GROWTH OF *STREPTOCOCCUS PYOGENES* IN MILK STORED AT ATMOSPHERIC TEMPERATURES

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(With 1 Diagram.)

Although the spread in epidemic manner of scarlet fever and septic sore throat has been sometimes clearly associated with the contamination of milk supplies, the essential source of such contamination has seldom been satisfactorily determined, because of the difficulty of co-ordinating with sufficient rapidity all the services necessary for tracing the root of the trouble. It is certain, however, that milk must be contaminated by *Streptococcus pyogenes*[1] in one of two ways: through the active infection of the udder tissue or by human agency during or after milking. Whilst intramammary contamination might reasonably be expected to be gross and of long duration, and thus responsible for widespread epidemics, contamination of milk supplies by human beings is more likely to be slight and intermittent in character. Consequently, extramammary contamination could cause widespread epidemics only if *S. pyogenes* is capable of multiplying rapidly in milk under ordinary conditions of commercial or household storage, but on this point very little information has been available. The object of the present investigation has been to ascertain whether *S. pyogenes* can multiply in milk stored under any normal conditions.

There is a considerable volume of evidence indicating that *S. pyogenes* is capable of invading the udder of the cow.

In the United States, Davis & Capps (1914) produced a clinical mastitis by introducing live culture into an artificial teat abrasion. The count of streptococci rose to 3000 per c.c., and these organisms were excreted in the milk for 3 weeks. Krumwiede & Valentine (1915), while investigating an epidemic of *Streptococcus* infection, isolated this organism from an udder sample of a cow which showed no clinical symptoms, though the milk itself was slightly altered in appearance. The *Streptococcus* appeared to be identical with strains isolated from man during the epidemic, producing a soluble haemolysin, fermenting the same carbohydrates, and being agglutinated to the correct titre with antiserum prepared against the human strain. Rosenau & Hesse (1917) reported a severe milk-borne epidemic of septic sore throat. Three cows supplying milk to the dairy concerned were found to be giving a purulent secretion, from which were isolated haemolytic streptococci pathogenic for mice and rabbits by injection, feeding, or swabbing of mucous membranes. A monkey was also infected by a combination of feeding and swabbing, and showed a marked febrile syndrome.

[1] Under the name *Streptococcus pyogenes* are included strains pathogenic for man which are now known to fall into the serological group A of Lancefield. Other names sometimes used include *S. scarlatinae*, *S. haemolyticus* var. *pyogenes*, *S. epidemicus*.

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Smillie (1917) reported a scarlet fever outbreak in a school, the source of the outbreak being traced to a cow which showed a slight mastitis. Haemolytic streptococci were isolated from the secretion of this udder and were identical with strains isolated from scarlet fever patients, in growth characteristics, carbohydrate fermentation, agglutination and pathogenicity. Brown & Orcutt (1920) identified a cow as responsible for an outbreak of tonsilitis, a *Streptococcus* being isolated from its milk which produced a wide zone of haemolysis in blood agar, had a *p*H of 5·1 in lactose, and was pathogenic for rabbits. The left forequarter of the udder was first involved and later, when this began to dry off, the left hindquarter became infected. The cow was excreting streptococci for over 4 months, counts as high as 25×10^7 being recorded. In the early stages no very obvious symptoms were noted, but signs of mastitis appeared as the quarters began to dry off. Frost & Carr (1927) investigated an outbreak of tonsilitis, isolating haemolytic streptococci from the bulk milk supply and also from one of the cows which showed mastitis and was excreting 36 million streptococci per c.c. The strain of *Streptococcus* isolated from this cow was capsulated, produced soluble haemolysin, had a *p*H of 5·1 in dextrose and failed to hydrolyse sodium hippurate. Benson & Sears (1923), whilst investigating an extensive outbreak of septic sore throat, found, among the cows supplying the milk, several which showed symptoms of mastitis. All affected cows were destroyed and examined post-mortem. From the secretion of an animal with consolidation of one-quarter of the udder, wide-zoned haemolytic streptococci were isolated, the count being 10 million per c.c. of secretion. This organism appeared to be similar to strains isolated from human patients, having a *p*H of 5·2 in glucose medium, producing acid but no clot in litmus milk, and being pathogenic for rabbits and mice. Jones & Little (1928) examined a cow excreting wide-zoned haemolytic streptococci from the left hindquarter of the udder. The streptococci were capsulated, produced a soluble haemolysin, had a *p*H of 5·0 in lactose medium and were pathogenic for rabbits. Organisms were excreted in numbers as high as $3\frac{1}{2}$ million per c.c. The injection of culture into the teat canal of the right forequarter led to a mild mastitis with *Streptococcus* counts up to 75 million per c.c. Subsequently the right hindquarter became involved naturally. A second cow was artificially infected with this strain of *Streptococcus* and acute mastitis with associated generalized symptoms resulted. Excretion of organisms continued for 47 days after infection, counts of 2–7 million streptococci per c.c. being recorded. Robinson & McComb (1932) found a cow with one quarter infected with haemolytic streptococci identical with those isolated from patients during an outbreak of septic sore throat. The streptococci were excreted continuously for 2 months, after which the cow was killed. A healthy cow was then artificially infected with haemolytic streptococci isolated from a child; this led to a slight change in the milk, but there were no symptoms of mastitis. Counts up to 20,000 streptococci per c.c. were recorded during the period of excretion, which lasted 4 months; thereafter the quarter went dry. When the cow came into milk, haemolytic streptococci were again excreted for about 6 weeks, but the text does not make quite clear whether the *Streptococcus* which reappeared after calving was identical with the human strain. In England a small outbreak of septic infection among members of a farmer's household and his staff resulted from the udder infection of two cows showing clinical mastitis (Golledge, 1932). In this case, epidemic spread was avoided because the herd milk was normally pasteurized before sale. Recently a serious scarlet fever epidemic at Doncaster has been traced to a cow infected with haemolytic streptococci of human type, and a similar epidemic in Denmark has also been traced to a cow having an infected udder (Bendixen, private communication).

The foregoing were all associated with outbreaks of disease among human beings, and from the evidence presented it seems very probable that the incriminated cows were in all cases excreting streptococci pathogenic for man. In addition, there are many more reports of the isolation of such streptococci from the milk of cows, but the exact identity of the organisms is less clearly defined. Minett & Stableforth (1931) and Minett (1935) showed that wide-zone haemolytic streptococci apparently identical with *S. pyogenes* can be isolated from

milk quite frequently, but that very few such strains can be considered to be of the true human pathogenic type as judged by the fermentation of sorbitol and trehalose. Out of twenty-one strains studied only three produced the human type of reaction in these media. These three strains were isolated from the udders of cows and constitute further evidence that streptococci pathogenic for human beings are sometimes disseminated by these animals.

From these records it may be seen that cows can have udder infections of *S. pyogenes* lasting some months; moreover—and this is a point of cardinal importance—the symptoms of mastitis may be quite mild. The general opinion is that in these cases the cow is primarily infected from a human source.

As long ago as 1911 Savage expressed the view that milk-borne *S. pyogenes* epidemics might be due to chance infection of the udder with streptococci of human origin, and this opinion was endorsed by Smith & Brown (1915). In spite of considerable experimental confirmation of this hypothesis and reaffirmation by Savage (1931) and Minett (1932), the opinion still appears to be widely held that milk-borne *Streptococcus* epidemics must be due to direct contamination of the milk by human carriers of infection. In the investigations of epidemics described by Winslow (1912), Rosenau & Moon (1915), Smillie (1917), Wilkinson (1931), Welch & Mickle (1933), the Chief Medical Officer of the Ministry of Health (1935), and Camps & Wood (1936), *S. pyogenes* was isolated from the bulk milk. The bacteriological examination of udder samples, where attempted at all, was done only after a long interval, during which an infected cow might have ceased to excrete streptococci, gone dry, or even changed hands.

There appear to be no experimental data to account for the ready acceptance of the idea that extramammary contamination of milk is the only type of contamination of epidemiological importance. Chance contamination by human beings can be the cause of prolonged or widespread epidemics only if *S. pyogenes* multiplies readily in market milk under normal conditions of storage. Davis (1914) reported that these streptococci began to multiply slowly in sterile milk after 20 hours' storage at 20° C., or rather earlier if stored at 26° C. In commercially pasteurized milk streptococci increased threefold during 20 hours of storage at 20° C., whilst at 26° C. they were overwhelmed by the rapid growth of saprophytes. No multiplication occurred at 10° C. Davis considered that "under no conditions met with in the ordinary handling of milk can there be any appreciable multiplication of haemolytic streptococci". Jones (1928) described a comparatively heat-stable and filterable factor present in fresh milk which at body temperature kills off *S. pyogenes* completely, and at lower temperatures leads to a considerable decrease in their numbers.

PLAN OF EXPERIMENTS

In planning an investigation of this type the bactericidal effect of fresh raw milk must be taken into consideration. This factor was described by Hesse (1894), and has since been studied by many investigators, including Rosenau & McCoy (1908), Hanssen (1924), Jones & Little (1927), Jones (1928), and Christiansen (1931). According to these workers, fresh milk is bacteriostatic and in certain cases possibly bactericidal. The bacteriostatic factor is relatively heat-stable, for, according to Jones, its potency is not altered if the milk is heated at 58° C. for 20 min., whilst, according to Hanssen, it survives 63° C. for 30 min. and 70° C. for 15 min. but is destroyed at 75° C. within 15 min. It would appear, therefore, that this factor can survive holder-pasteurization at 62·8° C. for at least 30 min., but not commercial sterilization (i.e. the heating of milk to 212° F. for 25 min. in open bottles, which are then sealed and heated at 235° F. for 12 min.). The observations of Hanssen and of Rosenau & McCoy indicate that the factor may remain active for 4–10 hours after milking. Its potency varies with the individual cow and with the season, possibly owing to variations in feeding (Hanssen, 1924). In addition to preventing multiplication of organisms in the milk itself, this factor may have a bacteriostatic effect when milk is inoculated into a medium such as

agar. Very little is known of the extent to which plating experiments can be influenced in this way, but according to Jones (1928) milk in a concentration of 1 : 20 in blood agar inhibits the development of zones of haemolysis by haemolytic streptococci.

From the foregoing it would seem likely that no simple answer could be provided to the question whether *S. pyogenes* would grow in milk. The result would not only depend upon the temperature and duration of storage but also upon the type of milk (i.e. whether raw or heated), its actual age, and its age when first contaminated with *S. pyogenes*.

For these reasons the main commercial grades of milk have been investigated, i.e. raw (graded and ungraded), pasteurized, and sterilized milk. Single cows stabled at the Institute were used as the source of raw ungraded milk. The disadvantage of this procedure was that the bactericidal power of milk from a single cow is more variable than that of pooled milk, but this was outweighed by the fact that the age and cleanliness of samples could be controlled at will. These types of milk were artificially contaminated with culture of *S. pyogenes*, and the number of the organisms was ascertained from plate counts carried out at various intervals.

Artificial inoculation of pasteurized and sterilized milk in the laboratory is comparable to what might occur under natural conditions. Such milk is sealed mechanically after processing and, except in the unusual event of a breakdown of the heating system with survival of streptococci, contamination with *S. pyogenes* can only occur after the bottle is opened by the consumer. Milk that is consumed raw may be contaminated at any stage from milking onwards, so that artificial inoculation of bottles of commercially produced milk some hours after milking simulates contamination by the consumer but not by the producer. Contamination by the producer was simulated by inoculating milk from a single cow immediately after withdrawal and also after short periods of storage at refrigerator and room temperatures. Milk from three different cows was used for this purpose, and all gave similar results.

To overcome contradictions which might arise out of variations in the initial number of *S. pyogenes* introduced into the milk, varying sized initial inocula were used in parallel experiments. Possible difficulties dependent upon the presence of too high a concentration of bactericidal substance in plating experiments were avoided by ensuring that the highest concentration of milk present in plate cultures was one part per hundred.

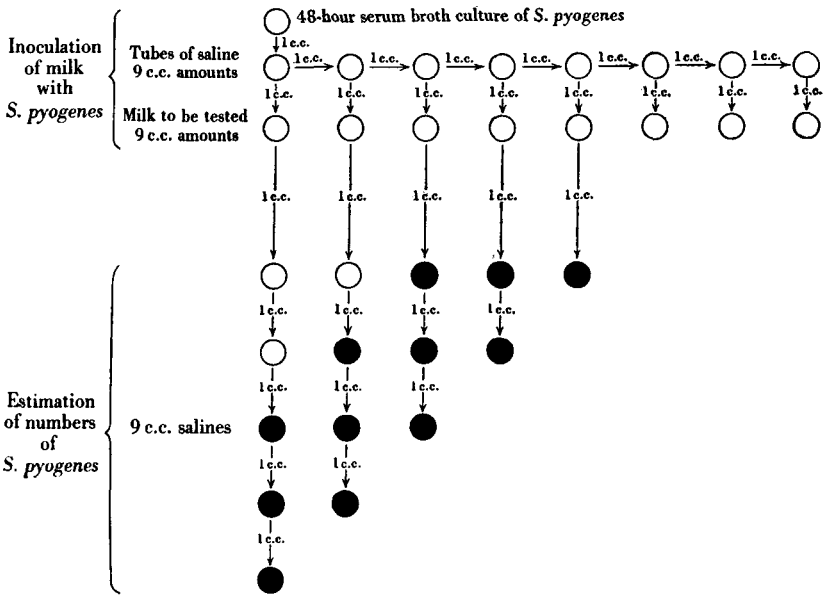
After inoculation, the milk was stored at 22, 18, and 15° C. as representative of average temperatures at which milk is frequently kept for household or commercial purposes at various seasons of the year.

Three strains of *S. pyogenes* were used: first the behaviour of an old laboratory strain (No. 1) in the various types of milk was ascertained, and the tests were then repeated with a strain freshly isolated from man (No. 2). All the confirmatory tests were carried out in the course of about 2 months, so that the second strain was used when still, comparatively speaking, newly isolated. Finally a strain of *S. pyogenes* (No. 3), newly isolated from the udder of the

naturally infected cow incriminated in the recent Doncaster outbreak[1] was examined for growth in ordinary fresh milk, and in fresh milk from the uninfected quarters of the cow concerned.

TECHNIQUE

Dilutions 10^{-1} to 10^{-8} of a 48-hour serum broth culture of *S. pyogenes* were made by transferring 1 c.c. volumes to 9 c.c. volumes of sterile saline. One c.c. amounts of each culture dilution were next inoculated into 9 c.c. quantities of



● = Saline dilutions used for plating, from each of which 5 plates were seeded with 1 c.c. amounts.

Diagram 1.

the milk under investigation. The number of streptococci added to each sample was then ascertained by carrying out colony counts. Serial tenfold dilutions of each milk sample were made in measured 9 c.c. amounts of sterile saline, and 1 c.c. quantities of these dilutions were inoculated into crystal violet blood agar in poured plates (Pullinger, 1935). Five plates were inoculated from each dilution from which it was decided to make counts, and throughout the whole procedure fresh pipettes were used for each manipulation. Experience quickly showed the number of dilutions it was necessary to plate in order to obtain reliable counts. The methods of inoculating the milk and plating it for the estimation of streptococcal counts are detailed in Diagram 1. Samples of milk were stored up to 96 hours after inoculation, provided the milk remained reasonably fresh and no conclusive result had already been obtained, plating being repeated every 24 hours.

[1] It is intended to publish some information regarding this cow at a later date.

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Table 1.

Type of milk	Storage prior to inoculation with *S. pyogenes*	Strain of *S. pyogenes* inoculated	Temperature of storage after inoculation ° C.	No. of cow	No. of experiment		Counts of organisms at hours shown: 0	24	48	72	96
HEATED											
Sterilized	Commercial for 18–24 hours	1	22	—	1	*S.**	6,020	∞	—	—	—
						T.†	23	60	—	—	—
		2	22	—	2	*S.*	52,600	∞	—	—	—
						T.	10	20	—	—	—
		1	18	—	3	*S.*	902	∞	—	—	—
						T.	—	—	—	—	—
		1	18	—	4	*S.*	75,400	∞	∞	—	—
						T.	40	104	135	—	—
		2	18	—	5	*S.*	636,000	8,240,000	∞	—	—
						T.	2	18	—	—	—
		1	15	—	6	*S.*	64,600	84,200	234,200	1,160,000	—
						T.	0	0	1,165	156,000	—
		2	15	—	7	*S.*	100,400	115,400	348,000	647,000	—
						T.	0	10	340	∞	—
Commercial pasteurized	Commercial for 18–24 hours	1	22	—	8	*S.*	3,400	3,020	Milk soured		—
						T.	12	∞	,,		—
		1	22	—	9	*S.*	7,600	5,720	,,		—
						T.	2,600	2,920,000	,,		—
		1	22	—	10	*S.*	4,420	4,080	,,		—
						T.	4,900	∞	,,		—
		2	22	—	11	*S.*	454,000	390,000	128,000	—	—
						T.	1,260	100,000	∞	—	—
		1	18	—	12	*S.*	12,120	12,680	8,360	—	—
						T.	3,600	Contam.	50,000,000	—	—
		1	18	—	13	*S.*	77,400	81,000	64,200	19,600	—
						T.	10,000	723,000	10,400,000	∞	—
		2	18	—	14	*S.*	28,020	24,080	16,600	—	—
						T.	34,430	246,600	∞	—	—
Laboratory pasteurized	None	2	22	—	15	*S.*	55,600	43,400	35,200	25,200	Milk soured
						T.	10	173	∞	∞	,,
		2	22	—	16	*S.*	69,000	46,000	39,600	30,600	,,
						T.	15	1,220	∞	∞	,,
		2	22	—	17	*S.*	32,000	30,000	15,000	13,800	,,
						T.	303	443	300,000	25,000,000	,,
		2	18	—	18	*S.*	25,180	18,860	15,720	—	—
						T.	146	3,100	∞	—	—
UNHEATED											
	None	1	22	T 189	19	*S.*	7,200	6,580	104,000	∞	—
						T.	1,966	192,600	∞	∞	—
		1	22	,,	20	*S.*	7,740	5,440	5,160	22,020	—
						T.	16	97,000	∞	∞	—
		1	22	,,	21	*S.*	18,040	13,180	13,360	59,200	∞
						T.	2,760	129,000	470,000	546,000	66,000,000
		2	22	,,	22	*S.*	16,680	3,120	1,120	0	—
						T.	283	296	1,003	1,620	—
		2	22	T 191	23	*S.*	198,200	14,200	2,400	—	—
						T.	2,830	23,000	∞	—	—

	None	1	18	T 191	24	*S.*	99,800	6,800	60,000	—	—
						T.	811	620	10,000,000	—	—
		1	18	,,	25	*S.*	52,000	53,400	45,400	49,600	—
						T.	66	—	233	4,073,000	—
		2	18	,,	26	*S.*	36,290	1,780	640	—	—
						T.	910	3,300	∞	—	—
		1	15	T 189	27	*S.*	360,000	214,800	185,000	—	—
						T.	356	403	500	—	—
		2	15	T 191	28	*S.*	91,500	78,000	34,800	36,000	—
						T.	1,833	1,046	5,700	∞	—
		3	22	,,	29	*S.*	80,800	51,800	6,500	—	—
						T.	276	83,300	∞	—	—
		3	18	,,	30	*S.*	346,000	178,000	182,000	275,000	22,800
						T.	157	196	200,000	∞	∞
Single cow ungraded	Room temperature for 4 hours	3	22	S 1	31	*S.*	91,800	43,300	Overgrown	—	—
						T.	2,576	∞	,,	—	—
		3	18	,,	32	*S.*	422,000	302,000	189,000	200,000	80,000
						T.	280	383	32,200	∞	11,330,000
		1	22	T 189	33	*S.*	980	676	182,000	Milk soured	
						T.	11,300	16,420,000	∞	,,	
		1	22	,,	34	*S.*	119,800	136,600	23,920,000	,,	
						T.	—	—	—	,,	
		1	22	,,	35	*S.*	356,000	251,200	243,000	83,800,000	Milk soured
						T.	36	8,660	68,300,000	∞	,,
		2	22	,,	36	*S.*	23,680	4,320	1,720	Milk soured	
						T.	186	403	88,600	,,	
		2	18	,,	37	*S.*	1,272,000	940,000	702,000	Overgrown	—
						T.	300	—	∞	∞	—
	Refrigeration for 4 hours	1	22	T 189	38	*S.*	354,000	134,000	Milk soured	—	—
						T.	903	∞	,,	—	—
		2	22	T 191	39	*S.*	164,000	106,000	,,	—	—
						T.	45,300	∞	,,	—	—
		1	18	T 189	40	*S.*	204,200	138,000	112,800	156,000	—
						T.	200	382	3,500,000	141,300,000	—
		1	18	,,	41	*S.*	878,000	510,000	500,000	404,000	—
						T.	190	350	∞	∞	—
		2	18	,,	42	*S.*	132,800	109,800	73,200	Overgrown	—
						T.	253	380	∞	∞	—
Commercial graded	Commercial for 12–18 hours	1	22	—	43	*S.*	7,100	2,000	Milk soured	—	—
						T.	310	∞	,,	—	—
		1	22	—	44	*S.*	16,300	17,000	,,	—	—
						T.	16,770	∞	,,	—	—
		1	18	—	45	*S.*	506,000	466,000	604,000	352,000	—
						T.	506	∞	∞	∞	—
		1	18	—	46	*S.*	11,200	101,600	75,400	63,800	—
						T.	1,906	∞	∞	∞	—

*S. = *Streptococcus pyogenes*. Counts recorded are the average of five plates.

†T. = total bacterial count of similar milk uninoculated with *S. pyogenes*. Count prepared on Standard agar, average of three plates recorded.

∞ = count innumerable.

Counts of the total bacterial content of identical samples of milk stored under similar conditions but uninoculated with *S. pyogenes* were also made, the standard technique being adopted except that three plates from each dilution were inoculated. Uninoculated milk was also plated in crystal violet blood agar to ensure that haemolytic streptococci were not already present in the milk before artificial contamination.

All plates were incubated in a hot room at 37° C. *Streptococcus* counts were carried out after 24 hours' incubation, when each colony was marked with ink on the bottom of the Petri dish. Plates were then reincubated for a further 24 hours, and re-examined for the appearance of more colonies. This double count was found necessary because sometimes haemolytic colonies were not clearly recognizable after 24 hours, whereas, if the total bacterial count of the sample was high, after 48 hours these colonies were masked by overgrowth of saprophytes. Total bacterial counts were made after the usual 48 hours' incubation.

Results

Results of these investigations are recorded in Table I. For the sake of brevity, only the average counts of a sample receiving a single inoculum are included. Representative results of the addition of high, medium, and low initial inocula are recorded in Table II.

Table II. *Effect of adding varying sized inocula of* Streptococcus pyogenes *to milk*

Exp.	Initial inoculum	*S. pyogenes* count at hours shown			
		0	24	48	72
1	Large	204,200	138,000	112,800	156,000
	Medium	1,360	1,298	1,262	1,632
	Small	142	88	22	178
2	Large	181,000	16,600	7,000	Milk sour
	Medium	36,290	1,780	640	
	Small	2,640	140	0	
3	Large	598,000	786,000	1,350,000	1,224,000
	Medium	7,920	9,020	10,800	46,800
	Small	56	84	100	360

From Table I it will be seen that *S. pyogenes* multiplies within 24 hours in sterilized milk stored at 22 and 18° C., but more slowly at 15° C. (Exps. 1–7).

Commercial pasteurized milk stored at 22° C. soured within 48 hours, but before souring the streptococci showed no tendency to multiply. At 18° C., the milk usually remained palatable for 48 hours, during which time the numbers of streptococci diminished gradually (Exps. 8–14). At 15° C. the streptococci show such a long lag period before they begin to multiply that growth in pasteurized milk at this temperature was not investigated. The poor keeping quality of commercial pasteurized milk, compared with freshly drawn milk from a single cow, is due partly to its greater age and partly to contamination with thermophilic or thermoduric organisms, an unavoidable characteristic

when very large volumes of ungraded milk are pooled. To give *S. pyogenes* a longer time in which to multiply, samples of freshly drawn milk from a single cow were pasteurized in the laboratory. In such milk also the numbers of streptococci diminished very gradually (Exps. 15–18).

Raw graded milk inoculated with *S. pyogenes* 12–18 hours after milking soured within 48 hours of storage at 22° C., but did not sour for 72 hours at 18° C. The numbers of streptococci showed no significant change during storage at either temperature (Exps. 43–46).

In all the above-mentioned experiments the newly isolated strain of *S. pyogenes* (No. 2) behaved like the old laboratory culture (No. 1), though it multiplied less readily and died out more quickly.

Raw milk from the Institute cows was inoculated with *S. pyogenes* immediately after withdrawal, and also after 4 hours' storage at room and refrigeration temperatures. When subsequently stored at 22° C., the old strain of *Streptococcus* (No. 1) began to show appreciable multiplication in such milk after 48–72 hours, whereas the newly isolated strain (No. 2) showed gradual diminution of numbers (Exps. 19–23). At 18 and 15° C., the numbers of organisms in both strains diminished gradually (Exps. 24–28). Whether the milk was contaminated immediately after withdrawal or after 4 hours' storage made no difference to the ultimate result (Exps. 33–37).

The strain of *S. pyogenes* of bovine origin (No. 3) showed an initial decrease which at 22° C. continued until the milk soured. At 18° C. the organisms began to multiply slowly after 48–72 hours of storage. Similar results were obtained when the streptococci were added to milk drawn from the uninfected quarters of the cow from which the strain had been isolated (Exps. 29–32). The colonies of this strain were difficult to count in plates heavily overgrown with saprophytes because only comparatively small zones of haemolysis were produced in blood agar.

In Table II are recorded typical results showing the effect of contaminating the milk with large, medium and small initial inocula of streptococci. It will be seen that the size of the initial inoculum does not determine whether multiplication or diminution of the numbers of streptococci will occur.

The relationship between the rate of multiplication of the streptococci and the saprophytic bacteria is shown in Table I.

DISCUSSION

The ready multiplication of *Streptococcus pyogenes* in sterile milk even at comparatively cool temperatures was expected, but it is of no importance in the consideration of milk-borne epidemics. Under normal conditions sterilized milk can be contaminated only after the container has been opened by the consumer and, except in the case of large catering establishments, such contamination can only endanger a relatively small group of people.

In clean fresh milk some strains of *S. pyogenes* may multiply slowly, whilst

others die out gradually. When growth occurs it only reaches significant proportions, from the point of view of extensive contamination of pooled milk, after storage for 48–72 hours at summer temperatures, and even later when the weather is cooler. Large volumes of milk are never stored so long except when designed for manufacture, while raw milk for liquid consumption is rarely kept by the consumer for as long as 48 hours after withdrawal. In samples of milk which sour rapidly there is no likelihood whatever of these streptococci multiplying.

The foregoing results have revealed a slight but unmistakable difference between the behaviour of different strains of *S. pyogenes* in milk. The important epidemiological fact is that whereas the old stock strain (No. 1), of doubtful pathogenicity for man, only multiplied gradually in raw milk under optimum conditions, a strain (No. 2) newly isolated from man, when stored under similar conditions, actually diminished in numbers. Moreover—and this point is significant—strain No. 3 of *S. pyogenes* immediately after isolation from a cow's udder actually diminished in numbers in milk during the first 48 hours of storage, and then began to multiply very gradually, whereas one might have expected that such a strain would be adapted to existence in cows' milk, and so multiply readily.

Since some strains of *S. pyogenes* can multiply in clean fresh milk, whereas in pasteurized milk having a relatively high initial bacterial count they gradually die, it would appear that the other bacterial flora have some inhibitory influence on the development of the streptococci. Whether this influence is a direct one, such as overcrowding, or is due to the accumulation of metabolic products such as lactic acid, or to a combination of the two factors, is a matter for speculation. Rapid multiplication of saprophytes generally curtailed the experiment by rendering the milk sour, but in certain cases before actual souring occurred, the total bacterial count was sufficiently great to make difficult the recognition of *S. pyogenes* colonies.

The failure of these streptococci to multiply appears, therefore, to be due to a combination of factors, namely, (*a*) the natural reluctance of pathogenic organisms to multiply at atmospheric temperatures, (*b*) the exaggeration of this reluctance to multiply by the bacteriostatic action of fresh milk, and (*c*) the facility with which saprophytes can multiply at atmospheric temperatures in spite of the bacteriostatic action of the milk.

While there is very convincing evidence that the udder of the cow is a potential reservoir of infection, there is a complete lack of experimental evidence to explain how chance droplet contamination could permeate large volumes of pooled milk. It would therefore appear that more attention should be paid to the examination of the individual cow in the investigation of milk-borne streptococcal epidemics. There seems little doubt that the ultimate source of infection is a human carrier, but if a cow is once infected she can excrete thousands of organisms per c.c. of milk and may continue to do so for weeks or even months. Such a cow would be a grave source of danger to public health.

The evidence available indicates that an infected cow has on several occasions been the essential factor in the spread of milk-borne epidemics, and such cows are undoubtedly far more often responsible for epidemics than is yet realized. It is essential that, in the investigation of outbreaks of *Streptococcus* infection showing the characteristic features of milk-borne epidemics, attention should immediately be directed to the bacteriological examination of samples of milk from individual cows concerned, since the successful isolation of the streptococci from dairy workers or from members of their households cannot be taken as conclusive evidence that the essential source of infection has been located.

SUMMARY AND CONCLUSIONS

1. *Streptococcus pyogenes* multiplies readily in sterilized milk stored at 22 and 18° C., and more slowly at 15° C. In fresh raw milk this organism begins to multiply slowly only after 48–72 hours of storage at 18–22° C. In laboratory pasteurized milk the result is similar, but both commercially pasteurized milk and raw graded milk bottled for distribution sour too rapidly for multiplication to take place after artificial contamination. These facts suggest that widespread epidemics are rarely due to extensive multiplication of *S. pyogenes* in milk during commercial or household storage.

2. The initial degree of contamination of milk with *S. pyogenes* has no influence upon this organism's ability to multiply during storage.

3. The failure of *S. pyogenes* to multiply during storage is due to (*a*) its natural reluctance to grow at atmospheric temperatures, (*b*) the bacteriostatic action of the milk, and (*c*) the readiness with which saprophytic bacteria multiply at atmospheric temperatures.

4. It is contended that infected cows play a major part in the spread of milk-borne *S. pyogenes* epidemics, and relevant literature is quoted to support this view. It is urged that routine investigation of apparent milk-borne epidemics should include the immediate bacteriological examination of an individual sample of milk from every cow concerned in the supply under suspicion.

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REFERENCES

BENSON, R. L. & SEARS, H. J. (1923). *J. Amer. Med. Ass.* **80**, 1608.
BROWN, J. H. & ORCUTT, M. L. (1920). *J. Exp. Med.* **31**, 49.
CAMPS, F. E. & WOOD, J. L. M. (1936). *Lancet*, ii, 756.
Chief Medical Officer of the Ministry of Health (1935). *Annual Report of the Chief Medical Officer*, p. 139.

CHRISTIANSEN, M. (1931). *Verh. internat. Milchwirtsch. Kongr.* Section 2, p. 107 (German edition).
DAVIS, D. J. (1914). *J. Inf. Dis.* **15**, 378.
DAVIS, D. J. & CAPPS, J. A. (1914). *Ibid.* **15**, 135.
FROST, W. D. & CARR, A. M. (1927). *Amer. J. Pub. Health*, **17**, 139.
GOLLEDGE, S. V. (1932). *Veterin. Rec.* **12**, 1499.
HANSSEN, F. S. (1924). *Brit. J. Exp. Path.* **5**, 271.
HESSE, W. (1894). *Z. Hyg. Infektskr.* **17**, 238.
JONES, F. S. (1928). *J. Exp. Med.* **47**, 965.
JONES, F. S. & LITTLE, R. B. (1927). *J. Exp. Med.* **45**, 319.
—— —— (1928). *J. Exp. Med.* **47**, 945.
KRUMWIEDE, C. & VALENTINE, E. (1915). *J. Med. Res.* **33**, 231.
MINETT, F. C. (1932). *Veterin. Rec.* **12**, 545.
—— (1935). *J. Path. and Bact.* **40**, 357.
MINETT, F. C. & STABLEFORTH, A. W. (1931). *J. Comp. Path. and Ther.* **44**, 114.
PULLINGER, E. J. (1935). *J. Dairy Res.* **6**, 369.
ROBINSON, E. S. & MCCOMB, J. A. (1932). *J. Inf. Dis.* **51**, 292.
ROSENAU, E. C. & HESSE, C. L. (1917). *J. Amer. Med. Ass.* **68**, 1305.
ROSENAU, E. C. & MCCOY, G. W. (1908). *Bull. Hyg. Lab. United States Pub. Health.* No. 41, 449.
ROSENAU, E. C. & MOON, V. H. (1915). *J. Infect. Dis.* **17**, 69.
SAVAGE, W. G. (1911). *Proc. Roy. Soc. Med.* **4** (Sect. Epidem.), p. 73.
—— (1931). *Ibid.* **24**, 1703.
SMILLIE, W. G. (1917). *J. Infect. Dis.* **20**, 45.
SMITH & BROWN (1915). *J. Med. Res.* **31**, 455.
WELCH, H. & MICKLE, F. L. (1933). *Amer. J. Hyg.* **17**, 229.
WILKINSON, E. (1931). *Brit. Med. J.* ii, 494.
WINSLOW, C. E. A. (1912). *J. Infect. Dis.* **10**, 73.

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