HISTOLOGY OF THE MAMMARY GLAND VII. HISTOLOGICAL AND HISTOCHEMICAL STUDIES, OF CELLS IN THE MILK OF DOMESTIC ANIMALS

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VII. HISTOLOGICAL AND HISTOCHEMICAL STUDIES OF CELLS IN THE MILK OF DOMESTIC ANIMALS

By

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Introduction

It has been well known that various kinds of cells appear in milk, and that they show the special type of degeneration at the stages of onset and stoppage of milk secretion. These cells in human and cow's milk have been carefully observed by several investigators, formerly from the point of view of public health, and recently in relation to the diagnosis of mastitis (1, 2, 5, 8, 9, 10 and 11). As for other domestic animals, only a few studies are reported using goat's milk (6 and 9).

In the present investigation, the author made a comparision among various kinds of cells appearing in the milk of domestic animals, using histological and histochemical methods.

Materials and Methods

The milk used in this investigation was obtained from healthy animals in the city and neighbourhood of Sendai. The number and variety of animals are given in Table 1, with the number of days after their parturition.

Table 1. The number of animals and the days after parturition.

	Horses	Cows	Sheep	Goats	Pigs	Dogs	Cats	Rabbits	Mice
Number of animals	3	7	11	9	3	3	3	5	5
Number of days after parturition	30~60	50~120	30~50	30~50	30~40	30~40	30~40	5~10	20~25

A certain portion of the milk obtained from each animal was used for the count of cells, and the rest for histological and histochemical studies.

Calculation of cells in milk was done with the materials treated with the

direct smear method of Prescott and Breed (3) modified by the author; that is, instead of toluidine blue, the milk smears were stained with hematoxylin-eosin or hematoxylin-eosin-Azure II for the differentiation of cells.

For the histological and histochemical observations, the milk smear was treated by one of the following fixatives; formalin, absolute methanol, 90 per cent ethanol, Regaud's fluid, Zenker-formalin and Aoyama's fluid. Then, the fixed materials were variously treated to demonstrate the different cellular components; by the Gomori's method for alkaline phosphatase, by the peroxydase stain, PAS method, propylene glycol-Sudan black B stain, Von Kossa's method and Gomori's method for calcium, Heidenhain's iron-hematoxylin stain for mitochondria, Aoyama's method for Golgi bodies, Masson's trichrome stain, carbol-fuchsin stain, toluidine blue stain, thionin stain, and Maximow's method. Identification of glycogen was made by the salivary test (37°C, 2 hrs), and that of RNA by ribonuclease treatment (37°C, 1 hr).

Mouse mammary glands, on the other hand, were also fixed with the same fluids as the above; embedded in paraffin and cut at 4 to 6μ, or at 20μ by a freezing microtome; and then they were stained by the same methods as mentioned above. Further, some mice were treated with vital stain, using trypan blue: 0.1 *ml* of 0.5 per cent trypan blue was intraperitoneally injected daily for five days. And their mammary glands were fixed with 10 per cent formalin, embedded in paraffin and cut at 4 to 6μ. For a further histological study of milk, some portion of the milk of every animal was treated with the supravital stain, using Janus green or neutral red, to compare with mouse blood cells also supravitally treated.

Results

The kind and number of cells in milk

The cells in milk are composed of epithelial cells and wandering cells without difference among the animals used.

The epithelial cells desquamated from the epithelia of the mammary glands and ducts are 12 to 16 μ in diameter, and possess a chromatin-deficient nucleus, in which, though rare, there might be one or two nucleoli. The cytoplasm of the epithalial cells, exhibiting an indistinct and irregular outline and containing several vacuoles inside, is weakly stained with eosin (Figs. 1 and 2). Among these cells, naked nuclei are frequently found, often containing nucleoli in them. During the degeneration of those epithelial cells, their nuclei lose stainability and begin to disappear from a part on the surface (Figs. 1 and 2).

The wandering cells are composed of mononuclear cells and polynuclear cells, the former including normal lymphocytes and large lymphoid cells measuring 15 to 20 μ in diameter and containing oval or kidney or horse-shoe shaped nuclei, and the latter including many of neutrophiles and only a few

of eosinophiles and basophiles (Figs. 3 to 6). The degenerative changes of these wandering cells are as follows: The lymphocytes and neutrophiles come to be more stainable with eosin, and their cytoplasm hypertrophies to some extent, bearing one or two vacuoles. The nucleus moves to a corner of the cytoplasm showing piknosis, decrease in size, and finally disappears. But some of neutrophiles do not show nuclear piknosis even when their cytoplasm becomes eosinophilic (Figs. 3 and 4). The cytoplasm of large lymphoid cells becomes basophilic as they hypertrophy in an early stage of degeneration, and then changes to be eosinophilic when the excentric piknotic nucleus breaks up into a few fragments and the cytoplasm vacuolates at the later stage of degeneration (Fig. 5).

The number of various cells in 1 *ml* of milk are given in Table 2, where the lymphocytes are included among the lymphoid cells.

Table 2. The number of cells in the milk (1 *ml*).

	Total No. of cells	Lymphoid cells	Neutrophiles	Eosinophiles	Epithelial cells
Horses	100± 30*	60± 50*	30± 20*	—*	10± 10*
Cows	300± 100	70± 40	180± 70	10± 60	40± 40
Sheep	100± 30	50± 10	30± 10	—	20± 10
Goats	750± 200	160± 60	520± 280	—	70± 60
Pigs	3060±1320	810±590	1260±1020	160±610	830±500
Dogs	960±1220	280±260	320± 400	180±310	180±110
Cats	830± 320	510±270	200± 230	20± 50	100± 40
Rabbits	250± 200	140±130	80± 60	—	30± 10

Unit of cell number; thousand
*... Confidence interval ($\alpha=0.05$)

As shown in Table 2, a large number of cells appear in the milk of all the animals: the cells in 1 *ml* of cow's milk were estimated to be 300,000 in the total, the cells in the horse's milk 100,000, the cells in the goat's milk 750,000, the cells in the sheep's milk 100,000, and the cells in the rabbit's milk 250,000 at the middle stage of milk secretion; the cells in the pig's milk 3,060,000, the cells in the dog's milk 960,000 and the cells in the cat's milk 830,000 at the later stage.

As the next step of this investigation, the ratio of lymphoid cells, neutrophiles, eosinophiles and epithelial cells to the total of the cells is calculated, and the results are given in Table 3.

As shown in Table 3, the lymphoid cells and neutrophiles are the main components of the cells in the milk of all the animals; neutrophiles appear abundantly in cow's and goat's milk, reaching 60 and 69 per cent of the cells respectively, but moderately in pig's and dog's milk, showing 41 and 33 per cent, and slightly in other animal's milk, reaching only to 20 to 30 per cent,

where, however, the lymphoid cells appear abundantly, showing about 50 per cent.

Table 3. The ratio of various cells in milk to the total.

	Lymphoid cells	Neutrophiles	Eosinophiles	Epithelial cells
Horses	60.0%	30.0	—	10.0
Cows	23.4	60.0	3.3	13.3
Sheep	50.0	30.0	—	20.0
Goats	21.3	69.3	—	9.4
Pigs	26.2	41.1	5.2	27.5
Dogs	29.1	33.3	18.8	18.8
Cats	61.4	24.2	2.4	12.0
Rabbits	56.0	32.0	—	12.0

Eosinophiles appear in small amounts if any: 3.3 per cent in cow's milk, 5.2 per cent in pig's milk and 2.4 per cent in cat's milk. The only exception is dog's milk which contained 18.8 per cent of those among other cells at the later stage of its secretion. In the case of eosinophiles, moreover, their appearance is limited only at the stoppage stage of milk secretion of all the animals except cows.

No difference is found in the ratio of epithelial cells to the total among the milk of animals used.

Histological and histochemical observations of the cells in milk

In this investigation, histological and histochemical features of lymphoid cells, neutrophiles and epithelial cells were observed, and the results of the lymphoid cells are given in Table 4, those of the neutrophiles in Table 5, and those of epithelial cells in Table 6. Further, the lymphoid cells and neutrophiles in the milk were compared with those in the blood and in the mammary interstitium of mice, and also with the epithelial cells of mammary gland of mice.

As shown in Tables 4, 5 and 6, there is no essential difference in the stainability of each kind of the cells among all the animals used.

The lymphoid cells and neutrophiles in the milk showed a weaker reaction of alkaline phosphatase and peroxydase than in the blood, and the number of their mitochondria and Golgi bodies was less than that of those in the blood and in the mammary interstitium of mice, all these respects resembling those of the lymphoid cells and neutrophiles desquamated into the lumen of the mammary glands. On the contrary, neutral mucopolysaccharides, lipids, acidophilic and basophilic substances in the cells in milk are more abundant than those in the blood and mammary interstitium of mice.

Table 4. Histological and histochemical features of lymphoid cells in milk.

		Horses	Cows	Sheep	Goats	Pigs	Dogs	Cats	Rabbits	Mice: Lumen of mammary glands	Mice: Mammary interstitium	Mice: Blood
Alkaline phosphatase		−(−～+)	−(−～±)	−(−～+)	−(−)	−(−～+)	−(−～±)	−(−～+)	−(−)	−(−～+)	−(−)	−(−)
Peroxydase		−	−	−, ±	−	−, ±	−	−, ±	−	−, ±		−, ±
Glycogen		−	−		−	−	−	−	−	−	−	−
Neutral mucopolysaccharides		−～±	−～±		−～±	−～±	−～±	−～±	−～±	−～±	−～±	−
Lipids		−～±	−～±		−～±	−～±	−～±	−～±		−～±	−～±	−
Calcium salts	Von Kossa	−～+	−～+		−～+		−～+		−～±	−～+	−	−
	Gomori	−	−		−～+				−			
Mitochondria		+～±	+～±	+～±	+～±	+～±	+～±	+～±	+～±	+～±	±	±
Golgi bodies			−～+		−～+				−～±	−～±	+～±	+～±
Acidophilic substances	Eosin	+, ±	+, ±	+, ±	+, ±	+, ±	+, ±	+, ±	+, ±	+, ±	−, ±	−
	Acid fuchsin	±～±	+～±	±～±	+～±	±	±	±	±	+～±	−～+	−～+
	Carbol fuchsin	±	+～±	±	−～±			±	−～±	+～±	−～+	−～+
Basophilic substances	Azure II	−, ±	−, ±	−, ±	−, ±	−, ±	−, ±	−, ±	−, ±	−, ±	−～±	−, ±
	Toluidine blue	−, ±	−, ±	−, ±	−, ±	−, ±	−, ±	−, ±	−, ±	−, ±	−, ±	−, ±
	Thionin		−, ±	−, ±	−, ±	−, ±		−, ±	−, ±	−, ±	−, ±	−, ±
Supravital stain	Neutral red	−～+	−～±	−～±	−～±			+～±	−～±	−～±		−～+
	Janus green	−	−	−	−			−	−	−		±
Vital stain (Trypan blue)										−	−～±	−～±

( )…Pseudo-alkaline phosphatase reaction.

Table 5. Histological and histochemical features of neutrophiles in milk.

		Horses	Cows	Sheep	Goats	Pigs	Dogs	Cats	Rabbits	Mice	
										Lumen of mammary glands	Mammary interstitium
Alkaline phosphatase		− ~ ++	− ~ ++	− ~ ++	− ~ +	− ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ ++	++
Peroxydase		+ ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ ++	++
Glycogen		− ~ ++	− ~ ++		− ~ ++	− ~ ++	− ~ ++	− ~ ++	++	− ~ ++	− ~ ++
Neutral mucopolysaccharides		− ~ ++	− ~ ++		− ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ +
Lipids		− ~ ++	− ~ ++		− ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ +
Calcium salts	Von Kossa		− ~ +		− ~ +		− ~ ++		− ~ +	− ~ +	−
	Gomori	−	− ~ +		− ~ +		− ~ +		− ~ +		
Mitochondria		− ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ ++	− ~ ++	++
Golgi bodies			− ~ +		−				− ~ ++	− ~ ++	++
Acidophilic substances	Eosin	++	++	++	++	++	++	++	++	++	+
	Acid fuchsin	++	+ ~ ++	++	++	++	+ ~ ++	++	++	++	+
	Carbol fuchsin	− ~ ++	− ~ +	− ~ ++	+ ~ ++			− ~ ++	− ~ ++	+ ~ ++	−
Basophilic substances	Azure II	−	−	−	−	−	−	−	−	−	−
	Toluidine blue	−	−	−	−	−	−	−	−	−	−
	Thionin		−	−	−	−		−	−	−	−
Supravital stain	Neutrald red	− ~ +	− ~ +		− ~ ++		− ~ ++	− ~ ++	− ~ ++	++ ~ ++	+ ~ ++
	Janus green	−	−		−		−	−	−	−	++
Vital stain (Trypan blue)										−	−

Table 6. Histological and histochemical features of desquamated epithelial cells into milk.

		Horses	Cows	Sheep	Goats	Pigs	Dogs	Cats	Rabbits	Mice: Lumen of mammary glands	Mice: Epithelium of mammary glands
Alkaline phosphatase		−	−	−	−	−	−	−	−	−~+	+~++
Peroxydase		−	−	−	−	−	−	−	−	−	−
Glycogen		−	−		−	−	−	−	−	−	−
Neutral mucopolysaccharides		−~+	−~+		−~+	−~+	−~+	−~+	−~+	+	−
Lipids		−~++	−~++		−~++	−~++	−~++	−~+	−~+	−~+	++~++
Calcium salts	Von Kossa	−	−		−		−		−	−	−
	Gomori	−	−		−		−		−		
Mitochondria		−	−	−	−~+	−~+	−	−	−~+	−	++~++
Golgi bodies			−		−				−	−	+~++
Acidophilic substances	Eosin	+	+	+	+	+	+	+	+	+	−
	Acid fuchsin	−~+	−~+	−~++	−~+	−~+	−~+	−~+	−~+	−~++	−~+
	Carbol fuchsin	−	−	−	−			−	−	−	−
Basophilic substances	Azure II	−	−	−~+	−	−	−	−~+	−~+	−~++	−~++
	Toluidine blue	−	−	−~+	−	−	−	−~+	−~++	−~++	++
	Thionin		−~+	−~+	−~+	−~+		−~+	−~+	−~++	++
Supravital stain	Neutral red	−	−	−	−			−	−	−	
	Janus green	−	−	−	−			−	−	−	

The epithelial cells desquamated into the milk showed more intense reaction of neutral mucopolysaccharides and acidophilic substance, as compared with those in the mammary glands of mice. On the contrary, they have less amount of lipids and basophilic substance and less number of mitochondria and Golgi bodies than those in the mammary glands do.

The histological and histochemical features of lymphoid cells, neutrophiles and epithelial cells are as follows.

1. Alkaline phosphatase

Treated by the Gomori's method, a weak reaction is found as fine granules in the cytoplasm and an intense one as irregular masses spreading evenly in the cytoplasm (Fig. 7).

Among the cells in the milk, only the neutrophiles show the alkaline phosphatase reaction; the cells in the pigs, dogs and cats are intense, and those in the cows, goats, sheep and rabbits are moderate. This reaction in neutrophiles generally found at the peripheral portion of the cytoplasm, and it rapidly decreases in the degenerating process.

The pseudo-alkaline phosphatase reaction, which is demonstrable without substrate, is found in a part of the large lymphoid cells except those in the goats and rabbits, showing irregular dark-brown granules (Fig. 8). This reaction in the large lymphoid cells is found as irregular granules in their cytoplasm, showing no alteration in the degenerating process.

No alkaline phosphatase reaction is found in the desquamated epithelial cells, except some of the mice, though this reaction is always found in the glandular epithalial cells.

2. Peroxydase

Treated by the benzidin-natrium-nitroprusside method for peroxydase, the neutrophiles and a part of lymphoid cells show blue granules as its reaction. The reaction appears abundantly at the peripheral portion and at the perinuclear portion of neutrophiles, and irregularly in lymphoid cells (Figs. 9 and 10).

Many of the peroxydase-rich neutrophiles appear in the milk of horses, pigs, dogs and cats, and the peroxydase-rich lymphoid cells abundantly in that of sheep, though rarely in that of pigs and cats.

The peroxydase reaction is found frequently in the large lymphoid cells of the blood, and always in the neutrophiles of the blood and mammary interstitium of mice. Further, this reaction is found in the neutrophiles just desquamated into the glandular lumen, but not in those during degeneration.

3. Glycogen

Stained by the PAS method, glycogen appears as red-colored coarse granules irregularly spread in the neutrophiles (Fig. 11).

The glycogen-rich neuctrophiles are abundantly found in the milk of

horses, dogs and cats, but rarely in that of cows and goats.

Glycogen is contained in almost all of the neutrophiles in the blood and mammary interstitium, as well as the neutrophiles in the milk of all the animals. Usually, the glycogen in the neutrophiles in the milk rapidly decreases in its amount at the onset of degeneration, but sometimes, it remains at a corner of the cytoplasm during degeneration.

No glycogen is found in the lymphocytes, large lymphoid cells and epithelial cells of any animals used.

4. Neutral mucopolysaccharides

Stained by the PAS method, red-colored substance, which is undigestible by saliva, appears in some of the lymphocytes, of the lymphoid cells and all of the neutrophiles, increasing in its amount during degeneration. This increase is especially remarkable in the lymphocytes. The large lymphoid cells contain a small amount of neutral mucopolysaccharides mainly composed of basophilic substance that will be described in Section 12. Their localization in the lymphoid cells differs from that in the lymphocytes and neutrophiles; in the large lymphoid cells, they are either localized in masses in the cytoplasm, or distributed near the peripheral portion in a shape of a band, and those in the lymphocytes and neutrophiles are stained diffusely throughout the cytoplasm (Fig. 12).

The lymphocytes, lymphoid cells and neutrophiles in the blood and mammary intertsitium contain no neutral mucopolysaccharides within them.

5. Lipids

Stained with propylene glycol-Sudan black B, black colored globules of lipids appear in all the lymphoid cells, neutrophiles and epithelial cells.

Although the lipids are not contained in the normal lymphocytes and neutrophiles, they appear with the progress of degeneration, at the perinuclear region, showing small globules, and afterwards, they spread throughout the cytoplasm, pushing the nucleus toward a corner of the cytoplasm. Especially a large amount of lipids come to be contained in the large lymphoid cells which happen to possess a small amount of neutral mucopolysaccharides during degeneration. The lymphoid cells frequently result in the occupation of their cytoplasm only by lipid globules after the disappearance of nucleus. It can be added that there are some vacuoles among the lipid globules in the lymphoid cells (Fig. 18). Lipids are also found in the desquamated epithelial cells (Fig. 19).

The lipids are rarely found in the large lymphoid cells of the mammary interstitium, and none in the leucocytes of the blood.

No differences are found in the morphological aspect and localization of lipids in the lymphoid cells, neutrophiles and epithelial cells among all the animals. However, the amount of lipids in them differs among the animals;

abundantly in pigs, cats and rabbits, but slightly in horses, cows and goats.

6. Calcium salts

Treated by the Von Kossa's method, there sometimes appear black or black-brown colored irregular granules of calcium salts in the lymphoid cells and neutrophiles in the milk of all the animals except sheep, pigs and cats. Treated by the Gomori's method, there might be found some calcium salts in the lymphoid cells of goats, and in the neutrophiles of cows, goats dogs and rabbits. Without exception among the animals, these salts appear only in the cells at the early stage of degeneration.

No calcium salts are found in the desquamated epithelial cells, nor in the wandering cells in the blood and mammary interstitium.

7. Mitochondria

Stained with Heidenhain's iron-hematoxylin, fine granules of mitochondria are found in every kind of cells in the milk ; abundantly in the lymphoid cells and neutrophiles, slightly in the epithelial cells (Fig. 13).

The mitochondria in lymphocytes and neutrophiles decrease in their number during degeneration, while those in lymphoid cells increase at the early stage of degeneration, and then decrease gradually.

In the blood and mammary interstitium of mice, the mitochondria in lymphoid cells and neutrophiles are granular or rod-like in shape, stainable with supravital stain of Janus green, but the cells in the milk are not stainable.

8. Golgi bodies

Treated by the Aoyama's method, Golgi bodies become visible in the lymphoid cells and the neutrophiles of goats, rabbits and mice. In almost all the cases, they are granular in shape, excepting that those in the rabbits and mice are sometimes network in shape.

The Golgi bodies in the form of network appear in all the epithelial cells of mouse mammary glands, but not in those desquamated into the lumen. Neither did they appear in the desquamated epithelial cells in the milk of other animals.

In the blood and mammary interstitium of mice, Golgi bodies in the lymphoid cells and neutrophiles are found at the perinuclear region in a form of irregular network, and rarely in the form of granules.

9. Eosinophilic substance

Eosinophilic substance is found in the lymphoid cells and neutrophiles of all the animals. The distribution of this substance in the cytoplasm and the fluctuation in its amount are corresponding to those of neutral mucopolysaccharides.

10. Acid fuchsin positive substance

Stained with Masson's trichrome stain, a large number of acid fuchsin-positive granules are found in the lymphoid cells and neutrophiles, increasing

with the progress of degeneration. Its distribution differs according to the kinds of cells and the kinds of animals; this substance is found abundantly in some lymphoid cells of cows and goats, but not in the others. In other animals, this substance is contained in all the lymphoid cells. When its amount is compared between the neutrophiles and lymphoid cells, it is contained more abundantly in the neutrophiles than in the lymphoid cells in the cats, rabbits and mice, whereas an opposite situation happens in other animals.

Acid fuchsin granules in the epithelial cells appear in the shape of masses, and their number is much less than in the lymphoid cells and neutrophiles (Fig. 17). First, these granules appear at the perinuclear region in the form of small masses, grow larger, move to the upper portion of the cells, and drop into the lumen, this aspect coinciding with that of epithelial cells desquamated into the milk.

11. Carbol fuchsin-positive substance

Stained with carbol-fuchsin, red colored granules appear in the lymphoid cells and neutrophiles of all the animals used. In normal cells, the granules are found at the perinuclear region and during the course of degeneration, they become irregular in shape and become spread in the cytoplasm (Fig. 16). In the large lymphoid cells of horses at the later stage of degeneration, the carbol fuchsin granules are found frequently among the vacuoles in the form of irregular network (Fig. 15).

No carbol fuchsin-positive granules are found in the epithelial cells of any animals used.

12. Basophilic substance

Stained with Azure II, toluidine blue or thionin, many of blue-purple colored fine granules of basophilic substance appear in the large lymphoid cells, which possessed a large amount of lipids and a little neutral mucopolysaccharides (Fig. 14). This basophilic substance is determined to be RNA, because it is easily digested by ribonuclease. At the early stage of degeneration of the lymphoid cells, in which lipid globules appear, the RNA increases in the amount, and at the later stage of degeneration, more lipids came to be stored, while the RNA decreases with the degeneration process, and finally disappears.

No difference is found in the amount of RNA in large lymphoid cells among the animals, excepting that the amount is less in the cows, goats and horses than in other animals.

The RNA is only rarely found in the epithelial cells. The amount of it is less than that in large lymphoid cells.

13. Supravital stain

When supravital stain is done using the mixture of Janus green and neutral red, neutral red granules are found in the lymphoid cells and neutrophiles.

The granules are larger in the former cells, one to several in number appearing, and those are smaller in the latter cells, spreading out irregularly. These granules are more in the cells of rabbits, cats and mice, and less in the horses, cows and sheep.

In the mouse blood, neutral red granules appear in most of the monocytes and neutrophiles. The granules are larger in the former cells, and smaller in the latter ones.

The Janus green demonstrates granular or rod-like mitochondria, appearing only in the leucocytes of the mouse blood, but not in the cells in the milk of any animals.

14. Trypan blue

The vital stain of trypan blue is used only to mice. This dye is stored in the blood monocytes and in the histiocytes of the mammary interstitium.

Discussion

Histological studies of cells in the milk have been made by Varrior-Jones (7), Kuboshima *et al.* (6) and Blackburn and Macadam (1). Varrior-Jones, using the Jenner's method, differentiated the cells in cow's milk into nine kinds as follows; finely granular eosinophiles, coarsely granular eosinophiles, basophiles, large epithelial cells, large neutrophiles, lymphocytes, red blood corpuscles, mononuclear eosinophiles, and large mononuclear leucocytes. Kuboshima *et al.* differentiated the cells in goat's milk into five kinds as follows; neutrophiles, eosinophiles, lymphocytes, epithelial cells and unknown cells that are large in shape, show nuclear piknosis and have pyronin granules in the cytoplasm. Further, they stated that monocytes and plasma cells are only rarely found. Blackburn and Macadam, using the method of Prescott and Breed, differentiated the cells in cow's milk into two; the granular leucocytes and non-granular cells, the former cells containing polymorphs, eosinophiles and basophiles, and the latter ones containing lymphocytes, non-vacuolated epithelial cells, vacuolated epithelial cells, monocytes, macrophages and plasmacytes. In this investigation, the lymphoid cells, neutrophiles, eosinophiles and epithelial cells are always found in the milk of any animals used, and the basophiles are rarely found.

It was noticed that the opinions diverge among the investigators mentioned above, especially with ragard to the differentiation between the epithelial cells and large lymphoid cells. In this investigation, the lymphoid cells and epithelial ones are clearly differentiated by the histochemical techniques; the former cells store granules of neutral red, acid fuchsin, carbol fuchsin and neutral mucopolysaccharides or RNA in the cytoplasm, and showed the nuclear piknosis during the degeneration, whereas the latter cells contained no substances described above, and showed no nuclear piknosis during the de-

generation.

Waite and Blackburn (8) reported that in Scotland, there are many cows which have a large number of the cells, especially the neutrophiles, in the milk, but not suffered from mastitis, and the investigators called these phenomena "nonspecific mastitis". They further stated that when the number of cells in the milk is less than 100,000 per 1 *ml*, the neutrophiles are also less, which, however, increase with the increase of the total cells in the milk.

In this investigation, it is suspected that the cows and goats usually suffer from non-specific mastitis, considering from two facts as follows: Firstly, there are a larger number of cells in the milk of the dairy cattle, such as cows and goats, as compared with other animals. Secondly, the ratio of neutrophiles is higher in the cows and goats, showing about 60 per cent, than in other animals.

Merchant and Packer (5) reported that the number of the cells in the normal cow's milk is 900,000 per 1 *ml* at the later stage of milk secretion, and 60,000 per 1 *ml* at the secreting stage.

In this investigation, the number of the cells in cow's milk is 300,000 per 1 *ml* at the secreting stage, clearly showing an abnormal value. The number of the cells in the milk of horses, sheep, rabbits and goats are 100,000, 250,000 and 750,000 respectively at the secreting stage showing higher values as compared with those of Merchant and Packer. The number of the cells in the milk of dogs, cats and pigs are 960,000, 830,000 and 3,000,000 respectively, at the later stage of milk secretion, showing normal values in dogs and cats, but abnormal value in pigs, as compared with those of Merchant and Packer.

Maximow (4) called lipids-rich cells in the milk "colostrum bodies". In this investigation, it was found that the lymphoid cells, neutrophiles and epithelial cells in the milk come to store lipids in their cytoplasm during the course of degeneration. Therefore, the colostrum bodies of Maximow are thought to be something transformed from the lymphoid cells, neutrophiles and epithelial cells. However, the lymphoid cells must be most intimately related to the formation of colostrum bodies, judging from the following two reasons: Firstly, the epithelial cells are much less in number and also store less amount of lipids than the lymphoid cells and neutrophiles during degeneration. And secondly, as already stated, the increase of neutrophiles should be caused by the mammary inflammation such as so-called non-specific mastitis of Waite and Blackburn.

Summary

The results obtained in this investigation are summarized as follows:

The lymphocytes, large lymphoid cells, neutrophiles and epithelial cells appear in the milk of the domestic animals used: the neutrophiles and

lymphocytes abundantly in the cow's and goat's milk, the lymphocytes and large lymphoid cells abundantly in the horse's, sheep's, goat's, cat's, rabbit's milk and the neutrophiles, lymphocytes and large lymphoid cells moderately in the pig's milk.

The histological and histochemical natures of each cell in the domestic animals are similar. The majority of the cells in the milk show degenerative aspects, and during this course, the lymphocytes, large lymphoid cells and neutrophiles store a large amount of neutral mucopolysaccharides, lipids and acidophilic substance, whereas they lose alkaline phosphatase, peroxydase, glycogen, Golgi bodies and mitochondria. The desquamated epithelial cells have no neutral mucopolysaccharides nor acidophilic substances. The lymphocytes, large lymphoid cells and neutrophiles show a piknosis of nucleus during their degeneration, whereas the epithelial cells do not.

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PLATES

Plate 1

Explanation of Figures

All figures show various degenerating cells in the secreted milk of various kinds of domestic animals. Maximow's stain. Drawn. ×2000.

Figs. 1 and 2. Desquamated glandular epithelial cells.
The cells in Fig. 1 have vacuolated cytoplasm which is weakly stained with eosin, but the cells in Fig. 2 have none. Nuclei of these cells show karyolysis in the degenerating process, and their cytoplasm gradually dissolves with the advance of degeneration finally to disappear.

Fig. 3. Neutrophiles.
Their nuclei show piknosis, and their cytoplasm becomes easily stainable with eosin, and swells with many vacuoles in their advanced degeneration.

Fig. 4. Small lymphocytes.
Their degenerating features are the same as the above.

Fig. 5. Large lymphoid cells.
Their nuclei show typical piknosis, and their cytoplasm is strongly stained with Azure II and swells with many vacuoles. When the degeneration advances, the cytoplasm loses its basophilic nature and becomes weakly eosinophilic.

Fig. 6. Eosinophiles.
Their nuclei also show piknosis, but their cytoplasm does not swell and contains no vacuoles. In a later stage of degeneration, eosinophilic granules in their cytoplasm are discharged outside and disappear.

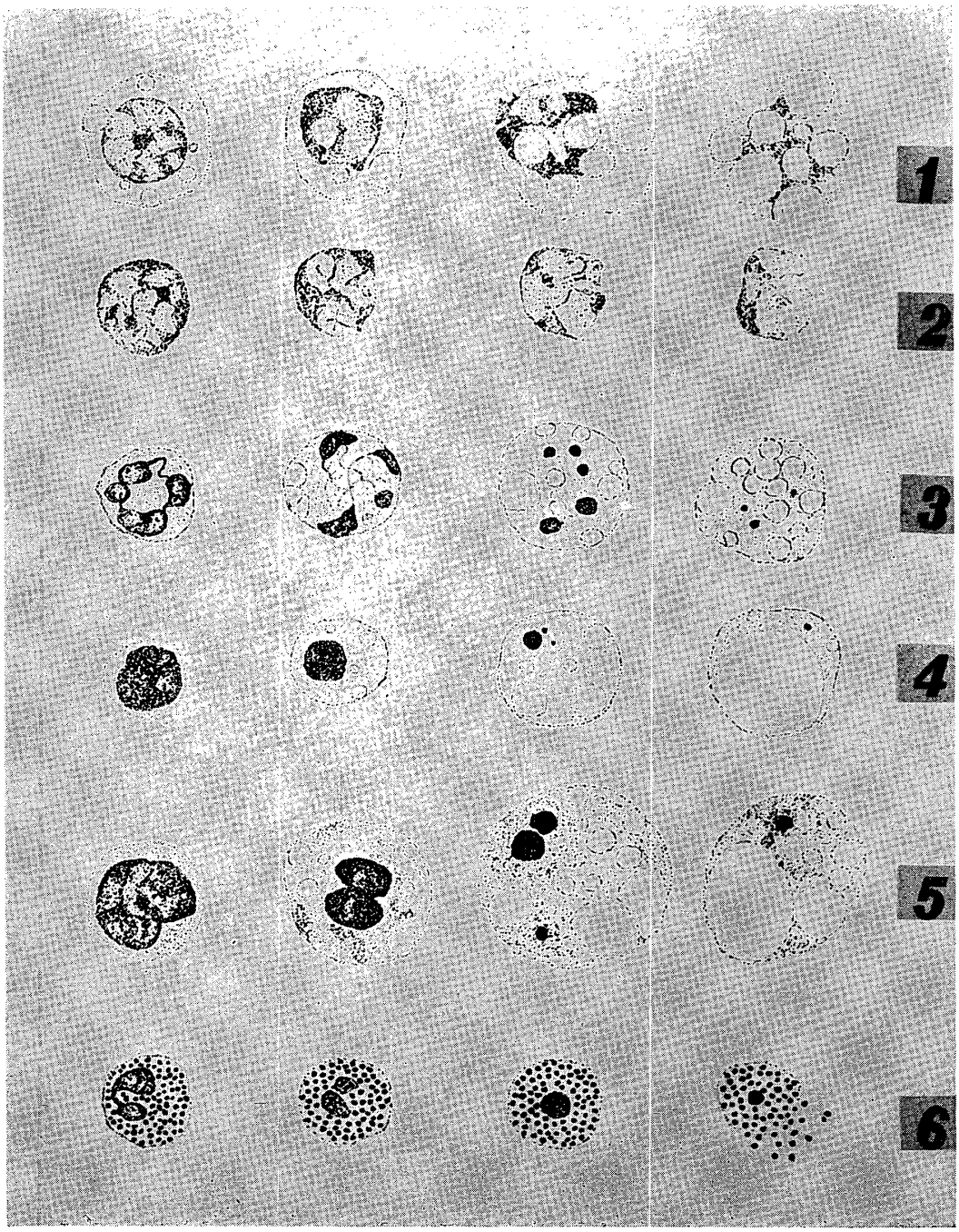
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Plate 2

Explanation of Figures

Fig. 7. Alkaline phosphatase in neutrophiles in dog's milk. Gomori's stain. ×2000.
The enzyme is prominent in the cytoplasm.

Fig. 8. Pseudo-alkaline phosphatase in a large lymphoid cells in horse's milk. Gomori's stain. ×2000.
This substance is irregularly spread in the cytoplasm in the form of coarse granules.

Fig. 9. Peroxydase in a neutrophile in cow's milk. Natrium-nitroprusside stain. ×2000.
The enzyme appears in the cytoplasm in the form of uniformly small granules.

Fig. 10. Peroxydase in a large lymphoid cell in sheep's milk. Natrium-nitroprusside stain. ×2000.
The distribution of this enzyme in this cell is the same as the above.

Fig. 11. Glycogen in neutrophiles in horse's milk. PAS stain. ×2000.
The glycogen appears abundantly in the cytoplasm in the form of irregular coarse granules.

Fig. 12. Neutral mucopolysaccharides in lymphoid cells in mouse's milk. PAS stain. ×2700.
These substances appear diffusely in the cytoplasm.

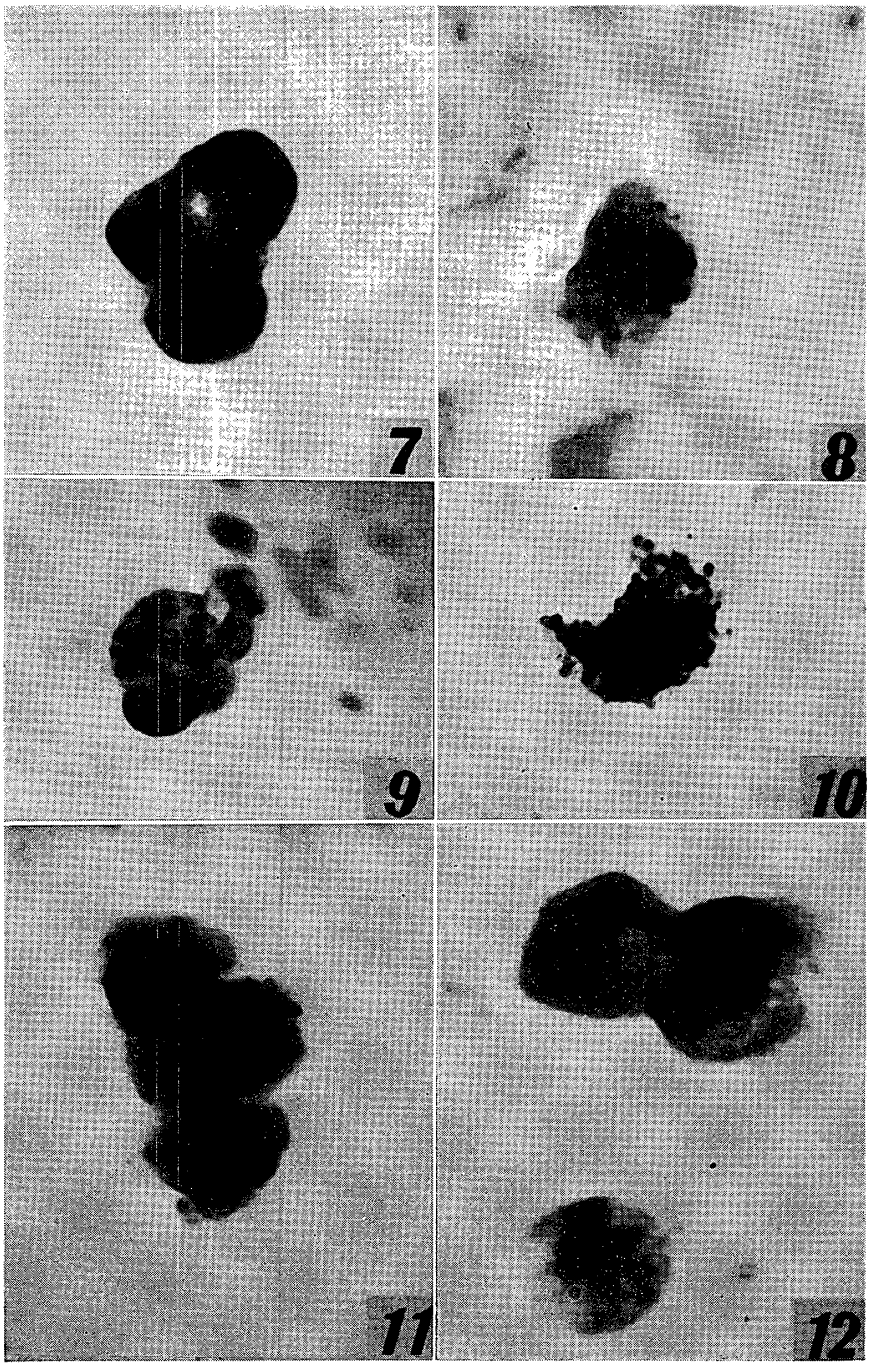
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Plate 3

Explanation of Figures

Fig. 13. Mitochondria in a large lymphoid cell in cow's milk. Heidenhain's stain. ×2000.
The mitochondria appear abundantly in the form of coarse granules.

Fig. 14. RNA in a large lymphoid cell in rabbit's milk. Toluidine blue stain. ×2000.
The RNA appears so abundantly in the cytoplasm that it seems to spread diffusely.

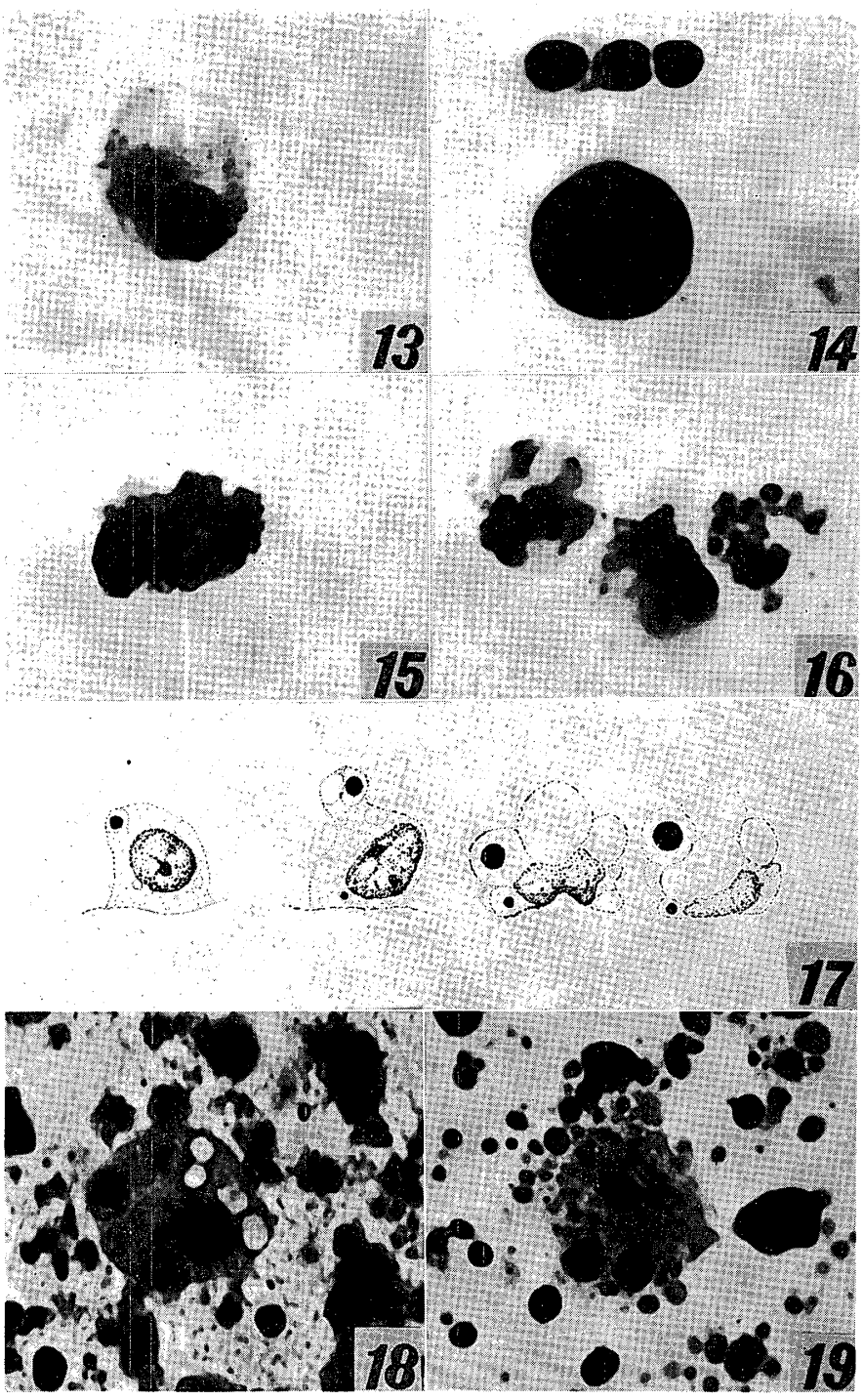
Fig. 15. Carbol fuchsin-positive granules in a lymphoid cell in horse's milk. Carbol fuchsin stain. ×2000.
The granules appear in the cytoplasm forming a frothy network.

Fig. 16. Carbol fuchsin-positive granules in a lymphocyte and some neutrophiles in goat's milk. Carbol fuchsin stain. ×2000.
The granules appear in the cytoplasm in irregular forms.

Fig. 17. Acid fuchsin-positive granules in the degenerating epithelial cells of mouse's regressive mammary gland. Masson's stain. Drawn. ×1500.
The granules first appear near the nucleus and gradually transmigrate to the upper border of the cell growing in size.

Fig. 18. Lipid globules in a lymphoid cell in pig's milk. Sudan black B stain. ×2000.
The globules increase in size and in number with the advance of degeneration.

Fig. 19. Lipid globules in a desquamated epithelial cell in pig's milk. Sudan black B stain. ×2000.
The outline of this cell is not clear, and the lipid globules in it gradually decrease in number with the advance of degeneration.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