UDDER INFECTION WITH STREPTOCOCCI OF THE SCARLET FEVER TYPE.

I. Spontaneous and Experimental Udder Infection.

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During the past four decades many outbreaks of scarlet fever attributed to contamination of milk supplies have been reported. This occurrence claimed considerable attention during the later '80's of the 19th century. Since then, although outbreaks attributed to milk have been recorded, the number has apparently declined. In certain particulars epidemics due to contaminated milk resemble each other. Their explosive nature—the bulk of the cases occur during a short period, usually within a week—is characteristic. The history that the sick have partaken of milk from a common source and the fact that the disease is not epidemic in other parts of the community having a different milk supply are equally significant.

The origin of milk-borne epidemics of scarlet fever has in the past led to much discussion.

Probably the most commonly held view is that of actual contamination of the milk by convalescents or persons actually sick with the disease. This view is supported by many recorded outbreaks in which persons known to have come in contact with scarlet fever or who were actually suffering from the disease milked the cows, mixed, handled, or bottled the milk. Among others, Hemenway (1) records a large epidemic attributed to milk thus contaminated. Other outbreaks regarded as milk-borne could be traced to actual cases who delivered the milk from door to door. Chalmers (2) reports such an outbreak. In addition the return of milk bottles from houses where scarlet fever was present was held responsible for the disease along particular milk routes.

The widespread and severe nature of certain outbreaks in England during the period from 1880 to 1900 led to investigations that directed attention to the cow as a source of infection. The difficulty of tracing human contamination strength-

ened the suspicion. Power (3) and Klein (4) as early as 1882 attributed one outbreak to the contamination of milk from a case of puerperal fever in a cow. Klein showed that cows inoculated subcutaneously with material obtained from the throats of human patients developed abscesses at the injection sites. Purulent material from such abscesses when injected into healthy cows also produced abscesses. Later Power (5), Cameron (6), and Klein (7) reported their findings in the Hendon outbreak in which they failed to find a human source of infection. Suspicion was directed to certain newly purchased cows suffering with a malady of the skin of the udder and teats. This they regarded as a specific infection transmitted by the hands of the milker. The condition was characterized by general constitutional disturbance, sore throat, discharge from the eyes and nose, vesicular eruptions of the skin of the buttocks and udder. They regarded the rupture of the teat vesicles as the source of the milk inoculation. Russell (8) encountered a similar outbreak in which 101 persons contracted scarlet fever; a malady similar to the Hendon disease affecting two cows was noted in the herd. A calf fed the milk of one of these developed a severe febrile reaction. Hill (9) likewise reported milk-borne scarlet fever; certain cows in the dairy from which the supply was drawn were found affected with Hendon disease. Hamar and Jones (10) also cited an outbreak in which a disease of the skin of cows similar to that noted by Power, Klein, and Cameron was present in the herd. They were inclined to attribute the human infection to the cows since the milk was known to be infective before human cases occurred on the farm. M'Fadyean (11) disagreed with them on the grounds that Hendon disease as a scarlatinal infection of cows is unproved and that the milker in whose family scarlet fever occurred may have been responsible for the epidemic through contamination. As a further criticism that Hendon disease is not a specific scarlatinal infection of cows he points out that although the disease was first recognized in 1882 and seemed to prevail for a year or two it had not attracted further attention until 1909 when a similar disease was reported.

It is apparent then that there are several views regarding the method by which milk may become infective: first, contamination of the milk during milking or handling; second, the return of contaminated bottles or utensils from the household in which the disease exists; and third, a scarlatinal disease of the skin and udder of cows from which discharges may enter the milk during milking.

With the change in the status of the streptococcus as the etiological agent of scarlet fever during the past few years, a fourth means of contamination becomes apparent. The findings of Savage (12), T. Smith and J. H. Brown (13), Davis and Capps (14), Brown and Orcutt (15), and Benson and Sears (16) in milk-borne epidemics of septic sore throat are of considerable interest in this connection. It

has been shown that *Streptococcus epidemicus* may be implanted in the udder and be shed in the milk in such numbers as to give rise to severe outbreaks of sore throat among those consuming the milk. That such is not unlikely in outbreaks of scarlet fever attributed to milk seems plausible. This is especially significant in many epidemics traced to dairies where no mention is made of clinical examination of the udder or bacteriological examination of the milk. The possibility of udder infection may have been overlooked.

Our problem concerns itself with udder infections with streptococci similar to those found in scarlet fever.

History of a Milk-Borne Epidemic of Scarlet Fever.

Through the courtesy of the New Jersey State Department of Health we learned of a sharp outbreak of scarlet fever in a small town. About 200 cases occurred. Of these 159 developed from May 20 to 25, 1927. The State authorities found that the bulk of the cases was confined to a certain milk route supplied by one distributor. Further information directed their attention to one of the farms supplying the dairy. On this farm a daughter had scarlet fever in March, 1927. In addition a young man employed as milker had visited his home shortly before the outbreak where there was a child sick of scarlet fever. Representatives of the State Department of Health made throat cultures from everyone suspected of contaminating the milk and obtained hemolytic streptococci in two instances from throats of men handling the milk at the distributor's. For these cultures we are indebted to Mr. J. V. Mulcahy, Chief of the Bureau of Bacteriology of the State Health Department. In addition he furnished us with a culture from the young man who milked the cows, but it was of the *viridans* type.

On May 25, Dr. I. H. Shaw, veterinarian for the State Department of Health, visited the farm and examined microscopically the milk sediment from each cow. He noted one chronic case of mastitis (Cow 11) and an acute injury of the teat of the left hind quarter (Cow 3). The milk sediment of Cow 3 contained leucocytes and cocci.

In the meantime the milk from this farm had been excluded from the supply and a pasteurizer installed in the distributor's with the

result that the epidemic rapidly subsided. At our suggestion, and through the courtesy of the State Department of Health, the farm was visited on June 4, 1927. Samples were drawn directly from the udder of each cow into separate sterile bottles.

Examination of Milk from Each Cow.

The samples were obtained on the evening of June 4, refrigerated at once, and plated late the same night. The normal appearing milk was plated in two dilutions, 1:20 and 1:100; that from abnormal quarters at 1:1,000 as well as the lower dilutions. All plates were prepared with 0.5 cc. of defibrinated horse blood and 10 or 12 cc. of melted agar. Thirteen cows comprised the herd. The udders of eleven were normal. The plate cultures revealed nothing of significance. Cow 11 had chronic mastitis of the left hind and right fore quarters. The plate cultures revealed non-hemolytic streptococci in enormous numbers both in the involved and apparently normal quarters. Cow 3 had a severe involvement of the left hind quarter. The teat had been injured, the quarter was swollen and could be milked with difficulty. The milk was yellow and of the consistency of heavy cream. All the blood in the plate containing as little as 1:1,000 cc. of milk was hemolyzed within 12 hours. The centrifuged sample revealed a large quantity of sediment consisting of packed masses of leucocytes and enormous numbers of short chained streptococci. After refrigeration for 12 hours the milk was further diluted and plated so that finally it was possible to estimate that it contained 345,000,000 streptococci per cc. Colonies sufficiently isolated were chosen for subculture. It may be said that the initial tests, such as those for the presence of capsules, the laking of blood in the test-tube, pathogenicity for rabbits, and a final pH of 5.0 in dextrose broth, indicated that this culture was of human origin. In addition to the human type a small proportion of the non-hemolytic bovine streptococci was found in the original sample.

The Spontaneous and Experimental Infection in the Cow.

Cow 3 was purchased by this Department and is hereafter referred to as No. 1452. 4 days after the first observation the quarter was swollen, firm, and the teat showed a healing scar. Yellow, purulent milk could be expressed only with difficulty. Bacteriological examination revealed relatively few hemolytic streptococci and enormous numbers of the non-hemolytic mastitis type. Evidently the bovine type noted June 4 had nearly replaced the hemolytic streptococcus. The inflammation continued in the quarter until the cow was slaughtered on June 23. During this time hemolytic streptococci were always present in small numbers and the mastitis type in enormous numbers.

On one occasion a single colony of the hemolytic type was obtained from the milk of the right hind quarter. This strain was identical in all respects with that obtained from the left hind quarter. Although the milk from the other quarters was plated frequently streptococci were not found.

On June 9, the right hind quarter was inoculated by means of a teat tube with 1/1,000,000 cc. of a 24 hour broth culture of the hemolytic streptococcus from the left hind quarter. No reaction occurred and examination of the milk during the next 3 days failed to show the organism.

On June 14, the right fore quarter was inoculated by means of a teat tube with 1/500,000 cc. of the hemolytic culture and the right hind quarter with 1/100,000 cc. of the same culture. 7 hours later the streptococcus could not be cultivated from the milk from either quarter. After 24 hours there was little to be detected clinically. The appearance of the milk was not greatly altered, and the quantity of sediment was not excessive but it contained leucocytes and a few diplococci. Plate cultures, however, revealed 25,600 hemolytic streptococci per cc. in the milk from the right hind quarter and over 1,000,000 per cc. in that from the right fore quarter. After 48 hours the right hind quarter was hot, the milk was yellow and contained large, irregular floccules. The right fore quarter revealed nothing abnormal except that the milk was yellow and flocculent. Samples from both quarters revealed an excessive amount of sediment composed of leucocytes and streptococci. The bacterial counts of the milk were: right fore quarter = 45,000,000 hemolytic streptococci per cc., and right hind quarter = 1,240,000 per cc. The next day 38,000,000 hemolytic streptococci per cc. were found in the milk from the right fore quarter and 1,240,000 per cc. in that from the right hind quarter. From this time onward the number began to decline until on the 14th day the milk from the right fore quarter failed to show streptococci while that from the right hind quarter revealed 26,000 per cc.

During the period of observation it can be said that the inoculation failed to produce well defined clinical disturbances in the inoculated quarters, although the milk was purulent.

The udder obtained from the abattoir at the time of slaughter revealed a pronounced atrophy of the left hind quarter characterized by severe degeneration of the secreting structures and hyperplasia of the interstitial tissue. The two inoculated quarters showed lesions of the mucosa of the lower portion of the udder involving the mucosa of the large milk ducts accompanied by a purulent exudate.

Since the spontaneous case of infection was complicated by injury and secondary infection with the usual type of mastitis streptococcus,

the question might be raised as to whether the factor of injury is a necessary precursor of infection. Further the experimental disease incited by inoculation failed to resemble the spontaneous disease. In order to throw more light on these questions a second series of inoculations was made.

Cow 1462.—A Holstein cow was injected by means of a teat tube inserted into the left hind quarter with 1/1,000,000 cc. of an 18 hour serum broth culture of the 6th transfer of the streptococcus obtained from the left hind quarter of Cow 1452. Plate cultures prepared from similar dilutions indicated that between 15 and 20 streptococci were injected. Plate cultures of the milk 7 hours after injection failed to show streptococci. 24 hours after injection the quarter still appeared normal. The milk contained only a little sediment composed of a few leucocytes and round cells and a few diplococci. The plate culture revealed 115,000 hemolytic streptococci per cc. After 31 hours the quarter was swollen, tense, hot, and

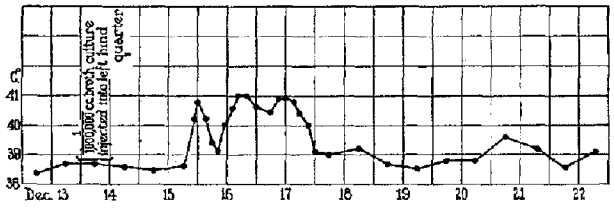

CHART 1. Temperature reaction, Cow 1462, following injection of left hind quarter.

painful. The milk contained an excess of fat but the amount of sediment was not excessive. There were 2,000,000 streptococci per cc. At 36 hours the quarter was greatly swollen. The milk was yellow and serous and contained large floccules. It coagulated on boiling. Plates revealed 8,320,000 streptococci per cc. 48 hours after injection there was a severe systemic reaction characterized by chills, fever (Chart 1), and depression. Swelling of the quarter was pronounced. The milk was seropurulent. It was estimated that 1,200,000,000 streptococci per cc. were being eliminated. On the 3rd day the quarter was distinctly reddened, the milk purulent, and 510,000 streptococci per cc. were recorded. A blood culture was negative. The cow had a fever. The reddening of the skin spread to the other quarters on the 4th day, persisted throughout the 5th day, and began to subside on the 6th day. It had disappeared by the 7th day. During this period the number of streptococci decreased until a minimum of 20,000 per cc. was reached. However they increased during the 7th, 8th, and 9th days, reaching the high point of 69,000,000 on the 8th day. There was a corresponding rise of temperature on the 7th and 8th days (Chart 1). By the 13th day their number

had declined to 2,000,000. From this time the acute inflammation gradually subsided with gradual atrophy and with a further decline in the secretion until on the 47th day only 25 or 30 cc. of purulent milk was obtained. Hemolytic streptococci were still present on this day. Chart 2 illustrates the number of streptococci per cc. of milk from the left hind quarter of Cow 1462 for 9 days following the inoculation.

It is to be noted that during the height of the attack the general reaction was severe, milk secretion was almost entirely suppressed, a fall from 8 pounds per milking to less than 1 pound being recorded.

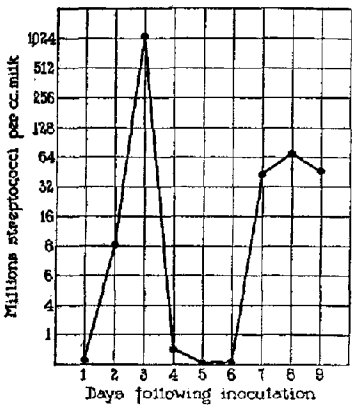

CHART 2.

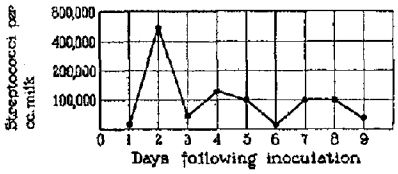

CHART 3.

CHART 2. Streptococci per cc. of milk during the first 9 days subsequent to injection of left hind quarter of Cow 1462.

CHART 3. Streptococci per cc. of milk during the first 9 days after injection of right hind quarter of Cow 1462.

27 days after the left hind quarter had been artificially infected one-millionth cc. of broth culture was instilled into the lower cistern of the right hind quarter. It was estimated that approximately 70 streptococci were injected. 7 hours later there was nothing in the appearance of the milk to arouse suspicion and 1 cc. failed to reveal the streptococcus. After 24 hours the milk appeared normal. The sediment contained a few leucocytes and plate cultures revealed 1,800 streptococci per cc. 2 days following the inoculation the quarter failed to show abnormalities. The milk was yellow and thickened; when centrifuged the sediment comprised about 1/20 of the total volume. It was composed of masses of leucocytes, diplococci, and short chained streptococci. The maximum count of 556,000 streptococci per cc. was reached on this day. During the next 9 days the udder and milk were examined daily. Lesions of the udder were not detected

clinically. The milk was always purulent and the sediment at times made up 1/10 of the volume. The number of streptococci varied from day to day, the minimum count recorded during this period being 18,000 and the maximum 136,000 per cc. On the 21st day the milk was still purulent and flocculent and contained 20,000 streptococci per cc. Chart 3 is included for comparison with Chart 2. It indicates the number of hemolytic streptococci eliminated per cc. during the first 9 days following inoculation of the right hind quarter. The two charts afford a sharp numerical contrast. In the primary inoculation the maximum of over 1 billion was reached on the 3rd day following injection, with a rapid fall until the 6th day and a subsequent sharp rise on the 7th, 8th, and 9th days. Chart 3 illustrates the elimination of streptococci subsequent to injection of the right hind quarter. Here the peak of 550,000 was reached on the 2nd day with a sharp decline on the 3rd day and irregularity thereafter.

It is true then that the streptococcus isolated from the spontaneous infection was of sufficient virulence in spite of cultivation for 6 months on artificial media to incite a severe mastitis. During the acute inflammation there were marked systemic disturbances characterized by fever, increased pulse and respiratory rates, congestion of the conjunctiva, inappetence, suppression of milk, and stiffness of the joints. We regarded the animal as critically ill during this period.

In data from both the spontaneously infected and the experimental cow evidence exists that the primary attack so increased the general resistance that the secondary injection resulted in entirely different manifestations. Here only mild local disease resulted; although the streptococcus established itself, its multiplication never reached the maximum recorded in the primary attack.

It may be argued that the spontaneous case (No. 1452) referred to is an isolated example of such infection.* While this is true to a certain

* While these data were being assembled, Mr. Friend Lee Mickle, Director of the Bureau of Laboratories, Connecticut State Department of Health, sent three cultures of streptococci for examination. The interest of the Health Department had been aroused because of an outbreak of mild scarlet fever which appeared among the customers along a certain milk route. One of the cultures was isolated from the udder of a cow in the herd supplying the milk, another from the throat of the owner of the herd, and a third from a case of scarlet fever on the milk route. These proved similar in cultural characters, pathogenic properties, and antigenic affinities to those obtained by us from the udders of two cows. In all probability the particulars of this outbreak will be published by the Connecticut State Department of Health.

extent, nevertheless material obtained from another case of mastitis associated with a similar organism suggests that such infections may occur at any time. Although at the time of isolation the significance of the bacteriological findings in this spontaneous case was not realized, nevertheless the organism was regarded as a human type other than *S. epidemicus*. A brief statement concerning this case follows.

Cow 4262.—Milk drawn from all four quarters into a sterile bottle May 11, 1925. When plated it was found to contain 720 colonies per cc.; 25 per cent were large zoned, hemolytic colonies. May 19, 1925, mastitis of the right fore quarter was noted. The milk was purulent and flocculent. 77,000 hemolytic colonies per cc. were recorded. On May 22 and 23, the milk from all quarters was examined with negative results. The hemolytic streptococcus had been replaced by the bovine non-hemolytic type. The milk was again examined on May 27 and 28, and the later findings confirmed. Little of significance could be obtained from the history of this cow except that it had been in the herd for 3 years. During the preceding lactation periods many attacks of mastitis had been noted. During the 2 months prior to our examination four attacks of mastitis of the right fore quarter had been reported. The hemolytic streptococcus was definitely of the human type as proved by the usual tests and, as it will be shown later, closely resembled those isolated from Cows 1452 and 1462. No record is available which indicates that this animal was responsible for an outbreak of scarlet fever.

DISCUSSION.

Heretofore contamination has been regarded as the usual means of spread of scarlet fever by milk. The findings in regard to septic sore throat, however, indicate that udder infection with the human streptococcus is far more likely. The same may be said of milk-borne epidemics of scarlet fever.

Unfortunately our examination was conducted after the epidemic had subsided. It is true though that when the milk from this farm was withheld from the general supply and the general supply pasteurized the outbreak subsided. When we made our examination the owner maintained that the milk from the left hind quarter of the infected cow was "all right" although he had withheld it from the general supply. It can be argued, however, that such milk was fully capable of causing severe illness among the consumers provided it entered the general supply. If 1 quart of milk drawn at the time when the streptococci were most numerous was mixed with the herd supply, the actual

dilution in this instance would amount to 1:100 since there were 12 cows contributing about 100 quarts. Our maximum count indicated well over 300 million per cc. in the spontaneous case and over 1 billion in the experimental inoculation. Assuming that the infective product was again diluted at least a hundred times at the distributor's the number of streptococci in even a small quantity of milk would be relatively high.

Spontaneous Case 1452 and experimental Case 1462 are examples of extremely severe infections and they indicate that the organism was highly virulent for the cow. The infection obliterated for practical purposes the primarily involved quarter. However the resulting general resistance in both instances was insufficient to protect other portions of the udder from infection. This argues for a prompt exclusion from the milking shed of cows with involved quarters since the organism may gain entrance to normal quadrants without exciting severe reactions.

In sharp contrast to these cases is that of Cow 4262 in which the organism was known to be present in the milk on two occasions 8 days apart. It probably inhabited the udder during this period. However it disappeared and was replaced by a bovine type and was not found subsequently. That this cow failed to cause trouble to consumers can be explained on several grounds, (1) that the milk contained relatively few organisms, (2) that the milk was mixed with that of a large number of cows (50 or 100) and sold in a large city where a few cases of scarlet fever would attract little attention, and (3) that when the milk became abnormal it was eliminated from the supply. This case argues for a prompt investigation of the milk of all cows when milk-borne infection is suspected, since a few days delay in instances of this kind may be sufficient for the disappearance of the streptococcus from the udder.

The period of incubation is of considerable practical importance. In regard to this period little is known in spontaneous infection. In all the experimental inoculations there was little to arouse suspicion during the first 24 hours although streptococci could be readily detected in the milk. After this time abnormalities in the appearance of the milk were apparent. It must be borne in mind that in these experiments the organisms were introduced into the milk cistern, and

although the number of streptococci administered was small, in all probability in the spontaneous disease the infective dose would be even smaller; and unless contaminated material was introduced by teat tubes or other means the organisms would probably be deposited at the meatus or about the ends of the teats. This indicates a somewhat longer incubation period than that observed in the experiments.

SUMMARY.

The clinical and bacteriological findings in two cows the udders of which became infected under natural conditions with hemolytic streptococci of the scarlet fever type are discussed. One of the cows was found in a herd supplying raw milk to a small town where a milk-borne outbreak of scarlet fever had occurred a short time before. When small numbers of the streptococcus obtained from this case were injected into the udder of a normal cow severe mastitis accompanied by a well marked general reaction resulted. Evidence leads to the conclusion that a sevère attack of mastitis due to this organism in one quarter does not sufficiently immunize the other quarters to protect them completely since the streptococcus can be readily implanted in them. The secondary infections were much milder than the original process.

We wish to acknolwedge our indebtedness to Mr. W. T. Eakins, Assistant Epidemiologist of the New Jersey State Department of Health, who furnished us with the history of the outbreak; also to Mr. J. V. Mulcahy who supplied us with cultures F. C. and M. B. obtained from the throats of milk handlers, and to Dr. I. H. Shaw for accompanying us to the farm and for other courtesies.

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