STREPTOCOCCI AND LEUCOCYTES IN MILK. I.

By WILLIAM G. SAVAGE, B.Sc. M.D. (Lond.),
Medical Officer of Health, Colchester.

THE undoubted fact that milk may act as a vehicle for the transmission of a number of diseases has directed considerable attention to the Bacteriological and Public Health aspects of milk. Yet it cannot be said, tuberculosis excepted, that the bacteriological examination of milk has afforded in the past much assistance in the prevention of disease, while only very occasionally has a subsequent examination served to elucidate the cause and origin of a milk-spread epidemic. A survey of the literature of the subject will show that although extensive work has been carried out upon the bacteriology of milk, the subject is so large and many-sided that what is known is but an insignificant proportion of what requires to be ascertained. A great deal of the work done has been in relation to tuberculosis. Almost all the milk examinations have been carried out with mixed milk samples, and not with quite fresh milk from individual cows.

At the present day, in my opinion, we are not in a position to frame satisfactory bacteriological standards for milk, and until more precise knowledge is acquired, it will not be possible for milk examination to take a place at all comparable to that which the bacteriological examination of water occupies.

The significance of streptococci in milk is of great practical importance, but published information is scanty and discrepant. Bergey[1], Conn[2], and other American bacteriologists have drawn attention to the prevalence of this class of organisms in American milk, and the first-

[1] Bergey, D. H. (April 1901), "The prevalence of streptococci in cow's milk," *American Medicine*, vol. I. p. 122.

[2] Conn, H. W. and Esten, W. M. (1904), "Qualitative analysis of Bacteria in market milk," *Rockefeller Institute Reprints*, vol. I.

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named showed their presence in milk drawn directly from the udder and collected into sterile tubes.

The significance of *pus* in milk is also of considerable importance. Authors usually dismiss it with the undoubtedly sound dictum that milk should not contain pus. This, however, leaves the question largely unanswered. That milk should not contain pus few will deny, but what constitutes pus in milk? All milk contains leucocytes. When does a leucocyte become a pus cell, and what distinguishes the one from the other? What number of leucocytes, or pus cells, constitute pus in milk? But few authors have defined what constitutes pus in milk, the only definitions with which I am acquainted being those of Bergey and Stokes and Wegefarth (cited below), both based upon the number of leucocytes in stained preparations.

More precise information is required, and with this object I have carried out a number of examinations, which will now be dealt with.

Methods of Investigation.

The majority of the samples were obtained from individual cows. They were all from cowsheds within the Colchester Borough and with the sanitary condition of which I was well acquainted. The samples were all collected at the afternoon milking, by the usual milker, and from cows in the sheds. The method of collection was throughout the same. The milk was milked into the pail in the ordinary way, at first, and from all four quarters, then after the ducts had been thoroughly washed out by the outflowing milk, samples were collected, a little milk being milked from each quarter direct into a sterile 2 oz. narrow-necked glass-stoppered bottle. I personally superintended the collection of all but four of the samples, removing and holding the stopper myself, and replacing after the sample was collected. No precautions were taken as to the cleanliness of milker or cow, but in every case the milk was only taken from udders which appeared clean to the naked eye.

All the samples were examined within two hours, the great majority within one hour, of collection.

As a rule no selection was exercised in picking out the cows to be examined. In several instances, however, cows recently calved and just readmitted to the herd for milking purposes were chosen; in one or two instances the cowkeeper was asked to select his finest and healthiest cows.

The udders of only a few of the cows were examined, but in all of

these they were apparently healthy and free from nodules. The cows were kept in 10 different cowsheds several of which were among the best in the Borough.

The mixed milk samples were from the sources indicated.

Those collected fresh at the farm were taken from the mixed milk of all the cows immediately after milking. The milk was well mixed and a small sample collected as above. Unlike the samples collected from separate cows these and all the mixed milk samples contained the fore milk and strippings as well as the middle milk.

The milk samples collected in shops were taken directly from the counter pan and poured into sterile glass-stoppered bottles; they were all examined within 1—1½ hours of collection at the shop, but the milk had usually been milked many hours previously.

The bacteriological examination included the following:—

Examination of centrifugalised deposit. 10 c.c. of the well-mixed milk were centrifugalised for 10 minutes in an electrically driven centrifugal machine running at about 1800 revolutions per minute. Part of the deposit was then spread out on a cover-glass, dried, fixed, and stained by methylene blue. As a rule it was not found necessary to dissolve out any fat present, with chloroform or ether.

Examination for streptococci. Different quantities of the milk (0·1, 1·0 and 10·0 c.c. usually) were added to glucose neutral-red broth tubes. After 24 hours' and also 48 hours' incubation at 37° C. the tubes were examined in hanging-drop preparation for streptococci chains. A positive result was recorded only when quite definite streptococci chains were detected, or in doubtful cases when stained preparations showed definite chains of cocci.

Owing to the large amount of milk in the 10 c.c. broth tube it was often difficult to see streptococci, so if none were detected in the 1 c.c. tube after 24 hours, 1 c.c. of the mixture of neutral-red broth and 10 c.c. of milk was transferred to a fresh neutral-red broth tube, which was then incubated and subsequently examined for streptococci. I believe the detection of definite chains in hanging-drop preparations is a more accurate method of estimating the numerical presence of streptococci, than direct plating on nutrient media. Streptococci do not always grow on agar plates, and are not easy to isolate.

B. coli were enumerated by adding different quantities of the milk to tubes of lactose sodium-taurocholate peptone and brushing the dilution containing the smallest quantity of milk which showed acid and gas, over plates containing Drigalski and Conradi's medium.

Method of leucocyte enumeration. A special method was employed. The presence of leucocytes and decision as to the presence of pus is, as far as I know, always determined by an examination of dried and stained films.

Thus Stokes and Wegefarth[1] state that in their method 10 c.c. of the milk was centrifugalised for $2\frac{1}{2}$ minutes in a Lentz centrifuge, the supernatant fluid poured off and the sediment spread evenly over an ordinary slide, then dried, ether treated and stained by methylene blue.

The preparations were all examined by means of a $\frac{1}{12}$ oil immersion lens, and the number of leucocytes in 10 microscopic fields were counted.

Bergey[2] has more recently adopted an almost identical method, the chief difference being that the sediment is spread on a cover-slip, while the period of centrifugalisation is not stated.

As a rule no definition is given as to what excess of leucocytes constitutes pus, each observer apparently having his own arbitrary standards. Bergey, however, counted the number of cells in fields of the $\frac{1}{12}$ immersion lens, and if they averaged more than 10 per field it was arbitrarily taken to indicate pus.

It is obvious that the results must vary with the size of the field of vision, and this will vary with the eyepiece used and the length of the microscope tube, as well as with the objective employed. In particular they will vary and vary enormously with the thickness of the film, and unless a very definite quantity of deposit, definitely proportionate to the whole, is taken, and unless spread with extreme uniformity, widely differing results are inevitable.

In my hands these methods have yielded most unsatisfactory results, the findings, in my opinion, being quite unreliable.

For this investigation therefore, a special method was adopted.

The ordinary Thoma-Zeiss blood-counting apparatus, in use in most laboratories, was employed. Direct counting of the leucocytes is impossible owing to the opacity caused by the large amount of fat.

One c.c. of the milk is put into a 20 c.c. centrifugal tube and freshly filtered Toisson's[3] solution is poured in, to almost fill the tube.

[1] Stokes and Wegefarth (1897), "The microscopic examination of milk," *Journ. of State Medicine*, vol. v. p. 439.

[2] Bergey, D. H. (1904), "Source and nature of bacteria in milk," *Pennsylvania Department of Agriculture Bulletin*, No. 125.

[3] This is the well-known indifferent solution used in blood enumerations. It does not injure the leucocytes but stains them enough to render them clearly visible. Its composition is methyl violet 0·025 grms., sodium chloride 1 grm., sodium sulphate 8 grms., glycerine 30 c.c., distilled water 160 c.c.

The two fluids are well mixed and then centrifugalised (in my machine at about 1800 revolutions per minute) for 10 minutes. The cream is then well broken up by a clean glass rod, to disentangle any leucocytes carried to the surface, and the mixture centrifugalised for a second 10 minutes. All the fluid is then removed except the last 1 c.c., great care being taken not to disturb the deposit.

In practice I aspirate off the fluid by means of a fine tube connected to an exhaust-pump. If it goes below the 1 c.c. mark, make up to 1 c.c. by careful addition of fresh Toisson solution.

Theoretically all the leucocytes present in the original 1 c.c. of milk are now present in the 1 c.c. of fluid, the object of the above manipulation being solely to get rid of the fat.

The following calculation is based upon such an assumption. The leucocytes are now thoroughly well mixed and distributed through the 1 c.c. A sufficient quantity is then placed on the ruled squares of the Thoma-Zeiss apparatus, the cover-glass put on, and the preparation examined exactly in the same way as for the enumeration of the red or white corpuscles in blood.

If they are very numerous the leucocytes can be counted on the squares, but nearly always the counting must be done by fields. To do this draw out the tube of the microscope until an exact number of squares spans a diameter of the field of vision. In my microscope this is always six with the tube out about an inch. Then count the leucocytes in a large number of different fields of vision, moving regularly from one field of vision to another. Never less than 20 fields should be counted. The number of leucocytes in all the fields counted, divided by the number of fields counted, will of course give the average number per field.

Let y = the average number of leucocytes per field of vision.

Let d = the number of squares which just spans the diameter.

Then the number of leucocytes per cubic mm. of milk

$$= \frac{56{,}000y}{11d^2}.$$

The method reads as though it were a troublesome one to carry out, but in practice it is extremely simple, and with a little experience four estimations can readily be done in 1 to 1½ hours, or not much longer than the dried film method.

d can be determined once for all by marking the draw tube. All that then has to be done is to count 20 fields of the preparation, and

divide the number obtained by 20; the resultant is y. Substitute d and y in the formula and an elementary calculation gives the result.

To give a simple illustration. The draw tube of the microscope is extended and it is found that six of the ruled squares exactly spans the diameter of the field. d therefore = 6.

A leucocyte-counting preparation is made and the leucocytes in 20 fields counted and found to be 48. The number per field of vision therefore is $\frac{48}{20} = 2{\cdot}4 = y$.

$$\therefore \text{ Number per cubic mm.} = \frac{56{,}000 \times 2{\cdot}4}{11 \times 6 \times 6} = 339.$$

A note on how the formula is obtained may be of interest.

d = the number of squares to span a diameter of the field of vision. Each square is $\frac{1}{20}$ mm.

Therefore the diameter of the field of vision = $\frac{d}{20}$ millimetre.

and the radius „ „ „ = $\frac{d}{40}$ „

The area of any circle is πr^2.

Therefore the area of the field of vision is πr^2.

$$\pi = \frac{22}{7} \text{ (near enough for practical purposes).}$$

The area of the field of vision in question is therefore $\frac{22}{7} \times \left(\frac{d}{40}\right)^2$.

The depth of fluid in the counting cell is $\frac{1}{10}$ millimetre.

Therefore the cubic capacity of the field of vision = $\frac{22}{7} \times \left(\frac{d}{40}\right)^2 \times \frac{1}{10}$ cubic millimetre.

y = the average number of leucocytes per field of vision,

i.e. in 1 field = y leucocytes.

$$\therefore \text{ in } \frac{22}{7} \times \left(\frac{d}{40}\right)^2 \times \frac{1}{10} \text{ cubic millimetre} = y \text{ leucocytes.}$$

Therefore in 1 cubic millimetre = $y \times \frac{7}{22} \times \left(\frac{40}{d}\right)^2 \times 10$ leucocytes

$$= \frac{56{,}000\, y}{11 d^2}.$$

In 1 cubic millimetre of the fluid = $\frac{56{,}000\, y}{11 d^2}$ leucocytes.

This formula is one which I have used for the last seven years for the estimation of leucocytes in blood and was described by me in the *Lancet*, Sept. 27, 1902, p. 866. For blood as ordinarily diluted 100-fold the formula would of course read

the number of leucocytes in 1 cubic millimetre of undiluted blood = $\frac{5{,}600{,}000\, y}{11 d^2}$.

This method is obviously not absolutely accurate. In the first place the assumption that all the leucocytes in the original 1 c.c. of milk are in the 1 c.c. of prepared fluid, can easily be shown to be inaccurate by removing the supernatant fluid to a fresh tube and estimating the number of leucocytes in it. I have done this several times and always found the proportion less than 12 per cent. of the whole. It is probably a fairly constant proportion and does not affect the comparative accuracy of the results.

There is also a certain amount of manipulative error inherent to all such enumeration methods, and in some cases the tendency to cohesion of the leucocytes is apt to introduce error. I have made a large number of comparative tests, examining different samples of 1 c.c. of the same milk, and have found that with care there is rarely more than an error of 20 per cent. between the different results.

With such a difficult matter as the estimation of leucocytes in milk, I do not think such an error vitiates the method greatly, since precise figures are not required but only roughly comparative data.

Streptococci in milk.

The results of the examination of samples from individual cows are shown in Table I (p. 130), and from mixed milk samples in Table II.

In the individual cows streptococci were very prevalent, being present in nearly half of them, when 1 c.c. of the milk was examined. Analysed more in detail we obtain the following figures:—

Streptococci present in	0·1	c.c. in	6	specimens, *i.e.*	15	per cent.
„	„	1·0	„	17	„	„	42·5	„
„	„	10·0	„	22	„	„	55	„
„	absent in	11	„	18	„	„	45	„

In the mixed milk samples streptococci are still more prevalent. In the 11 samples collected fresh at the farm and examined within three hours, so that it can be assumed that no multiplication of streptococci had taken place, streptococci were found in all. More in detail:—

Streptococci present

in 0·1	c.c. in	8	specimens (out of 10 examined),	*i.e.*	80	per cent.
in 1·0	c.c. in	11	„ „ „	*i.e.*	100	„

TABLE I. *Samples from individual cows.*

[No streptococci found on microscopic examination of the centrifugalised deposit.]

Cow	Cowkeeper	Number of leucocytes per cubic mm.	Streptococci in 0·1	Streptococci in 1	Streptococci in 10 c.c.	*B. coli* in 1	*B. coli* in 10 c.c.	Date since last calving
No. 1	A	1270	–	–	–	–	–	
2	,,	1800	–	+	+	–	–	
3	,,	47	–	–	–	–	–	
4	,,	65	–	+	–	–	–	
5	,,	570	–	+	+	–	–	
6	,,	1220	–	–	–	+	+	
7	,,	1020	–	–	–	+	+	
8	,,	770	–	–	–	+	+	
9	B	50	–	–	–	–	–	
10	,,	3580	–	–	+	–	–	3 weeks
11	,,	170	–	–	–	–	–	4 months
12	,,	340	–	–	–	–	–	4 ,,
13	C	620	–	–	–	–	–	6 ,,
14	,,	470	–	–	+	–	–	6 ,,
15	,,	4380	–	+	+	–	–	3 ,,
16	D	2240	–	+	+	–	–	3 ,,
17	,,	420	–	–	–	–	–	6 ,,
18	E	240	+	+	+	+	+	1 week
19	,,	1850	+	+	+	–	–	3 weeks
20	F	64	–	–	–	–	–	2 months
21	,,	113	–	–	–	–	+	3 ,,
22	D	410	+	+	+	–	–	
23	,,	2090	+	+	+	–	–	
24	,,	170	+	+	+	–	–	
25	G	660	–	+	+	+	+	6 months
26	,,	120	–	+	+	–	–	10 days
27	,,	1190	–	–	+	–	–	
28	,,	35	+	+	+	–	–	6 days
29	H	130	–	+	+	–	+	5 months
30	,,	70	–	+	+	–	–	5 ,,
31	,,	100	–	+	+	–	–	4 ,,
32	,,	1400	–	–	–	–	–	8 ,,
33	J	184	–	–	+	–	–	5 ,,
34	,,	330	–	+	+	–	–	
35	,,	85	–	–	–	–	–	
36	,,	690	–	–	+	–	–	4 months
37	K	270	–	–	–	–	–	15 days
38	,,	1700	–	–	–	–	–	6 months
39	,,	240	–	–	–	–	–	6 ,,
40	,,	1890	–	–	–	–	–	7 ,,

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TABLE II. *Mixed milk samples.*

Number	Number of cows	Source	Time between examination and milking	Number of leucocytes per cubic mm.	Microscopic examination of centrifugalised deposit	Streptococci 0·01	Streptococci 0·1	Streptococci 1	Streptococci 10 c.c.	*B. coli* 0·1	*B. coli* 1	*B. coli* 10 c.c
1	6	Fresh at farm	Under 2 hrs.	—	No strepto. chains. Very few organisms	+	+	+	+	−	+	+
2	14	,, ,,	1 hr.	—	No strepto. chains. No organisms.			+	+	−	−	−
3	18	,, ,,	¾ hr.	—	No strepto. chains		+	+	+	+	+	+
4	—	Private house. Afternoon milk	Unknown	—	Streptococci chains	+	+	+	+	+	+	+
5	—	,, Morning ,,	,,	—	,, ,,	−	+	+	+	+	+	+
6	20	Fresh at farm	1½ hrs.	—	No strepto. chains	+	+	+	+	−	−	
7	25	,, ,,	3 ,,	—	,, ,,		+	+	+	−	−	
8	7	,, ,,	8 ,,	—	,, ,, Diplococci		+	+	+	−	−	
9	18	,, ,,	8 ,,	—	No strepto. chains		+	+	+	−	+	
10	13	,, ,,	4—5 ,,	—	,, ,,	+	+	+	+	−	+	
11	17	,, ,,	4—5 ,,	—	,, ,,	−	+	+	+			
12	15	,, ,,	1½ ,,	1030	,, ,, No organisms	−	−	+	+	−	−	−
13	15	,, ,,	1 hr.	590	,, ,, ,,	−	−	+	+	−	−	−
14	17	,, ,,	1 ,,	830	,, ,, ,,		+	+	+	−	−	−
15	8	,, ,,	¾ ,,	630	No strepto. chains. Diplococci and numerous bacilli		+	+	+	−	−	−
16	13	,, ,,	1 ,,	330	Strepto. chains and diplococci	+	+	+	+	−	+	+
17	2	,, ,,	¾ ,,	1980	No strepto. chains. Some diplococci	+	+	+	+	+	+	+
18	—	Private house. Afternoon milk	8 hrs.	300	,, ,, ,,		+	+	+	−	+	+
19	8	Small retail shop	about 8 hrs.	250	Numerous strepto. chains and other organisms	+	+	+	+	+	+	+
20	—	,, ,,	,, 8 ,,	230	Numerous organisms. No chains	+	+	+	+	+	+	+
21	—	,, ,,	,, 8 ,,	360	Numerous strepto. chains and other organisms	+	+	+	+	+	+	+
22	—	,, ,,	,, 5 ,,	810	,, ,, ,, ,,		+	+	+	+	+	+
23	—	,, ,,	,, 6 ,,	21	,, ,, ,, ,,	+	+	+	+	+	+	+
24	—	,, ,,	,, 6 ,,	170	Diplococci and bacilli. No chains	−	−	+	+	−	+	+
25	—	,, ,,	,, 6 ,,	28	,, ,, ,,	−	+	+	+	−	−	+
26	—	,, ,,	,, 5 ,,	380	Some bacilli. No chains		+	+	+	+	+	+
27	—	,, ,,	,, 6 ,,	64	,, ,, ,,		−	+	+	+	+	+
28	—	Clean Dairy	,, 6 ,,	280	No streptococci. Numerous organisms		+	+	+	+	+	+

In the two instances in which they were not found in 0·1 c.c. the milk was from the same cowkeeper, one sample being collected three days after the other.

In the 17 samples collected from shops or at the farm, but in which there was a delay in examination, some multiplication possibly took place. The tables yield the following results:—

Streptococci present in 0·1 c.c. in 15 specimens, *i.e.* 88 per cent.
„ „ 1·0 „ 17 „ *i.e.* 100 „

Looking at these results comprehensively they show a striking prevalence of streptococci. In the whole 68 samples, streptococci were present in 45, or 66 per cent. when 1 c.c. of the milk was examined.

What is the source of these streptococci? All the cows from which they were obtained were as far as could be ascertained perfectly healthy, and many of them were among the best cows, and from the best kept cowsheds in the Borough. Some of them show a high leucocyte count, others a low one. The streptococci can only come from the interior of the udders or from unclean manipulation. If from the latter they must come from the milkers' hands, the teats of the cow, or from the entrance of dust during milking. The latter can practically be excluded since the bottles were quite narrow mouthed (internal diameter = $\frac{1}{2}$ inch) and were not opened until immediately before the milking. Almost invariably the bottle did not touch the teats, the milk being squeezed out in jets into the bottle. Undoubtedly since no precautions were taken to wash the udders or hands some organisms would sometimes gain access to the milk, but this is hardly likely to be the main cause for their presence. That manipulative dirt is not the cause is I think conclusively shown by the *B. coli* results. From these 40 cows in only seven were *B. coli* present in as much as 11 c.c. of the milk, and three of these were from one cowkeeper and out of four samples collected that afternoon. It is significant that in all these three samples and in one other sample streptococci were not present.

I regard the presence of *B. coli* as certain evidence of contamination during collection, and the fact that in only seven out of the 40 samples was it present shows that most of the samples were uncontaminated fluid from the udder.

In the same way *B. coli* are much less prevalent than streptococci in the mixed milk samples freshly collected at the farm.

These results show clearly that streptococci as a class are very

prevalent in pure cows' milk. Are they identical and do they possess characters which readily differentiate them from the streptococci of disease, particularly from the streptococci of the different infective mastitis lesions?

The differentiation of streptococci has until quite recently been attempted mainly on the basis of variations in morphology, growth in broth, litmus milk and gelatine and pathogenicity, while within the last few years agglutination characteristics have been employed. Such characters have afforded very little solid grounds for differential distinctions.

Gordon's[1] valuable work on the action of streptococci obtained from different sources upon the various sugars, alcohols, etc., has opened up another method of differentiation which may be of great importance. A number of the streptococci present were isolated and their characters fully worked out, but for only some of them were the tests selected by Gordon employed, and only the characters of these are set out in Table III (p. 134).

It was hoped that these organisms from healthy milk might have certain characters in common which would enable them to be readily identified and separated from the streptococci obtained from other sources in nature and in particular which would enable the streptococci of the different kinds of infective mastitis to be with certainty differentiated.

The differential tests selected for routine work by Gordon are clotting of litmus milk within three days at 37° C., the reduction of neutral-red broth during incubation anaerobically for two days at 37° C., the acid fermentation of saccharose, raffinose, inulin, salicin, coniferin, and mannite.

All these tests were employed with the exception of the anaerobic reduction of neutral-red, while in addition the fermentative action on maltose for all, and glucose and amygdalin for some, was determined.

Only 21 organisms were examined in this complete way. They do not show the prevalence of any one type, but formed no less than 12 groups, nine groups consisting of one organism each, the remaining 12 falling into three groups of six, four, and two organisms in each respectively. The most frequently present group (six organisms) behaved similarly as follows:—they clotted milk; they did not ferment saccharose, raffinose,

[1] M. H. Gordon (1903-04), "Characters by which Streptococci and Staphylococci may be differentiated and identified," *Report of Medical Officer Local Government Board*, p. 388.

TABLE III. *Characters of streptococci isolated.*

Number	Source	Growth in broth as regards clearness or turbidity	Morphology in 2 days' old broth cultures	Liquefaction of gelatine	Litmus milk: Acid	Litmus milk: Clot	Acid fermentation of: Glucose	Saccharose	Lactose	Raffinose	Inulin	Salicin	Coniferin	Mannite	Amygdalin	Maltose
1	Mixed milk No. 20	Uniform turbidity	Long chains	–	+	+	+	–	+	–	–	+	–	–	–	+
2	,, ,, 19	,, ,,	,,	–	+	+	+	–	+	–	–	+	–	–	–	+
3	,, ,, 16	Clear	,,	–	+	–	+	+	+	+	–	+	–	+	–	+
4	,, ,, 16	Uniform turbidity	Short and medium chains	–	+	–	+	+	+	–	–	+	–	–		+
5	Cow No. 18	,, ,,	Short chains	–	+	–	+	+	+	+	+	+	+	+	–	+
6	,, 19	Clear	Long ,,	–	+	+	+	–	+	–	–	+	–	–	–	+
7	,, 14	Uniform turbidity	Medium ,,	–	+	+	+	+	+	+	+	+	+	+	+	+
8	,, 14	Nearly clear	Long ,,	–	+	+	+	–	+	–	–	+	+	+	+	+
9	Mixed milk No. 14	,, ,,	Short ,,	–	+	+	+	+	+	+	–	+	+	+	+	+
10	Cow No. 16	Uniform turbidity	Medium and long chains	–	+	+	+	+	+	–	–	+	–	+	+	+
11	Mixed milk No. 15	,, ,,	Medium chains	–	+	+	+	+	+	+	–	+	–	+	–	+
12	Cow No. 22	Clear	Long ,,	–	+	+	+	–	+	–	–	–	–	–	–	+
13	,, 23	,,	Long ,,	–	+	+		–	+	–	–	+	–	–		+
14	,, 25	Uniform turbidity	Short ,,	–	+	–		+	+	–	–	+	–	+		+
15	,, 27	,, ,,	,, ,,	–	+	+		+	+	–	–	+	–	–		+
16	,, 31	,, ,,	,, ,,	–	+	+		+	+	–	–	+	–	–		+
17	,, 29	,, ,,	,, ,,	–	+	+		+	+	–	–	+	–	–		+
18	,, 33	,, ,,	Short and medium chains	–	+	+		+	+	–	–	+	–	–		+
19	,, 34	Turbid	Short chains	–	+	+		+	+	–	–	+	–	+		+
20	Mixed milk No. 23	Uniform turbidity	Medium ,,	–	+	+	+	–	+	–	–	+	–	–		+
21	,, ,, 12	,, ,,	Mostly short chains	–	+	+		–	+	–	+	+	+	+		+

inulin, coniferin, and mannite; they fermented glucose, lactose, salicin, and maltose.

The number of organisms isolated is quite too small for reliable deductions, but it is of interest to notice that their characters much more closely approximate to those of the streptococci most common in human faeces (Houston), than to those of the streptococci more frequently found in saliva (Gordon).

In particular they agree with Houston's streptococci in that they all (with one exception, possibly due to accidental failure to grow) ferment salicin while the great majority of streptococci from saliva, isolated by Gordon, do not.

If future work confirms this provisional deduction that all streptococci from cows' milk ferment salicin, then the finding of streptococci with this character in the throats of persons suffering from a milk-carried streptococcal outbreak may become valuable evidence as showing that these streptococci were of bovine origin and causally connected with the outbreak.

I have not up to the present been able to investigate the characters of streptococci from cases of mastitis, owing to lack of material, but such an investigation should be of particular value, and I shall be greatly indebted to anyone sending me some of the udder secretion of cows suffering from mastitis.

The pathogenicity of the streptococci described in Table III was not investigated, but several of the other streptococci isolated were tested upon mice by subcutaneous inoculation with uniformly negative results.

The presence of leucocytes in milk and their relation to streptococci is of considerable interest. The results of the leucocyte estimations is shown in Tables I and II.

In the first place these tables show that every specimen of milk contained leucocytes, but as regards the numbers present very great variations were met with. The number present in the samples from individual cows varied from 35 to 4380, those from mixed milk samples from 21 to 1980 per cubic mm. On the whole however the mixed milk samples showed, as might be expected, much greater uniformity than the samples from individual cows.

The date since the last calving is given in a number of instances, but there appears to be no relation between this time interval and the number of leucocytes.

The age of the cow, and, if pregnant, the period of pregnancy, are

probably more important influencing factors, but I have not definite data available for an accurate judgment.

Bergey (1904, *loc. cit.*) traced a connection between pus cells and the number of streptococci, finding (p. 25) that "the majority of the samples containing large numbers of streptococci also contained pus cells, that is more than ten cells per field." Such a relationship might have been anticipated, but my results show absolutely no such connection.

To illustrate this point Table IV was compiled from Table I. In this table the samples are arbitrarily divided into three groups according to the number of leucocytes, and the streptococci prevalence for each group is considered.

TABLE IV. *Samples from individual cows.*

Number of leucocytes per cub. mm.	Number of cows	Number of samples				Percentage			
		Containing streptococci in			Free from strepto-cocci	Containing streptococci in			Free from strepto-cocci
		0·1	1·0	10 c.c.		0·1	1·0	10 c.c.	
Less than 400	19	3	9	10	9	16	47	53	47
400—1000	8	1	3	5	3	13	38	62	38
More than 1000	13	2	5	7	6	15	38	54	46

The absence of any relationship between leucocytes and streptococci is clearly brought out by this table. In the 19 samples with leucocytes less than 400 per cubic mm. the percentages are practically identical with those in which the number of leucocytes is greater than 1000 per cubic mm., that is both as regards the actual presence of streptococci and as regards their numerical prevalence.

That these leucocytes are not all identical was readily apparent during the enumeration, but I have not at present made any differential estimations. I cannot differentiate between a leucocyte and a pus cell, and I am not prepared at this stage to lay down an arbitrary standard as to what number of leucocytes per cub. mm. is to be designated *pus* in the milk.

On the other hand the number of leucocytes, or pus cells, present in the case of milk from inflamed udders would be much higher than that given by any of the presumably healthy cows examined. The udder secretion of the cow which caused the extensive outbreak of sore throat in Colchester[1], April 1905, was practically thin pus, and streptococci were readily isolated from one loopful of the fluid. The number of

[1] Savage, W. G. (October 1905), "Outbreak of Sore throat at Colchester due to infected milk," *Public Health*, vol. XVIII. p. 1.

leucocytes was not then estimated, but an estimation was made exactly one month later, when the milk from this quarter was thin looking and had a yellowish tinge but otherwise was like milk. The number of leucocytes per cubic mm. was then 10,100. This quarter of the udder still showed a small indurated area.

An estimation made nearly two months after the initial attack showed 12,200 leucocytes per cubic mm. although the milk appeared fairly normal to the naked eye. There is therefore a wide difference between this milk from the inflamed quarter, clearly shown months after the occurrence of the mastitis, and any of the figures obtained with healthy milk. The milk from the same cow, but in this case from all four quarters, was examined Nov. 14th, 1905, or about seven months after the attack of infective mastitis, and the number of leucocytes was then 1190 (Cow 27, Table I).

All the results hitherto recorded were obtained with middle milk samples from individual cows. In all cases the fore milk and strippings were rejected. For this reason I examined the milk from four additional cows, from which three samples were collected in each instance, as follows: (*a*) the first milk milked, (*b*) milk obtained about half-way through the milking, (*c*) strippings only.

The results are shown in Table V.

TABLE V.

		Cow 41	Cow 42	Cow 43	Cow 44
Number of Leucocytes.	Fore milk	5345	3310	580	420
,, ,,	Mid ,,	3420	1840	470	300
,, ,,	Strippings	5020	3680	1590	410
Number of Streptococci.	Fore milk	Absent in 11 c.c.	Absent in 11 c.c.	Absent in 11 c.c.	Absent in 11 c.c.
,, ,,	Mid ,,	,,	,,	,,	,,
,, ,,	Strippings	,,	Present in 1 c.c.	,,	,,
Number of *B. coli*.	Fore milk	,,	Absent in 11 c.c.	,,	,,
,, ,,	Mid ,,	,,	,,	,,	,,
,, ,,	Strippings	,,	,,	,,	,,

In every case the middle milk contained less leucocytes than the fore milk or strippings.

I have not found the results of the examination of the centrifugalised deposit to be of much value. The prevalence of leucocytes in the deposit was only roughly, and by no means uniformly, parallel with the leucocyte estimations. In none of the fresh milk samples were actual

streptococci chains detected, although diplococci were occasionally met with. In 25 per cent. of the mixed milk samples definite chains were detected.

Numerical cultural investigation of streptococci in milk is far preferable to simple examination of centrifugalised deposits.

With regard to the presence of *B. coli*, as already mentioned, I consider this organism as always derived from outside the udder and a definite indicator of contamination during milk collection or storage. In the milk samples from individual cows *B. coli* were found in 17·5 p.c. of the samples. In the 11 mixed milk samples collected fresh at the farm and examined within three hours, *B. coli* were present in 36 per cent., and in the 16 shop samples and mixed milk samples not examined at once, they were present in 94 per cent. of the samples. The *B. coli* found were isolated in every case and their characters worked out.