind quarter.	
	Gross appearance of milk.	*S. epidemicus* per cc.	Gross appearance of milk.	*S. epidemicus* per cc.
1917				
Feb. 25	Thick; yellowish.	Large numbers.	Normal.	None.
Mar. 11	" yellow.	" "	"	30,000±
" 15			"	15,000
" 15–20	Left hind teat injured, probably by being stepped on.			
" 20			Curdled; yellowish.	872,000
" 25	Cow removed to animal hospital.			
" 26	Serous; yellow. 75 cc.	15,000	Normal. 275 cc.	375,000
" 28	Watery. Few cc.	39,300	" 375 "	200,000
Apr. 1	Serous; flocculent. Few cc.	22,000	"	23,000
" 8	Serous. Few cc.	175,000	" 250 cc.	28,500
" 15	" with white stringy particles.	32,000	"	57,000
			14 million leucocytes per cc.	
" 23	Serous; stringy. 10 cc.	90,000	Slightly flocculent. 250 cc.	75,000
			16 million leucocytes per cc.	
May 2	Serous.	665,000	Normal.	32,000
" 9	" stringy. 5 cc.	57,500	" 250 cc.	29,500
" 16	Serous; stringy. 8 cc.	2,085,000	Slightly stringy. 250 cc.	60,000
June 6	Serous; thick yellow masses. 5 cc.	250,000,000	Normal. 150 cc.	50,000
			5 million leucocytes per cc.	
" 20	Serous; thick yellow masses. 5 cc.	270,000,000	Slightly stringy. 150 cc.	2,000

the other three quarters was quite normal in appearance and amount. In the plate of milk from the left hind quarter, however, were found 30,000 colonies of *Streptococcus epidemicus* per cc. of milk. On gross

examination this quarter of the udder was apparently normal. On March 15 were found about 15,000 of the streptococci per cc. of milk from the left hind quarter. Some time between this date and March 20 the teat of the left hind quarter was injured. When examined on March 20 by one of us the teat was swollen and blood was dripping from it occasionally. There was a cut leading outward from the meatus. The milk collected at this time was thick, yellow, curdled, and slightly tinted with blood. Culture revealed 872,000 of the infecting organism in apparently pure culture per cc. of milk. Soon after this the cow was removed from the farm to the Angell Memorial Animal Hospital[2] where it was accessible for study from the laboratory at Harvard Medical School. To avoid transference of the infection from infected to normal quarters by the hands of the milker, he was instructed always to milk the normal quarters first. The milk from the four quarters was studied for many weeks. During this time the general condition of the cow was normal and the right fore and hind quarters of the udder remained uninfected. The results of the examination of the milk from the left fore and hind quarters are shown in Table II.

The point of greatest interest in as far as the epidemic is concerned is that there was detected bacteriologically an infection with *Streptococcus epidemicus* of the left fore quarter of the udder of this cow before it had attracted the attention of the herdsman and milkers at the dairy, that it was detected in the left hind quarter a week or more before the milk showed any gross change, and that the milk of this quarter again returned to almost normal appearance though the infection persisted for many weeks.

At the end of June the authors left Boston. Further study of Cow 108 was carried on by Dr. E. E. Tyzzer and Dr. Marshal Fabyan.

Study of Cultures.

Although *Streptococcus epidemicus* was isolated 80 times from patients, dairy employees, and Cow 108, all the strains were indistinguishable from one another. A general description therefore suffices for all.

[2] The cow was kept in this hospital through the courtesy of Dr. S. J. Mixter of Boston.

Appearance in Blood Agar.—In the blood agar plate composed of standard beef infusion agar plus 5 to 10 per cent of horse blood, the deep colonies after 18 to 24 hours incubation are biconvex, usually a little larger than those of typical *Streptococcus pyogenes*, and surrounded by a distinct clear colorless zone of hemolysis about 2 to 2.5 mm. in diameter unless the plate is crowded with colonies. There are no intact corpuscles next to the colony and no discoloration. The appearance is that of the beta type. The hemolyzed zone of the deep colony is essentially like that of *Streptococcus pyogenes*, but it may be a little slower in developing. The surface colonies on the blood agar plate or slant serve to distinguish this organism from *Streptococcus pyogenes*. They are large colonies, 1 to 4 or 5 mm. in diameter, watery when grown in a humid atmosphere but drying down rapidly to thin transparent films when exposed to a dry atmosphere; *e.g.*, that of the room. If the surface colonies are close together, as in a streak, they become confluent and amebiform. Hemolysis appears more slowly about the surface colonies than about the deep ones, and in fact may not be very noticeable after incubation over night. This is partly due to the rapid overgrowth of the surface colony, obscuring the zone of hemolysis beneath. Not infrequently the surface colonies at this stage have a greenish tint which, however, is not due to the methemoglobinization of underlying blood corpuscles, though it may possibly be due to the formation of methemoglobin from released hemoglobin which has diffused into the substance of the colony. It is easily distinguished from the alpha type of appearance produced by pneumococci and *viridans* streptococci. The surface colony itself is like that of *Streptococcus* (*Pneumococcus*) *mucosus*, the Type III pneumococcus of Cole, but the latter organism produces the alpha appearance in blood agar and is further distinguished by fermentation and immunological reactions.

Morphology.—If some of the growth from a fresh watery surface colony is examined microscopically there are found diplococci and short chains of streptococci with large capsules enveloping the entire group. These capsules are best seen by suspending the material in a droplet of bouillon and a suitable India ink, covering with a cover-slip, and examining the moist preparation under the microscope. They are also revealed by the Huntoon capsule stain. The cocci themselves

are round or slightly flattened, closely packed together within the chain. They are Gram-positive and when seen encapsulated closely resemble *Streptococcus (Pneumococcus) mucosus*.

Appearance in Bouillon.—In bouillon there is nothing to distinguish *Streptococcus epidemicus* from *Streptococcus pyogenes*, though the former is likely to produce more clouding and less sediment than the latter. Usually the bouillon is fairly well clouded and there is a moderate amount of finely flocculent sediment which is easily disintegrated and suspended by shaking. Microscopically there are found moderately long chains of streptococci. A small amount of capsular substance may or may not be present.

Fermentation Reactions.—The fermentation reactions of all the strains were determined after incubation for 1 week in large test-tubes by titration of the total acidity of cultures in fermented bouillon plus 5 per cent of sterile horse serum and 1 per cent of the test substance.

In Table III the titratable acidity of representative strains is expressed as per cent normal acid. No subtraction has been made for the reaction of the medium. The fermentation reactions are the same as those of *Streptococcus pyogenes*. All the strains fermented saccharose, lactose, and salicin, but not raffinose, inulin, or mannite. It is to be assumed that they ferment dextrose and maltose also.

Comparison with Bovine Strains.—For comparison with the other strains there are included in Table III two strains of bovine hemolytic streptococci, H-Cow 71 and H-Cow 72. They produce a higher titratable acidity in saccharose and lactose media than do the strains of *Streptococcus epidemicus*. These two strains came from the milk of apparently normal cows. In blood agar they produce the beta type of hemolysis, but the zones show minor differences from those of the other strains. The zones of hemolysis develop rather slowly, those of H-Cow 71 remaining small, and those of H-Cow 72 becoming broad but with a hazy outer portion. Neither strain produces capsules. The individual elements are large. These minor differences between such non-pathogenic bovine strains and pathogenic hemolytic streptococci of human origin may easily escape the attention of one without considerable experience in the study of streptococci, but they are convincing when recognized. In 1915 it was said that:

"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" (1). This is still true and something

TABLE III.

Fermentation Reactions.

Strains from.	Titratable acid (per cent normal).					
	Saccharose.	Lactose.	Salicin.	Raffinose.	Inulin.	Mannite.
Patients.						
H-3	3.9	3.15	2.65	0.9	1.05	1.05
H-10	3.55	3.1	3.0	0.95	1.1	1.1
H-11	3.4	2.9	2.7	0.4	0.3	0.3
H-14	3.6	3.3	2.55	0.6	0.65	0.3
H-16	3.6	3.1	2.5	0.6	0.7	0.6
H-17	3.6	3.0	2.85	0.6	0.7	0.85
H-18	4.1	3.05	2.55	0.3	0.55	0.2
H-20	4.05	2.9	2.7	0.7	0.5	0.75
H-21	3.15	2.9	2.65	0.6	0.85	0.45
H-23	4.05	2.95	2.7	0.35	0.55	0.5
H-40	3.7	3.3	2.4	1.0	1.15	1.1
Employees.						
H-W	3.8	3.15	3.25	0.9	1.2	0.9
H-C	3.6	3.2	2.6	0.95	1.05	1.1
H-S	3.65	3.0	3.2	0.85	0.6	0.9
H-K	3.7	3.25	3.3	0.8	0.65	0.85
H-M	3.55	3.7	3.55	0.8	0.6	0.85
H-D	3.75	4.1	3.3	0.9	0.6	Lost.
H-J	3.6	3.0	2.55	0.6	0.5	0.4
Dairy cows.						
H-Cow 108	3.6	3.2	3.2	1.0	1.0	0.8
H-Cow 71	4.9	5.0	3.3	0.55	0.55	0.65
H-Cow 72	4.95	4.85	3.25	0.55	0.7	0.5

The titratable acidity of the medium was 0.5 to 1 per cent normal. A titratable acidity of less than 1.5 is regarded as a negative fermentation reaction.

further has been done to facilitate the recognition of these minor distinguishing characteristics. Ayers and his associates have called attention to the differences in final hydrogen ion concentration which serve to distinguish streptococci from different sources and many pathogenic from non-pathogenic streptococci (5, 6). Avery and

Cullen (7) have reported the usefulness of the determination of hydrogen ion concentration in the differentiation of hemolytic streptococci of human and bovine origin. They found that in dextrose bouillon "the human type of *Streptococcus hæmolyticus* reaches a final hydrogen ion concentration of pH 5.2 to 5.0, and the bovine type of pH 4.5 to 4.3." Among the strains of streptococci which they studied were the three strains from dairy cows listed in Table III. Their results for these strains were as follows:

Designation of strain.		pH	Diagnosis.
Brown and Orcutt.	Avery and Cullen.		
H-Cow 108	V 10	5.1	Human type.
H-Cow 71	V 8	4.5	Bovine "
H-Cow 72	V 9	4.5	" "

The fact that streptococci of bovine origin produce in carbohydrate media more acid than do pathogenic streptococci of human origin has been noted by various authors (Broadhurst (8), Stowell, Hilliard, and Schlesinger (9), and Smith and Brown (1)) employing the titration method, and has been utilized as a means of differentiating streptococci from these two sources. In our experience more than 90 per cent of bovine streptococci of the beta type produce in dextrose bouillon from 1 to 1.5 per cent more normal acid than do human streptococci of the beta type, and the method of titrating the total acidity against 0.05 N sodium hydroxide with phenolphthalein as an indicator is reliable in the hands of an individual worker using a medium of fairly constant composition for the comparative study of strains from both sources.

Blood-Salt Solution Test.—In another article (10) is described the behavior of strains from this epidemic in a suspension of blood in salt solution. It is sufficient here to call attention to the fact that also by means of this test Strains H-Cow 71 and H-Cow 72 fall into the bovine group while Strains H-Cow 108 and H-M fall into the human group.

Animal Experiments.—Soon after isolation 1 cc. of bouillon culture of a number of strains from representative sources was injected intravenously into rabbits, with results as indicated in Table IV.

The organism was not particularly virulent as judged by the mortality of rabbits injected. The most conspicuous lesions produced were those of the external ears and testes. The lesions of the ears appeared on the 4th to the 9th day and resembled erysipelas, sometimes affecting the whole pinna simultaneously, but more often

TABLE IV.

Inoculated Rabbits.

Rabbit.	Sex.	Culture.	Maximum temperature.	Weight (variation).	Localizations.	Result.
			°F.	gm.		
A	M.	H-10	105.5 (4th day).*	1,220–1,610	Apparently well throughout.	
B	"	H-C	107.3 (2nd day).	1,310–1,070	Both ears, 9th day. Left testis, 10th day.	Recovered (40 days).
C	"	H-S	105.1 (3rd day).	1,270–980	Right ear, 9th day. Lame in left hind leg temporarily, 10th day.	Recovered (40 days).
D	F.	H-M	106.0 (2nd day).	2,090–1,770	Endocarditis.	Died (4th day).
E	M.	H-M	106.6 (2nd day).	2,180–1,700	Both ears, 5th day. Both testes.	Very sick. Chloroformed (7th day).
F	"	H-Cow 108	106.2 (2nd day).	1,330–1,150	Both ears, 4th day. Left eye, 4th " ". testis, 4th day. Right testis, 14th day. Right hind foot, 14th day.	Died (29th day).
G	"	H-Cow 108 (19 mos. later).	106.5 (3rd day).	1,325–1,140	Left ear, 5th day. Right fore foot, 8th day.	Recovered (17 days).

* The day on which the injection was made is counted as the 1st day.

starting in one area and migrating to the remaining parts. The ear became red, hot, considerably swollen, and heavy so that it drooped. In a day or two there appeared little droplets of blood-stained serum exuding at points on either surface of the ear. When cultured in blood agar plates these droplets were found to contain large numbers

of the streptococcus injected. There often appeared areas of purpura, blisters or bullæ, and occasionally a necrosis, dry gangrene, and sloughing off of a part of the ear. If the rabbit lived the inflammation gradually subsided after a week or more, the skin became dry, underwent desquamation, and finally the ear regained its normal appearance. A section of an ear from Rabbit E revealed the following changes.

The cartilage and narrow strip of dense fibrous tissue on either side of it are intact. The looser vascular connective tissue and subcutis are greatly distended and in large areas obliterated by a serofibrinous exudate containing much cell debris (necrotic connective tissue and degenerating cell nuclei) but very few leucocytes and not many endothelial cells. In the tissue on the dorsum of the ear the blood vessels contain plugs of fibrin and some are plugged with streptococci, but most of the streptococci are massed in the lymph spaces and tissue spaces surrounding blood vessels. A few streptococci are seen scattered in the tissue spaces just beneath the cutis. The tissue on the ventral surface of the ear is less vascular and here the streptococci are seen quite generally scattered about throughout the distended connective tissue and fibrinous exudate. In the section studied the epidermis was intact and there were no blisters or bullæ.

It is to be noted that in three out of five rabbits both ears showed similar lesions, whereas the injection was made into the vein of only one ear. In no case did the infection appear to spread from the site of injection.

The next most common lesion was orchitis which occurred in three of five male rabbits injected with freshly isolated cultures. The scrotum enclosing the testis became hot, purplish red in color, swollen, and tense, the testis remaining in the sac. In Rabbit B the process subsided after a few days and there was a desquamation of the skin of the scrotum. In Rabbit F the orchitis persisted until death and at autopsy both testes were found adherent to the scrotum. The left testis which had been longest diseased was simply a homogeneous yellow caseous mass. The right one was a sac of fluid yellow pus. Cultures from the right testis revealed *Streptococcus epidemicus* in pure culture. In Rabbit E the orchitis was not detected until the animal was chloroformed on the 7th day. Both testes were freely movable from scrotum to abdomen but had a mottled appearance more noticeable in the left testis. This testis was sectioned and showed the following changes.

Transverse section of the testis shows on one side a distinct area of necrosis involving the tubules and interstitial tissue, and extending at one point into the tunica vasculosa. In the center of this area the outlines of the tubules are distinct and the parenchymatous cells are distinguishable but structureless and without nuclei. At the periphery of the necrotic area the appearance is that of coagulation necrosis with numerous disintegrating nuclei. Polymorphonuclear cells are scattered throughout most of the tunica vasculosa. On the opposite side of the testis from the necrotic area is a mass of fibrin containing in its meshes large numbers of polymorphonuclear leucocytes and large mononuclear cells. This mass is apparently outside the tunica vasculosa but was probably inside the tunica albuginea which has been stripped away from most of the testis and is not present in the section though remnants of it are seen. On one portion of the epididymis a fibrinous exudate between the tunica vasculosa and tunica albuginea is plainly seen, while scattered through the connective tissue of the epididymis are many polymorphonuclear cells. The rete testis and the ducts of the epididymis are apparently normal as is also the connective tissue of the septula and that between the tubules of the testis. A few streptococci are seen within leucocytes.

Other lesions encountered and studied were an endocarditis in Rabbit D and a keratitis and conjunctivitis in one eye of Rabbit F. Rabbit D apparently died suddenly as a result of the fresh cardiac lesion. One of the cusps of the tricuspid valve was enormously thickened, forming a large tumor that could be seen through the heart wall. A stained section showed an edematous and necrotic valve filled with fibrin and bearing a large subendothelial hemorrhage at its free edge. Small clumps of streptococci were visible in the tissue. Rabbit F had an infection of the cornea of one eye which spread outward into the conjunctiva and inward into the anterior and posterior chambers of the eye, also into the choriocapillaris and retina. *Streptococcus epidemicus* was isolated repeatedly from the conjunctiva during life.

The lesions of the ears and testes appearing in such a large percentage of the rabbits injected with cultures from this epidemic seemed remarkable and more than mere coincidence. In 52 rabbits similarly injected with streptococci from other epidemics by Smith and Brown similar lesions of the ears were encountered four times and orchitis not at all. The tendency to produce these lesions in rabbits was apparently one of the "distinguishing characteristics" of strains isolated in this epidemic, and one which tended to identify the strain

from Cow 108 with those from throats. In contrast to the results reported by Rosenow (11) at various times in the elaboration of his theory of "selective localization," however, it is to be noted that erysipelas and orchitis were not reported in any of the human patients during the epidemic. The organism produced certain characteristic lesions in patients, and others equally characteristic in rabbits. It was interesting to find that the strain from Cow 108, 19 months after isolation, was still able to produce the ear lesion in Rabbit G, but orchitis was not produced.

Subsequent Study of the Dairy.

The contamination of the milk with the streptococcus from Cow 108 was something which could not be detected by current routine methods for the examination of milk. One of our objects, therefore, was to devise methods for safeguarding the production of raw milk against such accidents. With this end in view milk from the cows of Dairy H and swabs from the throats of the employees were studied for 2 or 3 months after the subsidence of the epidemic. There is nothing particularly difficult about culturing throat swabs from the employees in blood agar once a week. This, we believe, is the most important measure for the prevention of milk-borne streptococcus epidemics. Second in importance is the culturing of milk in blood agar. Obviously it is not practicable to culture the milk of individual cows each week. It was found, however, that if a mixed sample of the milk from a group of ten to fifteen cows was plated out in blood agar it was easy to detect strains of bacteria which formed the characteristic flora of milk from individual cows of the group. In the examination of milk from individual cows it was found that certain cows gave almost sterile milk while others gave milk containing large numbers of bacteria. These high counters, as we called them, seemed to harbor a characteristic flora in their milk ducts. These organisms were commonly streptococci of the alpha or gamma type in blood agar, sometimes micrococci, and more rarely bacilli or streptococci of the beta type. A small amount of the milk of Cow 108 added to a group sample was easily detected in the blood agar plate. Many times we were able to detect the withdrawal of a cow or the addition of a cow

in a certain group by a change in the group flora. If an organism appeared in a group sample which was at all suspicious, samples from the individual cows of that group were cultured. By the use of this procedure it would be easy to lower the bacterial count of a dairy milk by gradually eliminating certain cows which are high counters. In the purchase of a dairy cow there is no reason why this should not be taken into consideration.

In correlation with the regular examination of throat swabs from the milkers and group samples from the cows, the confining of a certain milker to a certain group of cows is highly desirable from a sanitary standpoint. In Dairy H this was considered impracticable but we believe that the ability to fix the responsibility for a certain grade of milk from a certain group of cows with a certain milker would have economic as well as sanitary advantages.

As a minimum requirement for dairies producing raw milk we would recommend the regular examination of throat swabs from the milkers and the use of blood agar rather than plain agar for making milk counts.

SUMMARY.

A streptococcus epidemic of moderate extent and severity was characterized by clinical symptoms different from the usual septic sore throat, though the organism found was culturally *Streptococcus epidemicus*.

The infection was traced to the milk from a single quarter of the udder of a cow in a dairy of 112 cows producing an otherwise excellent grade of raw milk.

A number of the milkers on the dairy farm were found infected. It was impossible to trace the infection of the cow's udder to any one of the milkers, though such an infection seems probable since the streptococcus isolated from the cow was in every respect like streptococci isolated from patients and milkers, and different from those usually found in normal cows or cows with garget.

Certain recommendations are made to safeguard producers of raw milk against the occurrence of such epidemics.

Addendum.

The authors are indebted to Dr. E. E. Tyzzer and Dr. Marshal Fabyan of the Department of Comparative Pathology of Harvard Medical School for the following notes regarding Cow 108 after she had passed from under our observation.

Cow 108 was kept under observation until Jan. 3, 1918, when, since lactation had ceased, she was slaughtered for beef. During this time the following bacteriological examinations of the milk were made.

Aug. 27, 1917. Milk from the left hind quarter showed the hemolytic streptococcus previously isolated.

Aug. 29. Milk from the right hind quarter, no hemolytic organism present in plates.

Aug. 30. Milk from the right fore quarter, hemolytic streptococcus not present.

Sept. 11. Right hind quarter negative.

Sept. 13. Right fore quarter negative.

Sept. 25. Both right hind and right fore quarters negative.

The infected quarter was the first to become dry and the secretion of milk had ceased in other quarters by the middle of November. The localized induration which had become apparent soon after the cow came under observation, persisted up to the time of slaughter. It was situated at some distance above the teat in the anterior portion of the left hind quarter of the udder and appeared as an ill defined mass of about the size of a hen's egg.

Autopsy.—The udder was first sliced in various directions by the inspector who made his examination before turning the material over for further study. The affected portion after incision showed remarkably little difference from the normal portion. The involved tissue, however, was slightly firmer and less flabby than the normal gland. The animal showed also an early tuberculosis involving the bronchial lymph nodes and a small portion of the lung.

Microscopic Examination.—Stained sections of various samples of the mamma showed definite inflammatory changes in the indurated part, which were absent in other portions. There appeared to be an increase in the interglandular connective tissue, although this is rather difficult to determine and may be an open question. The chief abnormality consisted of collections of lymphoid cells mingled with which were few plasma and large mononuclear cells. The larger foci were distributed in the walls of the large ducts but there were also similar foci including gland acini. The ducts showed eosin-staining secretion in which an occasional mononuclear cell was apparent, and this material differed in no way from the secretion found in the ducts of the normal quarters.

Cultures taken from the indurated portion of the gland were negative. The inoculation of rabbits with a suspension obtained by grinding this tissue in salt solution resulted negatively.

It is evident from these findings that the infection was present in the left hind quarter as late as Aug. 27, 1917. At the time of autopsy no evidence was obtained of the presence of a virulent streptococcus. The absence of polynuclear leucocytes in both the tissues and the secretion indicates that there was at this time no active process present.

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EXPLANATION OF PLATE 9.

FIG. 1. The first blood agar plate culture of milk from Cow 108 showing large numbers of colonies of *Streptococcus epidemicus*, after incubation over night. The plate was too thickly seeded for the colonies and zones to attain their full development.

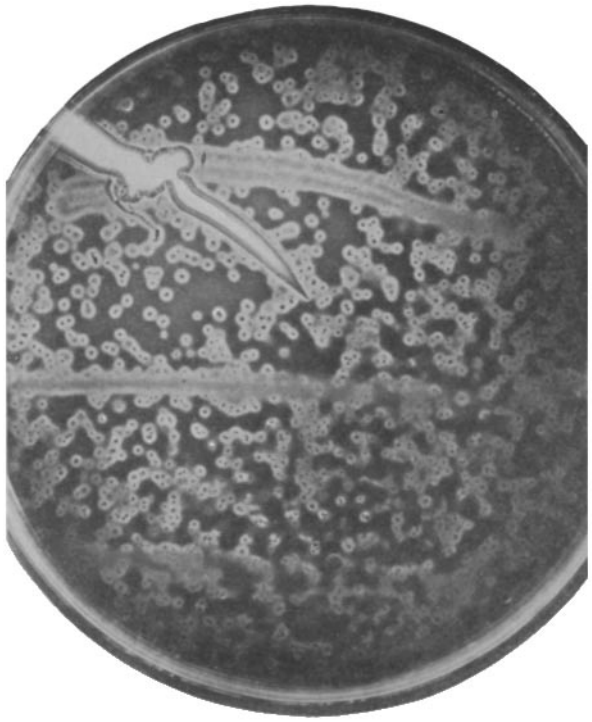

FIG. 1.

(Brown and Orcutt: Dairy infection with *S. epidemicus*.)