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HISTOPATHOLOGICAL STUDIES ON BOVINE MAMMARY GLAND I.
HISTOLOGICAL FINDINGS OF MASTITIS

Saburo Yamagiwa*, Takeshi Ono**, Tamio Uemura*** and Taiji Ida**

(Received for publication, July 25, 1957)

PREFACE

Specialists in public health are well aware of the importance of the economical and of the food hygienic significance of bacterial mastitis. With regard to this disease, comparisons between the superiority and inferiority in diagnostic methods and research on chemotherapy have been actively conducted. A number of reports have already been made in this line. Accepting an opportunity to participate in a mastitis research group in Japan starting from last year, the authors have immediately launched out to collect research materials in Hokkaido, and have investigated the disease as described in veterinary literature. In consequence, they unexpectedly reached a conclusion that basic histopathological knowledge concerning the mammary gland of dairy cows is commonly inadequate. In continuation of this report, the authors intend hereafter to describe the findings obtained through their researches. They sincerely hope that the reports will be of assistance in the research on mastitis.

In this report, the authors have tried to describe the histological findings which they encountered as precisely as they can with the aid of the attached photographs. Frankly speaking, they must confess that they have run into a difficulty in reaching a solution to their own question in the first step of microscopical investigation—just what is mastitis? The authors realized that their knowledge so far gained from the text books proved itself unsatisfactory. As Spencer and Pattison have daringly pointed out, mastitis can not be simply explained in terms of either "acute exudative" nor "chronic productive" in describing and classifying the histological changes. In any case, the present authors in this report wish to place emphasis upon the analytical investigation of microscopical inflammatory changes in mammary gland tissue.

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C: diffuse mastitis
MATERIALS AND METHODS

During May and October 1956, mammary glands were collected indiscriminately from more than 120 cows at slaughter houses in various places in Hokkaido. Of the above specimens, the findings on the 30 cases collected in the Sapporo slaughter house form the subject matter of the discussion in this report. It is to be regretted that the authors can not provide precise clinical and other data concerning the cows because they were slaughtered cases. Bacterial cultivation was conducted on 3 cases among materials.

After the animal carcasses had been flayed, the mammary glands for study were totally removed and observed, then were sliced dorso-ventrally in parallel with the long axis of the body containing the teats of the posterior and anterior quarters. From the teats, milk sinuses, mammary gland tissue, etc. in the Sapporo 30 cases, 297 tissue blocks were collected in order to provide section preparations regardless of the existence of macroscopic changes (Table 1).

The section preparations were embedded with paraffin-celloidin, and were stained with hematoxylin-eosin (H.-E.) and Gram-Weigert. In addition, silver impregnation was employed when necessary.

SURVEY OF THE LITERATURE IN CONNECTION WITH THE HISTOPATHOLOGY OF MASTITIS

The mastitis subjected to the discussion in this report, is only limited to the common classical one caused by streptococci and micrococci as reported in the literature. On this occasion, the authors omitted from study not only those cases which belonged to so-called specific productive inflammation, but also severe suppurative and gangrenous mastitis caused by pyogenic bacilli, botryomyces, gas gangrene bacilli and others. What the authors will discuss in this report has been called in text books "interstitial and exudative mastitis" (SHOLL, L. B.; 1946), "subacute and chronic focal mastitis and chronic diffuse mastitis" (RUNNELS, R.A.; 1953), "die unspezifische diffuse Mastitis" (NIEBERLE, E.; 1954), and "chronic contagious mastitis" (DAVIES, G.O.; 1955). The authors consider that reference should also be made to "interstitial mastitis" (SHOLL, L. B. and J. P. TORREY; 1951), "the patchy nature of the streptococcus mastitis" (MORRILL, C. C.; 1938), "acute and subacute catarrhal mastitis and chronic mastitis" (CHU, S. J.; 1949) and "local mastitis" (EDDS, G. T. and J. C. TEACK; 1954). In addition, as to references published in Japan, the authors should quote "catarrhal suppurative mastitis" (KITAURA et al.; 1955), "circumscribed interstitial and fibrous mastitis" (SUGANO; 1956).

Putting aside any discussion concerning the interpretation of name, what the authors are most interested in is the microscopical small focus. There are quite a few authors who have undertaken to discuss this change. In this regard, the present authors can point out, in addition to RUNNELS, MORRILL and EDDS, such name as TURNER and REINECKE (Involu·tion of the normal caprine mammary gland; 1936) ANDERG and WEIRETHER (Normal bovine udders inoculated via the teat canal with Novoxil and Tyrothricin; 1943). CHU, MCFARLANE and RENNIE (Productive change in inoculated tissue; 1950) and PATTISON (The patchy nature of the disease; 1951). Among these just mentioned, PATTISON describes
the change with the most careful considerations. He himself, together with his co-workers, has conducted an experiment in inoculating a large quantity of *Str. agalactiae* into the teats of goats. In carrying out his investigations on the changes of mammary glands, he prepared 741 block-pieces of tissue from the mammary glands of 39 goats where the inoculation was made and 105 from the non-inoculated. To their regret, the present authors could not attain their intention to study the characteristic of small foci but they have had to depend exclusively on text figures presented by earlier authors. It was possible, however, to obtain a summarized idea on microscopical small foci with the assistance of descriptions given by Runnells and Pattison centering around the text figures of Edds and Trace, Chu and Pattison. Information on such focal changes seems to be still in a stage in which further discussion and study are necessary. The authors, however, would like to offer comment on findings of former authors chiefly from the morphological point of view in addition to presenting their own findings as follows.

**HISTOLOGICAL FINDINGS**

As is shown in table 1, so far as the present materials are concerned, present authors consider their materials, from the viewpoint of histological findings, to be diagnosed as mastitis in all cases. The following data are histological findings which were classified according to the authors' own way (Mastitis circumscripta and mastitis diffusa). However, two udder quarters (TM 29 right anterior and TM 17 left posterior) in which *Corynebacterium pyogenes* was isolated through cultivation will be described in another report.

*Mastitis circumscripta* (*Mastitis alveolaris* and *Mastitis lobularis*)

It is the most striking characteristic that the focus is microscopically minute and usually it cannot be identified macroscopically whether or not a pathological change exists.

*Mastitis alveolaris* (Figs. 1~13) Viewing the appearance of foci in figure 1, the authors notice that the section preparation, in all parts of the area, is packed with parenchymal tissue in the period of lactation. A detailed observation, however, reveals the existence of minute foci within lobules (Arrow marking). The foci can be divided into two types. The one includes some

*With each figure is printed, case No., the portion from where the section preparation was obtained, staining method and magnification in order. L indicates left side, R right side, A anterior quarter of mammary gland and P posterior quarter.*
Acini in the focus which contain leukocytes and detrited substances; in this case cellular infiltration can hardly be found on the acinar epithelia. In the other types acini rather show an atrophy and the acinar wall is rich in infiltrated cells, proliferated fibrocytic element and so forth.

Figure 2 shows a preparation obtained from another case showing minute focus which appears intralobularly similarly as in figure 1. Acini and small ducts are generally normal in the portions other than the pathological focus. Affected acini contain leukocytic lumps mingling in detrited substances. Some acini have epithelial cells thickened showing a leukocytic infiltration. Interstitial tissues, surrounding the above acini, accumulated leukocytes and fibrocytic element. Such circumstances can be obviously explained by reference to figure 3 preparation of another case, and figure 4—magnified picture of figure 3.

Figure 5 and its magnified picture figure 6 are presented to show a quantitative difference of changes which have a similar quantitative nature. Of 3 acini in the central portion of figure 5, the left two acini show thickened epithelial cells and a rather remarkable indication of necrobiosis and leukocytic cell infiltration. Acini are losing their figure because of detrited substance and exudates which filled acinar lumina. In the right acinus there appears a strong cell infiltration of epithelial layer and epithelial cells also show an extremely increased thickness (Fig. 6). Leucocyte infiltration is also notable in the focus and surrounding interacinar tissue, and the dilatation of blood capillaries and increase of fibrocytic cells are recognized simultaneously. Ductules, however, showed no changes.
FIG. 3. *TM 18, L, A. H.-E.* × 150

FIG. 4. *TM 18, L, A. H.-E.* × 300

FIG. 5. *TM 23, L, A. H.-E.* × 150
Histopathological Studies on Bovine Mammary Gland I.

Fig. 6. TM 23, L, A. H.-E. × 600

Fig. 7. TM 18, L, P. H.-E. × 150

Fig. 8. TM 26, R, A. H.-E. × 150
Figures 7 and 8 and magnified figure 9 of the latter show advanced changes respectively. In other words, both two portions in the upper left part of figure 7 and one portion of the upper left part of figure 8 show the obscure location and shape of acini and the surrounding interacinar tissue shows a considerably developed inflammatory reaction increasing its width. Inasmuch as the constituent cells in this portion are degenerated, the identification is not easy but the participation of histiocytic cells is obvious when one considers the results of staining and other items. At the same time the recessive leukocytic cell infiltration can be regarded as one of the characteristics.

In addition to the above findings the authors also have noticed, among minute foci, some which provide the following indications (Figs. 10-13). The observation conducted on these preparations using magnifier has proved the foci to be a roughly reticulated structure sporadically intralobularly formed by the interacinar tissue though they showed normal duct system. Figure 10 shows the magnified lobule located in such portion. In the same lobule, the lower half and the
right and left upper half show different character to some extent. The acinar epithelial layer, however, indicated common nature in general, i.e., epithelial cells decrease in number as shown in magnified picture—Fig. 11, the arrangement of nuclei becomes irregular, small lumps which mingled into desquamated cells and leucocytes are recognized in several places of acinar lumina. The outstanding discrepancies in the three portions previously described concern the acinar wall, namely the lower half and upper right half are abundant in cells and organized mesely formation which looks coarse outwardly. Cell element chiefly consisted of fibrocytes accompanied by plasma cells and leucocytes. Impregnated preparations enable the authors to notice such changes more clearly (Figs. 12 and 13). Such preparations were made from the same lobule with the cytological preparations previously presented, but they show the increase of argyrophile fibers clearly in the lower half and right upper portions of lobule.
FIG. 12. TM 5, L, P. Bielschowsky × 90

FIG. 13. TM 5, L, P. Bielschowsky × 150
Mastitis lobularis (Figs. 14–24) Changes are observed in lobular character. In case changes have occurred in intact mammary gland tissues, they as well as acinar ones are recognized as minute focus. The majority of changes are liable to take possession of glandular tissue to a considerably wide extent even though they are different in size (Figs. 14 and 15). To cite an example, many atrophic acini are observed existing in between interlobular fibrous tissues as shown in figure 14. Among the lobules with lesions, secreting lobules also exist. Both lactiferous duct and small duct show a dilatation having no contents. Careful observations on atrophic lobules obviously reveal an intralobular ramiform structure mainly with the ductules there. In figure 15, one-third of the area of the picture also indicates pathological tissue. Lobules which are embedded in proliferated interacinar connective tissue are atrophic. A microscopical investigation enables recognition of intralobularly proliferated fibrous tissue accompanied by infiltrated cells which are composed of lymphocytes, leucocytes and particularly of plasma cells in quantity. The acinar lumina contain serous substances slightly stained with eosin. In this section preparation, as the ductule contains the same substance, it is hard to recognize any ramiform structure in the lobule. The writers, however, are able to notice the focus of mastitis lobularis (Arrow marking) in the closely adjacent posterior udder (Cf. Fig. 14). Furthermore, pictures of mastitis lobularis are often seen together with diffuse mastitis to be discussed below (Figs. 16 and 17). In both pictures, the left half and the right half show groups of acini which respectively belong to the focus of diffuse mastitis and the focus of lobular mastitis. Between the above two foci there exists connective tissue
lacking in cell element. This connective tissue is annexed to proliferated periductal connective tissue.

In the acini located in the above-mentioned focus of diffuse mastitis, lumina are filled with exudates, while the epithelial layers increase their thickness and are infiltrated by leucocytes (Similar to Figs. 3 and 4). On the contrary, interstitial tissue indicates slight cellular reaction and argyrophile fibers show no increase. Among acini in lobular mastitis shown in the right of picture, the necrobiosis of epithelial cell layer and the infiltration of leucocytes are observed. In addition, interacinar tissue shows an active inflammatory reaction. Argyrophile fibers also show an outstanding increase.

In figures 18 and 19, only lobules which have fallen into mastitis lobularis-lesion are observed adjacent to each other. This can be regarded as partial magnified picture of figure 14. Slender lumina are the ductules. Each acinus shows atrophic state and low cylindrical epithelial cells line the wall of atrophic acini arranged rather regularly. In acini and ductule, desquamated cells are only observed sporadically and there is no indication of the emigration of inflammatory cell elements. The increase of interstitial tissue in its width and the increase of cells are of importance to interstitial changes. The greater part of cell components are fibrocytic elements and permit the participation of a few plasma cells and leucocytes. As shown in figure 19, increase in the cell elements is accompanied by an increase of argyrophile fibers, but not very dense. The pictures of figures 20 and 21 are also similar to two pictures above presented. Necrobiosis and leucocytic cell infiltration of epithelia are clearly observed in both acini and small ducts.
Fig. 18. TM 14, L, P. H.-E. × 90

Fig. 19. TM 14, L, P. Bielschowsky × 90

Fig. 20. TM 19, L, A. H.-E. × 90

Fig. 21. TM 19, L, A. Bielschowsky × 90
under high power magnification. The cell increase in the interstitium is greater than that of previous figures and histiocytic element is mingling. In addition, in several places—the central portion of the picture—small hemorrhages are shown. The density of argyrophile fibers is higher as compared to that in figure 19.

If it is permitted to confer the name of subacute to these changes in the six pictures mentioned above, it is also allowable to give an interpretation to the following three pictures (Figs. 22~24) as they show chronic changes. First of all, as for figure 22, a few lobules and ducts shown in the picture are those existing among the lobules which manifest changes similar to that in the lower half of lobule shown in figure 10. In the lobule

Fig. 22. TM I, L, A. H.-E. × 90

presented in figure 23, small ducts are dilated cystically, acini have either become atrophied or have disappeared. The increase of fibrocytic element shows a strong tendency and phagocytes and plasma cells are sporadically observed. There is no leucocytic infiltration. The argyrophile fibers show an conspicuous increase. The authors are of opinion that it may be reasonable to diagnose the change in figure 23 as mastitis lobularis cysticus. In figure 24, acini and ductules approximately do not show their lumina, which are densely lined by oval-shaped epithelial cells (inactivity atrophy).
Histopathological Studies on Bovine Mammary Gland 1.
The present authors would tentatively attempt a clarification of "mastitis circumscripta" summarizing the above findings. They consider that what was meant by the expressions "the patchy nature of the disease" (Morrill), "small foci" (Sholl), "focal distribution" (Runnells), "local mastitis" (Edds and Trace), "mild and transient disturbances" (Davies), etc., which have been used in textbooks, would coincide with "the foci of mastitis circumscripta" in the present report. Frankly speaking, it is because the phrase "non-specific mastitis" is often likely to remind the student of mastitis diffusa immediately, of which description is given later, that the authors have here presented a detailed description of mastitis circumscripta believing it is necessary. Since the materials used in the present study came from the slaughter house, the authors are unable to present the findings with an ample foundation, different from those of Pattison's experimental case of goats, regarding the development of the disease. On this occasion it would be naturally necessary to take into consideration also the kinds of causal bacilli, their favorite locus and other conditions (the authors have used the findings concerning Sholl's Br. abortus infection and Davies, Chu and Wilson's Str. agalactiae and uberis infection as references. So far as the foci of mastitis circumscripta in this report are concerned, the authors could not prove the existence of bacterial agent). The present authors would like to point out that some of the findings presented by Pattison quoting the experimental results of Andberg and Weirether who used Novoxil and Tyrothricin should not be made light of. In consequence, Pattison has concluded that the changes—similar to mastitis alveolaris described by the authors—represent a subsequent non-specific reaction which follows the instillation of any kind of foreign body, including the findings of cases inoculated with heat-killed streptococci which were examined by himself.

However in terms of causal genesis, it may be possible for us to confirm that minute inflammatory foci can at least be found anywhere multicentrally under the microscope. At this time it should be stressed that the mastitis changes have variety in development such as those of acinus, lobule and collective lobules, and either interstitial and parenchymal changes generally have mild and progressive characters in terms of quality.

Furthermore the authors still have another theme to be discussed on circumscripted mastitis particularly on lobular mastitis. It is related to the pathogenesis of changes in the final stage. For instance, McFarlane and Rennie regard such atrophied gland tissue embedded in the fibrous tissue of bovine secreting mammary gland tissues as a passive change attributable to the influence of mastitis changes. Pattison opposes above conception with an interpretation that atrophic gland tissue is resulted from defence reaction and plays an indispensable part in mastitis changes. Even though the authors' cases were obtained from materials of
slaughtered animals as well as materials provided by McFarlane et al., the greater parts of the degenerated gland tissue embedded in fibrous tissues were, as they have attempted to give detailed explanations at the time of describing their findings, the results of actively occurring inflammatory changes.

**Mastitis diffusa (Figs. 25~35)**

As already shown in the above table (Table 1), the mastitis of this type in all cases that can be clinically diagnosed, is the classical and the most important type. Its character has been clearly described pathologically. In an attempt to assist the interpretation of the characteristics of previously described mastitis circumspecta, the authors will present their own findings in short, with the intention to supplement previous authors' findings.

The characteristic of mastitis of this type is the inflammatory reactions occurring in duct system, periductal and its surrounding portion. Two sections of ducts with large diameter are exhibited in figure 25 and, on the stained preparation, detrited substances accompanied by numerous bacilli can be found filling the lumina of these ducts. Their wall is remarkably thickened owing to proliferation of collagenous fibers. Slight increase of argyrophile fibers in the interlobular interstitial tissue, which communicates with the wall, is seen but the increase cannot be observed in the gland tissue (Cf. Fig. 17). The black-stained substance in acini is the serous substance with small quantity of detrited substance. On the preparation stained with hematoxylin-eosin, the larger duct shows irregular epithelial cell arrangement, necrobiosis, desquamation and even metaplastic changes. Leucolytic cell infiltration is slight degree and the fibrocytic cells and plasma cells slightly increase in the interstitial tissue.

The picture presented in figure 26 shows the appearance of lobules in an acute inflammatory stage. The things which attract attention are the dilatation and thickened wall of intralobular small duct. In the lumen of such small duct there exist a large quantity of exudate which is mainly composed of leukocytes. The arrangement of epithelial cell layers has fallen into disorder and severe leukocyte infiltration is observable in them. Although the leukocytic cell infiltration can be found in the interstitial tissue, it showed very slight infiltration there. A few histiocytic cells are also distributed among infiltrated cells. The lobular inflammatory picture reproduced in figure 27 shows a developed case as compared to the previous picture. In other words, the wall cells of a dilated ductule are extremely irregularly arranged, bubble-form swollen nuclei are stratified and there

**FIG. 25. TM 30, L, P. Bielschowsky × 375**
FIG. 26. TM 4, R, P. H.-E. ×20

FIG. 27. TM 28, L, A. H.-E. ×20
is a participation of the infiltration of lymphocytes. The lumen contains condensed serous fluid with a small number of desquamated epithelial cells. The surrounding portion adjacent to the basement membrane increases its width narrowly, becomes coarse and contains two or three layers of fibrocytic cells. The periductal tissue is in contact with interacinar tissue where inflammatory cells mainly composed of plasma cells are accumulated; leucocytes are not present there. Acini are atrophied and their epithelial cells show a remarkable increase of thickness. The majority of acini contain bubble-form nuclei. There are some cells with bubble-form nuclei which broke out into lumen desquamatedly. Many of the acini contain hyaline droplets in the epithelial cells. Figure 28 also shows the inflammatory picture of a lobule. The characteristic shown in this picture is productive change of the epithelial cells, ductule and acinus. In other words, the proliferation is taking place together with the necrobiosis, desquamation and others of epithelial cells.

**FIG. 28. TM 15, R, P. H.-E. \( \times 90 \)**

Numerous atrophied acini are seen which are inclined to be obstructed in the lumen by proliferated cells. Argyrophile fibers are also increased as shown in figure 29. Such circumstance can easily be explained by reference to figures 30 and 31. In response to such parenchymal changes, interstitial changes also show an outstanding development which results in increasing of plasma cells and fibrocytic cells to a certain extent and in conspicuous increase of argyrophile fibers.

The authors have picked a large duct to start with (Fig. 25), but they again would like to describe changes of duct. Figures 32 and 33 show the inflammatory changes of duct in the same case of diffuse mastitis. Epithelial cells are very irregularly arranged as they are in the previous three pictures. There are to be seen necrobiosis, hyaline droplet
FIG. 29. TM 15, R. P. Bielschowsky × 90

FIG. 30. TM 30, L. P. H.-E. × 90

FIG. 31. TM 30, L. P. Bielschowsky × 90
Fig. 32. TM 15, R, P. H.-E. × 90

Fig. 33. TM 15, R, P. H.-E. × 90
degeneration (conspicuous in Fig. 33), desquamation and abnormal proliferation (squamous arrangement is presented in Fig. 33). The lumen is packed with detrited substances mainly consisted of exudated cells. In the periductal tissue and its surrounding portion, cell elements mainly consist of plasma cells and fibrocytic cells show an increase, but the fibrosis is still in a stage showing a slight tendency. The case presented in figure 34 is also regarded as being in a pathological stage similar to the former two pictures but it shows immature proliferated tissue which projects into the duct. This tissue, however, is lacking in cell elements, but is coarse and contains a small number of infiltrated leucocytes in its main component—fibrocytes. The inflammatory change in the periductal tissue of the duct is not conspicuous just as in the previous two pictures. The last figure, 35, shows a part of the inner wall of milk sinus. The left side of wall surrounding the lumen shows a beautifully stratified epithelialization. An active proliferation of granulation tissue is observed in the portion enveloping the sinus. The upper and right walls are lacking in epithelial cell layer and immature granulation tissue is disclosed nakedly. The authors would like to draw attention to the newly formed blood capillaries which are arranged simultaneously taking a direction toward the lumen.
DISCUSSION

In this report, the authors have described the histological findings of mastitis classifying it into two types based on the extent of the lesions. There are some cases which showed similar wide distribution to that of the multilobular type among those which are classified as mastitis circumscripta; these cases may give an impression to someone that the difference from mastitis diffusa is obscure. In this regard, the authors particularly would like to point out that important discrepancies of the characteristics of pathological changes between the two types still can be stressed as they do exist. At this time, they have no intention to adhere the titles of classification. In short, the authors are of opinion that the differentiation is good enough if the cases only prove to have the following characteristics:

1. Microscopical minute foci develop multicentrally in mastitis circumscripta.
2. The minute foci, secondary, terminate their pathological changes with interstitial inflammatory reactions in lobule or groups of lobule following the fairly mild progression of the disease.
3. Furthermore, the duct system does not provide a location for the lesion.

Pattison extracted patchy lesion in a case which showed slight clinical symptoms based on the findings on experimental goats. He placed emphasis on
the fact that injected *Str. agalactiae*, in these foci, attacked only acinar epithelia leaving the duct system intact. Even though present authors’ materials do not provide sufficient data for them to carry on etiological discussions, they, however, consider it indubitable that inflammatory reaction is clearly limited to the acinar tissue in mastitis irrespective of causes. The pathological changes which belong to mastitis diffusa, conversely, show a peculiar characteristic in the participation of clear inflammatory reaction of ductal tissue.

In such a case of experimental mastitis diffusa as was conducted by Pattison, the authors consider that clinically marked or moderate symptoms must be detected. In fact, the authors’ cases of mastitis diffusa have all been clinically diagnosed as mastitis.

In conclusion, the writers wish to call renewed attention to the fact that there are two types of affections on acinar tissue only and on acinar and ductal tissue successively. Even under the same condition, as prevailed in Pattison’s experiment, it attracts the authors’ attention that animals have proved there are two-way-reactions. There are some authors who, aiming at discriminating reactions in animal body, depend on the kind of streptococci. In this connection, the present authors, however, think they had better not to make any comment.

**SUMMARY**

In this paper there are described patho-histological findings on thirty cases of bovine mastitis. Further discussion is offered. As a result of this examination, the writers place emphasis on two-way inflammatory tissue reactions which exist in bovine mammary glands. At the same time they also pay special attention to the types of mastitis of which the foci were located in the acinar tissue of acini, lobules and groups of lobules.
Histopathological Studies on Bovine Mammary Gland 1.

REFERENCES