THE SANITARY SIGNIFICANCE OF LEUCOCYTES IN MILK

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ABSTRACT

It is difficult to understand the true significance of variable numbers of leucocytes (white blood corpuscles) and other types of cellular materials in milk without an understanding of the reason for their presence. The fat laden gland cells and gland cell nuclei present in limited numbers in normal milk represent wastage from the active gland and have no sanitary significance. Leucocytes even in fairly large numbers, i.e., millions per cc, may be present in milk that is normal so far as is evident from ordinary chemical or bacteriological examination.

On the other hand, excessive numbers of leucocytes are usually associated with some abnormal condition, particularly mastitis. The usual form of mastitis is one caused by an infection with streptococci and these bacteria are usually evident among or in the leucocytes when the milk has come from a cow suffering from an inflamed udder. Pathogenic streptococci are sometimes difficult to distinguish microscopically from saprophytic streptococci. Other bacterial infections of the udder may also cause increased numbers of leucocytes to appear in milk.

INTRODUCTION

The problem of the sanitary significance of leucocytes (white blood corpuscles) in fresh milk attracted more attention and discussion 15 to 20 years ago than it has in recent years, altho it is a problem of continual interest. This Station frequently receives requests for information regarding the matter. For this reason it has been thought desirable to rewrite the material presented before the Laboratory Section of the International Association of Milk Dealers at their 19th Annual Convention in Detroit in 1926, and to present it in bulletin form.

THE PROCESS OF MILK SECRETION

In order to understand this problem, it is necessary to understand the origin of all body or tissue cells (including leucocytes) that may
Fig. 1.—Section thru the teat and one-quarter of the udder of a cow.  (A) Region of teat and milk cistern. (B) Region of the larger collecting ducts. (C) Main secretory portion. (From museum specimen prepared by Dr. G. S. Hopkins. Cornell University Agr. Exp. Sta. Bul. No. 158.)
occur in milk as drawn from the udder. The cells that are derived from the tissues of the udder should be distinguished sharply from the extraneous bacterial cells, some of which also occur in the udder so that they are present in milk as drawn. Each individual bacterial cell constitutes a bacterium as these organisms are unicellular in nature. The bacteria found in the udder normally live as harmless parasites, tho they may at times be pathogenic, i. e., cause disease, as well as parasitic in nature. They do not belong to the tissues of the udder as do the leucocytes, epithelial cells, and the like whose sanitary significance is to be discussed in this bulletin.

Returning then to a study of the origin of the body cells in the mammary gland tissues, it becomes necessary to explain the nature of the process of milk secretion itself, a subject that is and has been discussed very little in agricultural bulletins issued in the United States.¹

In order to understand milk secretion, it is necessary to describe the gross anatomy of the udder as well as the microscopic structure. Normally, the udder of a cow is composed of four distinct glands or quarters each with a teat. The teat itself is hollow (Fig. 1), the interior cavity being known as the milk cistern. Opening into this cistern are the main collecting ducts which bring the milk to the cistern from the body of the gland. These ducts branch, becoming smaller and smaller as they are traced back to the secreting portion of the gland. The structure of the secreting portion may be likened to the structure of a bunch of grapes in which the grapes and stems are hollow, and the whole embedded in a mass of white fibrous connective tissue. The hollow grapes would then be the secreting portions of the gland, the so-called alveoli, while the hollow stems of the individual grapes would represent the smallest of the ducts, and the main stem one of the collecting ducts.

¹ The only reports on the subject that the author recalls are two publications issued by the Purdue Agricultural Experiment Station at Lafayette, Indiana, and now out of print. These are "The Udder of the Cow," by C. S. Plumb, Purdue Agr. Exp. Sta. Bul. No. 62, 1896; and "The Mammary Gland," by A. W. Bitting, Purdue Agr. Exp. Sta. Rpt., 1899, 36–43, 5 pls., 1900.

Studies in this field are now being made at the Beltsville Station of the Bureau of Dairying of the United States Department of Agriculture, and the following reports have been published: Relation of conformation and anatomy of the dairy cow to her milk and butterfat producing capacity, by W. W. Swett, Jour. Dairy Sci., 10, 1–14, 1927; and Comparison of conformation, anatomy, and skeletal structure of a highly specialized dairy cow and a highly specialized beef cow, by W. W. Swett, R. R. Graves, and F. W. Miller, Jour. Agr. Res., 37, 687–717. 1928.
If this grape-like structure is cut in cross section (Fig. 2), the hollow individual alveoli would show in various ways; but if cut squarely across would show as rings in which the walls would be composed of the secreting or gland cells. Alveoli which are distended with milk would show with thinner walls than alveoli in the resting condition (Fig. 2). The structure of the individual

gland cell is much like that of gland cells in other similar secreting glands. The gland cell in the resting alveolus is polyhedral in shape with diameters nearly equal. Cell membranes are not always distinct in sections prepared for microscopic examination, but can usually be distinguished. The secreting cells in the distended alveoli are flat and more like pavement blocks.

In preparing the gland for cutting thin sections for microscopic examination, the milk in the distended alveoli is coagulated by the fixing fluids used. In stained slides this shows (Fig. 2) as a more or less granular mass with circular spaces or vacuoles which indicate
the original position of milk fat drops, the latter being dissolved out of the curdled milk by the treatment used in preparing the slide. The remaining granular mass is composed of curdled casein and lactalbumens. The milk sugar (lactose) being soluble does not appear in the microscopic section.

In a complete section of one quarter of the udder, many thousand of these hollow alveoli would be shown, each with its lining of gland cells. Between the oval and circular sections of these alveoli, there would be triangular spaces filled with white fibrous connective tissue (Fig. 2). This tissue not only serves as a connecting tissue, but also has other important and complicated functions. It carries the nerves and blood vessels, and has an intimate relationship to the very important lymphatic system. Reserve food materials may be stored in some parts of this tissue as body fat. It is essentially fibrous in nature, the ordinary fibers being very tough and inelastic. In young animals one of the important constituents of these fibers is gelatin, a substance that is readily extracted with hot water. In older tissues this soluble gelatin is transformed into insoluble material. Among the bundles of fibers, the flattened nuclei of connective tissue cells may be seen in stained preparations (Fig. 2). Certain special cells in this tissue which contain granules readily stainable in certain aniline dyes, e. g., orange G, are known as mast cells (Fig. 2).

The open spaces between the bundles of fibers are filled with lymph, a liquid that is essentially blood without the red blood corpuscles. Some leucocytes (white blood corpuscles), are present in lymph and appear in this connective tissue (Fig. 2). In some of the triangular spaces blood vessels are seen usually in pairs, one a small artery and the other a small vein (Fig. 2). Where cut in cross section the artery shows with the thicker wall and contains no blood, the vein shows with thinner walls and contains blood which shows primarily as red blood corpuscles, with few white blood corpuscles. Blood capillaries can be demonstrated (Fig. 2) with proper stains also. These are embedded in the connective tissue just back of the gland cells lining the alveoli.

In the process of milk secretion, blood serum oozes thru the walls of the capillaries; and occasionally white blood corpuscles, which are amoeboi in nature, make their way thru these same walls. The lymph so formed oozes into and thru the gland cells, being transformed into milk during its passage thru the gland cells. No micro-
scopic evidence can be found of the chemical changes that go on in these cells, except that fat drops form in the protoplasm of the distal end of the gland cells. These dissolve out in making microscopic preparations and leave holes or vacuoles some of which may be seen in the gland cells of the resting alveoli shown in Fig. 2. The generally accepted theory of their release into the milk in the lumen is that they move to the surface and break out of the cell. There has been much discussion over the question whether these fat drops take with them a coating of albuminous protoplasmic material, or whether they are naked as they are released from the cell.

It is quite well established that the milk sugar (lactose) is made in the gland cells by a transformation of the glucose present in the blood and lymph. The casein, lactalbumens, and other substances in the milk are made from constituent parts of the blood and lymph in a way that is poorly understood.

The formation of these nitrogenous constituents of the milk may be passed over hastily as they do not concern us at this time. The history of the gland cells and leucocytes is to be followed more closely. During the transformation of the lymph into milk, the amoeboide leucocytes migrate thru the tissues and may pass thru between the gland cells into the lumina of the alveoli with the milk. As shown in Fig. 2, these characteristic cells may be found rather commonly both in the fibrous connective tissue and in the milk in the lumen of the alveolus. It is more difficult to find one in the act of passing thru between the gland cells, a fact that suggests that the act of migration is carried thru rather quickly. Because it is difficult to find leucocytes in their passage thru the glandular epithelium, some histologists have raised a question whether the polynuclear cells in the milk are actually leucocytes.

There is good reason for thinking them to be leucocytes, as the resemblance between the cells in the connective tissue and in the alveoli is much closer than can be shown in a line drawing. Leucocytes as found in the blood are cells of a very characteristic type unlike any other cells in the body. They show as lymphocytes and as transitional, polymorphonuclear, and polynuclear leucocytes all of which types may be found in milk (Fig. 3). Moreover, they show certain characteristic granulations, some being so-called eosinophiles, others neutrophiles, etc. These names are used because of the reactions that the granules in the cytoplasm show to certain aniline dyes. When appropriate blood stains are used on freshly drawn milk, all of these special
types of cells may be found in the milk. While it is not possible to furnish incontestable proof of the migration of these leucocytes from the lymph into the blood, as it is not possible to watch the process under the microscope in the living tissues, the similarities between these cells that have been pointed out would seem to furnish very satisfactory proof that this migration takes place. The proof is stronger because it is well known that leucocytes also migrate thru the intestinal epithelium, or into the secretion in the salivary and other glands.

In addition to the nucleated leucocytes normally found in all milk, it is usually possible to find in well-prepared sections of udder tissues bare nuclei of cells in the coagulated milk in the alveoli (Fig. 2). There is not much question but that these should be regarded as nuclei of gland cells that have been torn out of the cells with the discharge of the fat drops. The process of milk secretion is a very active process, and it is easy to see how such conditions might arise. The number of these epithelial cell nuclei in the milk is usually very much less than the number of leucocytes.

At other times, particularly during the colostral period, entire gland cells or even groups of gland cells may become loosened from the wall of the alveolus and be discharged with the milk (Fig. 2). These fat-laden gland cells, so-called colostral corpuscles, have been known for nearly a century. They were first discovered by a French

![Fig. 3.—Microscopic appearance of high-grade milk containing large numbers of leucocytes where there was no evidence of any bacterial infection. 600X.](image)

![Fig. 4.—Microscopic appearance of high-grade milk showing a single epithelial cell (colostral corpuscle). 600X.](image)
physician, Donné, in 1837. He called them "corps granuleux," or granular bodies. As a matter of fact, in spite of the common statement in textbooks that they are characteristic of colostral milk, they occur in milk in fair numbers throughout the lactation period. Such an epithelial cell as seen in ordinary market milk is shown in Fig. 4. These cells have a large granular nucleus, and their cytoplasm is filled with fat vacuoles.

In other words, during active secretion, gland cells and nuclei of gland cells are being used up and discharged with the milk. These epithelial cells and nuclei are waste and apparently have no other significance. Similar waste epithelial cells are found in other body secretions, as for example the saliva.

In microscopic preparations of milk as drawn from the udder, these epithelial cells and nuclei can still be recognized by the characteristics mentioned. Likewise, the leucocytes can be recognized in all of their varied forms—lymphocytes, transitional, polymorphonuclears, polymorphonuclears, neutrophiles, eosinophiles—if fresh milk is stained with proper blood stains.

SIGNIFICANCE OF BODY CELLS IN MILK

With these facts in mind, the original question—What is the sanitary significance of these cells in cow's milk?—can now be discussed. As already stated, the gland cells and free gland cell nuclei apparently have no sanitary significance. Their presence is an entirely normal phenomenon. There would appear to be no more harm in consuming a gland cell or its nucleus in milk than in eating the same type of material in sweet breads or in a deliciously cooked piece of calves liver.

The significance of the leucocytes cannot be dismissed so simply, however. What is it that brings them into the milk? They occur in entirely normal secretions, such as saliva, so that it is not surprising to find them in variable numbers in normal milk (Bulletin No. 380 of this Station). Normal numbers of these migratory cells probably then have no sanitary significance whatever. The normal numbers may vary greatly.

Fifteen to 20 years ago the author and several associates made an extensive survey of the number of these cells in the normal milk of individual cows in various herds of cattle, using a more accurate method of counting than that employed by previous workers. The results showed that these cells were much more abundant in milk
than had been supposed up until that time. The average number of cells found for 122 cows giving milk generally regarded as normal was 868,000 per cc, and 27 of these cows gave milk with a count over 1,000,000 per cc. These findings practically ended the claim that had previously been made that any milk containing leucocytes in excess of 500,000 per cc should be rejected as containing "pus." Leucocytes are far more abundant in milk as secreted than are bacteria. Where bacteria occur in hundreds per cc, these cells occur in thousands. They differ from bacteria in that they do not grow in the milk so that their number does not increase after the milk is drawn.

Wide variations occur in the numbers present, and the milk of the four quarters of a single udder may show as large variations in numbers as the milk from four different cows. Some quarters give milk with a cell count less than 5,000 per cc. The highest cell count obtained in apparently normal milk was in strippings from the udder of a single teat about a week after the cow had freshened. This milk contained about 54,000,000 per cc. The milk was examined carefully as many sanitarians at that time claimed that milk of this type was unfit for use. No streptococci or other types of bacteria that could be regarded as undesirable were found in the milk. The sample was eventually consumed with no ill effects. The same investigation confirmed previous reports that milk drawn at the end of milking contained more cells than that at the beginning, probably because the udder is massaged somewhat at the end of the milking. At the same time, it was found that increasing the vacuum used in machine milking did not increase the number of cells. Taken alone, the number of cells, whether leucocytic or epithelial in nature, seems to have little significance.

RELATION BETWEEN BACTERIAL INFECTIONS AND NUMBER OF LEUCOCYTES PRESENT

In some cases, truly pathological conditions cause the presence of excessive numbers of leucocytes and other cellular debris; and it is the presence of leucocytes under these abnormal conditions that may give these cells a sanitary significance. The most common type of pathological condition is a streptococcus infection of the udder producing a mastitis. Where the udder becomes infected with

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2An average cell count of 657,000 per cc for 40 cows by the same technic has been reported by Copeland, Lynn, and Olsen, T. M., The bacterial flora of normal cow's udder. *South Dakota Agr. Exp. Sta. Bul. No. 218.* 1926.
streptococci, they occur in the lumina of the alveoli (Fig. 5). The streptococci grow in the milk in the alveoli, and in their growth undoubtedly ferment the lactose as they do in milk cultures in the laboratory. This fermentation produces acid. The surprising thing is, however, that the milk as drawn from the infected quarter has a more alkaline reaction than normal milk. The bacteria certainly produce acid, and yet the milk from streptococcus-infected quarters as drawn has a pH of 7.0 to 7.2, while normal milk has a pH of 6.5 to 6.7.

The explanation of this condition seems to lie in the fact (Technical Bulletin No. 80 of this Station) that the infection causes the gland cells to lose their vitality so that they no longer transform the blood serum into milk, and the more or less unchanged blood serum enters directly into the interior of the alveolus. This serous exudate, as it would be called by pathologists, is more alkaline than normal milk, and if it enters in sufficient quantity over neutralizes the acid formed by the bacteria. As a matter of fact the neutral to alkaline reaction of the milk (pH 7.0 to 7.2) is practically the same as that of unchanged blood serum. Possibly this is one way in which the healing processes of the body tissues combat the bacterial infection. Certainly the growth of the streptococci is not favored by the increased alkalinity.

Fig. 5.—Drawing of a microscopic section of the udder of a sheep with acute streptococccic mastitis. Alveoli containing streptococci. Glandular epithelium disorganized. (From Ernst, Milchhygiene für Tierarzte.) 1000×.

Fig. 6.—Microscopic appearance of milk from a cow suffering with streptococccic mastitis. Phagocyte with engulfed streptococcus in center of picture. 600×.
Excessive numbers of leucocytes also enter directly into the milk with this serous exudate, causing an increase in the cell count. Some of the leucocytes act as phagocytes, that is they engulf and digest the bacteria (streptococci). When such phagocytes are found by microscopic examination of the milk (Fig. 6), it is practically sure proof that the milk has been derived from an infected udder. If this cellular material and the serous exudate become predominate, then the nature of the milk secretion is so changed in appearance that it is usually termed "pus." The microscopic appearance of milk from an udder infected with streptococcic mastitis is shown in Fig. 7. Frequently it is possible to detect fibrin, a natural constituent of blood, in mastitis milk. This substance is absent from normal milk.

Other bacterial infections of the udder may occur and quite probably disturb the normal milk secretion in a similar way. Thus, Cooledge\(^3\) reports that he finds indication of increased numbers of leucocytes in milk from udders infected with *Brucella abortus*, the organism of contagious abortion of cattle. It seems probable that infection with any pathogenic organism may cause increased numbers of leucocytes to enter the milk.

Possibly there is some connection between the presence of the certain types of bacteria normally present in udder tissues and the number of leucocytes present; and Steck\(^4\) has secured evidence that indicates such a relationship. It must always be kept in mind that the number of leucocytes in the milk may also be influenced by physiological disturbances in the milk secretion having no connection whatever with bacterial infections.

Research is needed to determine whether significant relationships exist in regard to these things which may be compared to the relationships that have been discovered between variations in numbers

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of white or red blood cells in human blood and specific pathological conditions. Certain English and French investigators have in recent years made differential studies of leucocytes in milk in relation to the period of lactation, congestion caused by delayed milking, and bacterial infections of the udder which give a good idea of the value of such investigations. Bourgeois finds the leucocytiastic formula of normal milk, i.e., the ratio between the number of mononuclear leucocytes and number of polynuclear leucocytes, to be similar to that of normal blood. Delayed milking increases the proportional number of polynuclears, which proportion becomes still higher in streptococccic infections of the udder.

CONCLUSIONS

In other words, the relationships between the number and types of leucocytes in milk and the conditions that give rise to variations in numbers and types of these cells are so complex that they cannot be summarized in any simple way. The only relationship that stands out sufficiently distinct to have been utilized in a practical way in routine microscopic examinations is that excessive numbers of leucocytes predominantly of polynuclear types, accompanied by long-chain streptococci and particularly when phagocytes containing bacteria are present, indicate streptococcic udder infections with practical certainty. If one or more of the specified conditions are lacking, then the indications become less certain. On the other hand, milk containing small numbers of leucocytes of any kind and no indication of abnormal bacterial infection may certainly be regarded as normal so far as microscopic examination is concerned. When conditions intermediate between these two extremes are found, interpretation of microscopic findings are difficult and uncertain.
