Relationships of Leucocytes and Streptococci to Fibrosis of the Udder*

G. J. HUCKER, PH.D.

New York State Agricultural Experiment Station, Geneva, N. Y.

THE significance of the presence of long-chained streptococci and cells in milk has long been a problem for milk control officials who are interested in detecting udder infections. The problem has received considerable attention by various investigators who have studied the flora of the so-called " normal " udder in an attempt to determine definitely the types of streptococci harbored in udders that are apparently normal to the casual observer. In many instances also the cell content of the milk has been studied in an effort to learn whether milk from udders which have been considered normal in most respects contain an appreciable number of cells. In such investigations milk has been selected which was normal in appearance and from cows which to all appearances were normal.

It has been pointed out by Udall and Johnson (1930) that cows may give milk that is regarded as normal although the udder may show signs of infection in the form of indurated tissue. This indurated or fibrotic tissue which is thought to be a result of the breaking down of the glandular structure and its replacement by white fibrous tissue, imparts hardened areas to the udder which may be detected by manual examination. It has been assumed that individuals showing this scar tissue have at some time been infected, and that these indurated or fibrotic conditions found upon physical examination are the result of this infection. Certain investigators believe that once a quarter has been infected and these indurated areas developed, the quarter will never become entirely free of the infection. Under such conditions the infection is liable to re-occur at certain intervals. Other workers have preferred to believe that this scar tissue is only the result of an infection. Under such a condition it would be possible for an infection to run its course and the udder become entirely free from the invading organism and its effects, viz., leucocytes, the scar tissue remaining as an inert evidence of the breaking down of the secreting tissue. If this view is correct, the presence of scar tissue would have little significance as a means of detecting cows in a herd that may harbor infection and serve as foci of infection of the entire herd. Under such conditions scar tissue must be interpreted as meaning that the cow either is or has been infected.

On the other hand, if it is found that once a cow is infected the infecting organism always remains in the udder awaiting the proper condition for a subsequent attack upon the tissues, the presence of scar tissue becomes an important criterion for detecting cows with chronic mastitis. The question resolves itself into a study of the significance of the indurations. Does a

* Read before the Laboratory Section of the American Public Health Association at the Sixty-first Annual Meeting in Washington, D. C., October 25, 1932.

fibrotic condition indicate a circumscribed lesion or rather a mass of inert white fibrous tissue which may contain no evidence of an infecting organism?

It has been pointed out by Hucker, Trudell and Jennings (1932) that all studies pertaining to udder flora and the constituents of milk should be carried out by using milk known to come from cows which have been subjected to a careful physical examination. If the results are to be considered as studies of milk from "normal" udders or udders known to be free from previous infections, these normal udders should be found to be free from fibrotic tissue.

The purpose of the present investigation has been to learn the relationship between the presence of cells and streptococci in milk and the presence of indurations or scar tissue in the udder.*

METHODS

Regular weekly quarter samples of milk from approximately 150 cows have been examined by the methods outlined by Hucker, Trudell and Jennings (1932) over a period of 10 months. The udders from which these samples have been secured have been examined at irregular intervals for the presence of white fibrous tissue, by the methods proposed by Udall and Johnson (1930). Such a routine not only gave information regarding the flora and leucocyte content of the milk but the pathological condition of the udders.

DEFINITION OF TERMS

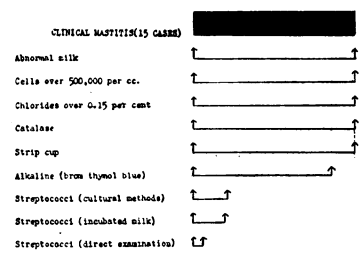
As pointed out by Hucker, Trudell and Jennings (1932) two types of mastitis are recognized based upon external evidences, viz., clinical and sub-clinical. The clinical type, referred to by many as active or acute, is relatively rare and involves an extreme toxic condition with occasional systemic disturbances. In sub-clinical (latent, chronic) type the individual may appear normal in most respects but the milk reacts in varying degrees to laboratory tests for mastitis and demonstrable scar tissue appears in the udder. Although the sub-clinical type may become more acute at certain intervals the condition never assumes the extreme conditions noted in clinical mastitis. In aggravated cases of sub-clinical types the milk may become broken down with little evidence of toxemia in the individual.

DATA

THE EFFICIENCY OF THE VARIOUS LABORATORY TESTS IN DETECTING CLINICAL MASTITIS

Clinical Mastitis—The condition of the individual suffering from acute mastitis is so obvious that no tests are required for its detection. If milk can be obtained from the affected quarter it is (Figure I) always broken down with either a watery or serous appearance. The reaction is pH 6.9 to 7.4 and the catalase content is high while the amount of chlorides is always above normal.

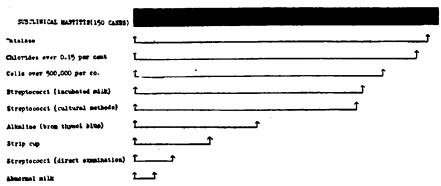
FIGURE I—EFFICIENCY OF VARIOUS TESTS UPON MILK TO DETECT MASTITIS



It is only with difficulty that organisms can be isolated from the secretion of the infected quarter. Direct

* It is realized that voluminous literature exists dealing with the relationship of leucocytes and streptococci in the udder and their significance in udder infections. A complete bibliographic review is being prepared and will subsequently appear. For a complete published discussion of this subject reference should be made to Breed (1914).

FIGURE II—EFFICIENCY OF VARIOUS TESTS UPON MILK TO DETECT MASTITIS



microscopic examination, even of samples previously incubated, rarely reveals long chained streptococci, while cultural methods add little more possibility of isolating organisms.

THE EFFICIENCY OF CERTAIN LABORATORY TESTS IN DETECTING SUB-CLINICAL MASTITIS

The relation of fibrous tissue in the udder to the presence of cells in the milk—The significance of cells in milk has received considerable attention. Breed, after a detailed study of a series of cows, concluded that there were several unknown factors that may influence the number of cells in milk and that care should be taken in interpreting the significance on their presence in milk.

In the present investigation (Table II, Figure III) it has been found that over a period of 10 months no quarter that was free of indurations or fibrotic tissue discharged at any one time more than 150,000 cells per c.c. On the other hand, even this number were found in only one isolated instance. Ninety-nine per cent of the normal quarters, *i.e.*, quarters free from scar tissue, showed less than 60,000 cells per c.c., while a great majority, viz., 87 per cent, never gave a higher count than 30,000 cells per c.c.

On the other hand, 63 per cent of the quarters showing fibrotic tissue gave milk showing more than 150,000 cells per c.c. It is also interesting to note that 73 per cent of the quarters with a marked fibrosis showed over 500,000 cells per c.c. in the milk.

Hucker, Trudell and Jennings (1932) have pointed out that all quarters showing a cell count greater than 3,000,000 per c.c. give definite evidence of infection. From the results of the present investigation it is safe to conclude that all quarters showing cell counts of more than 150,000 per c.c. should be considered as suspicious, if not definitely proved to be infected with streptococci.

It was also found that not all quarters

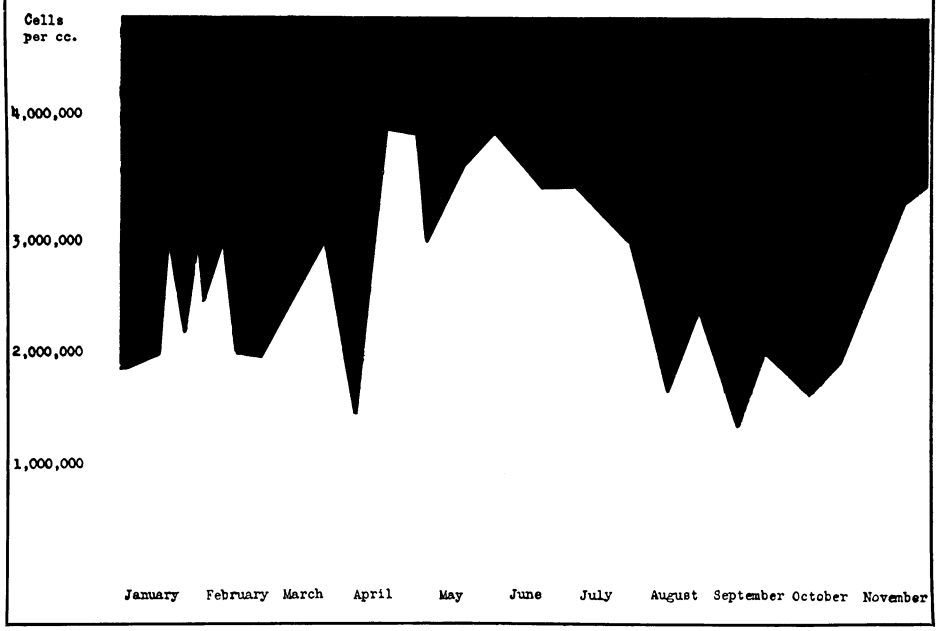
TABLE I

RELATION OF NUMBER OF CELLS PER C.C. IN MILK TO THE PRESENCE OF FIBROSIS IN THE UDDER

Amount of fibrosis	Number of quarters studied	Number of tests on each quarter	Per cent showing cells per c.c.						
			Less than 30,000	30,000 to 60,000	60,000 to 150,000	150,000 to 300,000	300,000 to 1,000,000	1,000,000 to 5,000,000	More than 5,000,000
Absent	63	20	87	12	1	0	0	0	0
Slight	68	20	60	3	7	9	7	9	5
Distinct	109	20	25	5	7	11	19	25	8
Marked	24	20	22	5	0	0	18	34	21

Total tests = 5,080.

FIGURE III—MINIMUM NUMBER OF CELLS PER CUBIC CENTIMETER IN MILK FROM 124 "HIGH CELL COUNT" QUARTERS



which show white fibrous tissue discharged an appreciable number of cells. About 25 per cent of the quarters classified as showing distinct or marked fibrosis failed in any instance out of a series of examinations extending over 10 months to show cells in a greater number than 30,000 per c.c. It seems evident that there are two distinct groups of fibrotic quarters, one which never throws off cells and one which constantly, over a long period, will give milk in which the cell count is relatively high. At present it is safe to conclude that cell counts in excess of 150,000 per c.c. are always from quarters which show some degree of fibrosis. However, not all indurated quarters give excessive cell counts.

It has been further observed (Figure III) that once a quarter begins to throw off a large number of cells in the milk the numbers of leucocytes remains relatively large for months or even longer. Over a period of 10 months of observations on so-called "high count" quarters the minimum number of leucocytes never dropped below 1,000,000 per c.c. In other words, once a large number of leucocytes appear in the milk from any quarter, they rarely diminish.

The same observations were true regarding the milk containing a small number of leucocytes. The maximum (Figure IV) number found in the milk from quarters evidencing no scar tissue when studied over a period of 10 months, never exceeded 200,000 per c.c. The individuals remained over the entire test period as "low cell count" cows.

A certain amount of variation (Figure V) between the leucocyte content of the milk from the 4 quarters of the same individual was noted. One of the most interesting observations in this connection was the fact that a greater variation could be found in the milk from quarters showing an excessive number of leucocytes than milk containing a minimum number.

TABLE II

RELATION BETWEEN H-ION CONCENTRATION OF THE MILK AND THE PRESENCE OF FIBROSIS IN THE UDDER

Amount of fibrosis	Number of quarters studied	Number of tests on each quarter	Per cent showing reaction with Brom thymol blue							
			Light green		Blue green		Blue			
			pH 6.6	pH 6.7	pH 6.8	pH 6.9	pH 7.0	pH 7.1	pH 7.2	pH 7.4
Absent	60	20	95	5	0	0	0	0	0	0
Slight	70	20	81	11	1	3	0	0	0	4
Distinct	85	20	80	3	7	6	0	0	0	4
Marked	22	20	44	5	6	4	9	13	8	11

RELATION OF FIBROTIC TISSUE TO THE BROM THYMOL BLUE TEST

For some time the brom thymol blue test has been used in detecting cows that are infected and that should be classed as having mastitis. Its widespread use has raised many questions of interpretation of the results secured.

In ordinary use the results secured by this test have been classed in three groups, viz., light green, which includes the cows giving normal milk, blue green or the suspicious cows, and dark green which always signifies abnormal conditions. Certain workers have endeavored to make finer distinctions and interpret the results as H-ion readings. The latter although applicable in the laboratory is not recommended for practical barn use.

The results of the present investigations (Table II) with the brom thymol

FIGURE IV—MAXIMUM NUMBER OF CELLS PER CUBIC CENTIMETER IN MILK FROM 112 "NORMAL" QUARTERS

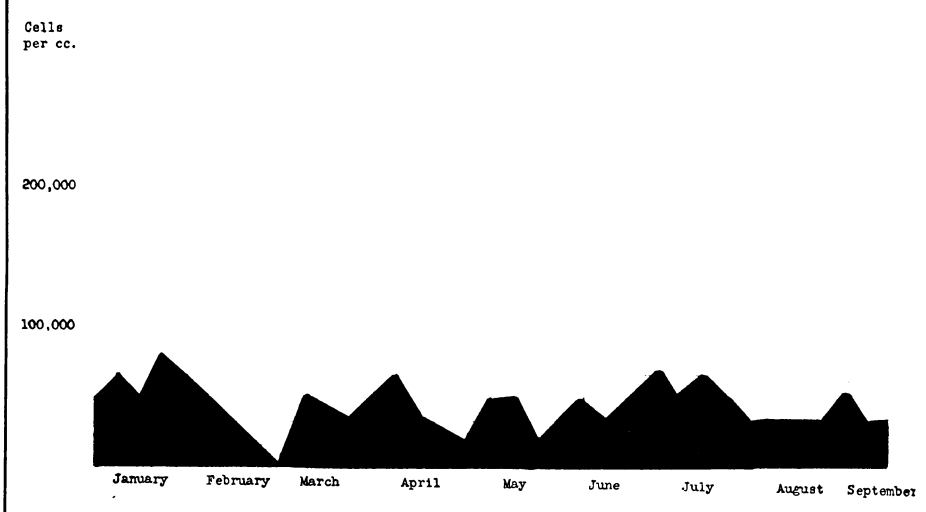
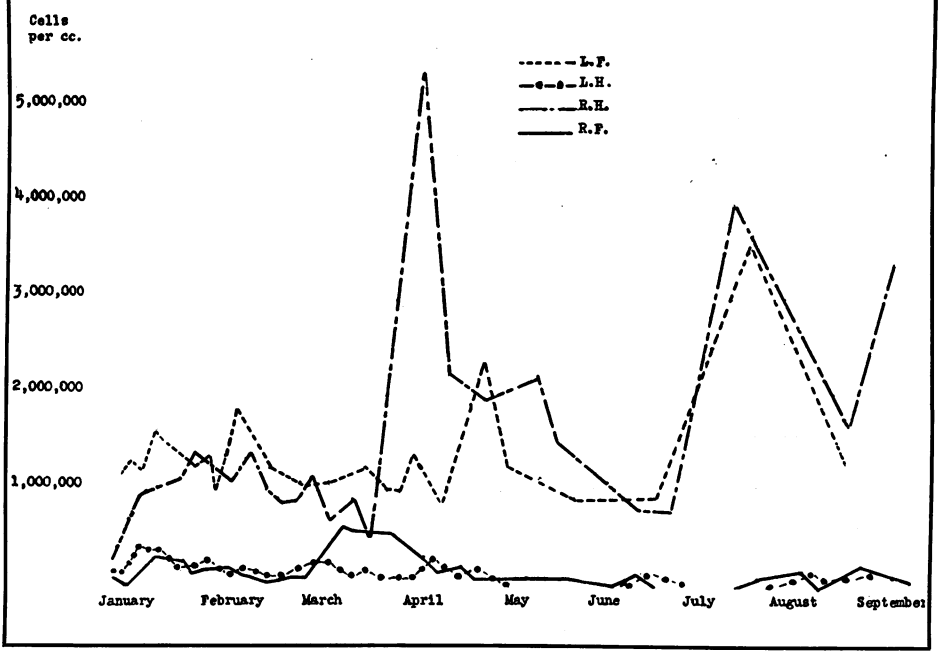


FIGURE V—VARIATION WEEKLY IN NUMBER OF CELLS IN MILK FROM 4 QUARTERS OF THE SAME INDIVIDUAL



blue test are very similar to those secured from a study of the cells and streptococci present. In no instance during the 10-month study did a quarter free of indurated tissue give a positive reaction to brom thymol blue, though not all of the indurated quarters gave positive reactions. It is concluded that a positive reaction by this test always signifies indurated tissue in the udder while fibrosis free quarters never show it.

RELATION OF FIBROTIC TISSUE IN THE UDDER TO THE PRESENCE OF STREPTOCOCCI IN MILK

A study of 264 quarters which extended over 10 months showed that only approximately 11 per cent were free from indurations or fibrotic tissue. The remaining 90 per cent showed affected tissue to some extent. The most interesting observation (Table III) in this connection is the fact that no streptococci were found during this

TABLE III

RELATION OF FIBROSIS IN THE UDDER TO THE PRESENCE OF STREPTOCOCCI IN THE MILK

Amount of fibrosis	Number of quarters studied	Per cent showing streptococci
Absent	30	0
Slight	76	5
Distinct	100	24
Marked	58	10

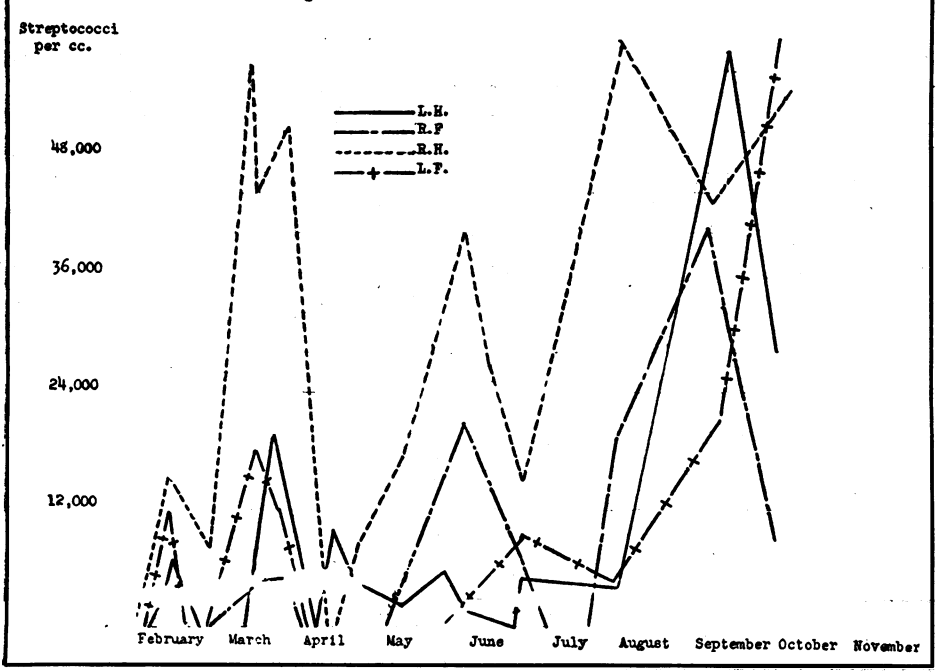
period in the milk from the quarters that were free of fibrotic tissue. The 30 quarters which by physical examination were found to remain pliable with no hardened areas or indurations were found to remain free of streptococci. From these results it appears probable that where previous investigators found streptococci in the freshly drawn milk an examination of the udder would have shown the presence of fibrotic tissue.

These observations raise the question of the nature of the so-called "normal" flora of the udder. Some workers have assumed that a certain number of streptococci are normal inhabitants of the udder. The above data, however, indicate that such is not the case if normal udders are those that are free from fibrotic tissue. On the other hand, the conclusion should not be drawn that all udders that show indurated tissues will throw off streptococci.

Of the 234 quarters that showed indurations on physical examination, only 39 per cent were found to discharge demonstrable streptococci in the milk. It is also of interest to note that, of the quarters which discharged streptococci, the milk from the majority contained streptococci in all samples, indicating that once streptococci are thrown off, the presence of these organisms is more or less constant. The indurated quarters in which no streptococci were found remained as free from these organisms over the period in which the observations were made as the quarters in which no fibrotic tissue was found. It is concluded that streptococci are found only in milk from udders that have developed fibrotic tissue. Not all indurated udders, on the other hand, eliminate streptococci.

The weekly variation in the number of streptococci in the milk from the four quarters of one individual was found to be excessive. It was noted that at certain intervals the milk from

FIGURE VI—VARIATION WEEKLY IN THE NUMBER OF STREPTOCOCCI IN MILK FROM 4 QUARTERS OF THE SAME INDIVIDUAL



a given quarter (Figure VI) was practically free of long chain streptococci while subsequent examinations showed relatively large numbers of them.

RELATION OF STREPTOCOCCI IN MILK TO THE NUMBER OF LEUCOCYTES

A study over 10 months of a composite sample of all four quarters of 133 cows (Table IV) has shown that 78 discharged consistently more than 500,000 cells per c.c. in their milk. A further study of these cows also showed that all contained streptococci at some period during this interval. On the other hand, of the 55 cows showing less than 500,000 leucocytes per c.c. only 4 showed streptococci at any time during these observations. In other words, if kept under observation the milk of cows eliminating large numbers of leucocytes will generally contain long chain streptococci at some period during lactation.

A study of the relationship of the simultaneous presence of cells and streptococci in quarter samples reveals somewhat different results than are found in the case of the composite sample. A study of 117 quarters which (Table V) showed a pronounced fibrosis revealed that 20 threw off more than 500,000 cells per c.c. and streptococci were not observed in the milk of any of these quarters.

Observations revealed that cows with one quarter discharging an excessive number of leucocytes will show streptococci in one of the four quarters some time during lactation. However, these streptococci are not always in the quarter showing the large number of cells. On the other hand, not all high cell producing quarters will throw off streptococci.

This peculiar relationship needs investigation to determine whether it is a fact or merely a coincidence.

A further interesting (Table V) observation is the fact that over a period of 10 months' observation 27 of these 117 quarters with pronounced fibrosis have shown neither cells nor streptococci in the milk.

TABLE IV

RELATION OF STREPTOCOCCI TO THE NUMBER OF LEUCOCYTES IN MILK

Number of cows	More than 500,000 cells per c.c.		Less than 500,000 cells per c.c.	
	Streptococci present	Streptococci absent	Streptococci present	Streptococci absent
133	78	0	4	51

TABLE V

RELATION OF STREPTOCOCCI TO THE NUMBER OF LEUCOCYTES IN MILK FROM QUARTERS WITH MARKED FIBROSIS

Number Of quarters	More than 500,000 cells per c.c.		Less than 500,000 cells per c.c.	
	Streptococci present	Streptococci absent	Streptococci present	Streptococci absent
117	47	20	6	27

CONCLUSIONS

1. Milk containing more than 500,000 cells per c.c. always indicates an abnormal or pathological condition in the udder.

2. Milk containing large numbers of cells will generally continue to contain excessive numbers while milk with a small number of cells rarely shows more than 200,000 per c.c.

3. Variation in number of cells per c.c. is greater in milk containing large numbers of cells than milk showing a smaller number.

4. Alkaline reaction to brom thymol blue is never obtained on freshly drawn milk from a fibrosis free quarter.

5. When streptococci are found in aseptically drawn milk, the quarter is always indurated.

6. All quarters free from scar or indurated tissues never show demonstrable streptococci or cells in excess of 150,000 per c.c. in the milk.

7. Not all indurated quarters show streptococci or a significant number of cells.

8. Milk from a normal quarter or free of fibrosis does not contain long chained streptococci or cells in excess of 150,000 per c.c.

BIBLIOGRAPHY

Breed, R. S. Cells in milk derived from the udder. *New York State Agri. Exper. Sta. Bull.*, 380:61, 1914.

Hucker, G. J., Trudell, F., and Jennings, William. Detection of sub-clinical mastitis. *New York State Agri. Exper. Station, Tech. Bull. No. 199*, 1932.

Udall, D. H., Johnson, S. D. Diagnosis and control of mastitis. *Cornell Vet.*, 21:190–206, 1930.

Meals for School Children—Yugoslavia

THE central committee of the Junior Red Cross of Yugoslavia has been active in establishing lunch rooms for school children, particularly in the villages where children live at long distances from school and do not receive sufficient food for lunch. Part of the necessary funds is provided by central and local committees of the Junior Red Cross on the condition that its members actively participate in the work of the lunch rooms and that they cultivate their own vegetable gardens, the products of which are used for the lunch rooms. Subsidies are also granted by the local government and by private relief agencies. A recent law on primary schools orders the establishment of lunch rooms in all village schools.—*Nouvelles de l'Union Internationale de Secours aux Enfants*, Geneva, Sept., 1932.