HISTOLOGY OF THE MAMMARY GLAND III. WANDERING CELLS IN MAMMARY TISSUES AT THE FARROWING AND WEANING STAGE AND THEIR RELATION TO CIRCULATING BLOOD LEUCOCYTES IN MICE

HISTOLOGY OF THE MAMMARY GLAND
III. WANDERING CELLS IN MAMMARY TISSUES AT THE FARROWING AND WEANING STAGE AND THEIR RELATION TO CIRCULATING BLOOD LEUCOCYTES IN MICE

By

Mitsuo OKADA

Department of Animal Husbandry, Faculty of Agriculture, Tohoku University, Sendai, Japan

(Received December 1, 1957)

Introduction

It is well known that the occurrences of the colostrum bodies at the pre- and post-partum and at the weaning stage are closely related to an increase of the wandering cells in the mammary tissues (10). Emmel *et al.* (3) studied the relation between leucocytes and lactation in the albino rats, and reported that a definite leucopenia occurred during active nursing and that an increased passage of the lymphoid cells into the mammary alveoli occurred in correlation with a decrease of the same elements in the circulating blood. There remains, however, questions as to the relation between the behavior of the wandering cells that migrated into the mammary tissues and the circulating blood leucocytes at the farrowing and weaning stage, when the colostrum bodies appeared abundantly. Moreover, no one has investigated the relation between the wandering cells which appeared in the mammary tissues at the pre- and post-partum and weaning stage and the circulating blood leucocytes at the same time.

The scope of the present investigation is to determine the variation in differential leucocyte counts of the circulating blood through the stages of non-pregnancy, pregnancy, lactation and post-weaning, as well as the cytochemistry and the behavior of the cellular elements which appeared in the mammary tissues, as the third step of the investigation concerning the histology of the mammary gland.

Materials and Methods

Adult female mice of Swiss strain, four to five months old, were used. Each animal was kept under the same feeding and management. The first day

of pregnancy was determined by the presence of the vaginal plug. The youngs were weaned at the 22nd day of post-partum.

Blood samples were obtained, at 9.00 to 9.30 A. M. before feeding, by lancing the tail veins with a sharp razor edge. The first flow of the blood was avoided and the second flow was used for the differential leucocyte counts. For differential cell counts, blood smears were stained by the Mäy-Giemsa method.

The animals which were at the immediate post-partum, mid-lactation and at the second day of post-weaning were anaesthetized with ether, and pieces of the thoracic, abdominal and inguinal mammary glands were taken. They were fixed in Zenker-formol solution, embedded in paraffin, and cut at the thickness of 6μ.

The staining methods were as follows: For the wandering cells, hematoxylin-Azure II-eosin (Maximow's) stain; for polysaccharides, PAS method modified by Lillie. The identification of glycogen was made by means of the salivary test (37°C, 2 hours). For ribonucleic acid, toluidine blue stain. Ribonucleic acid was confirmed by the ribonuclease treatment (37°C, 1 hour).

Results

1. *The variation in the differential leucocyte counts during the resting, pregnant, lactating and weaning stage.*

The results obtained for the blood leucocytes are presented in Table 1 and Fig. 1. As shown in Table 1 and Fig. 1, monocyte counts showed a slight

Table 1. Changes in leucocyte counts during the resting, pregnant, lactating and post-weaning stage.

Stages		Number of animals used	Number of leucocytes counted	Proportion of various kinds of leucocytes (percentage)			
				Monocytes	Lymphocytes	Neutrophiles	Eosinophiles
Resting		10	3191	9.0(10.0-8.0)*	69.2(70.9-67.4)*	19.5(20.0-18.3)*	2.3(2.9-2.0)*
Pregnancy	3rd	7	1362	9.0(10.5-7.7)	71.0(73.2-69.1)	17.6(19.3-15.9)	2.4(3.3-1.9)
	7th	7	1922	9.1(10.7-7.2)	70.9(74.0-69.6)	17.5(18.5-14.7)	2.5(3.4-1.9)
	11th	6	1181	9.6(11.7-8.3)	67.6(70.0-65.6)	20.1(22.0-18.7)	2.7(3.7-2.1)
	15th	6	1279	10.3(11.9-8.9)	64.3(66.1-61.7)	24.0(26.3-22.0)	1.4(2.0-1.0)
	19th	13	3079	9.4(11.3-9.3)	66.3(66.5-66.3)	23.9(24.0-22.6)	0.4(0.7-0.3)
Lactation	1st	11	2712	8.8(9.6- 7.8)	69.1(70.8-69.2)	21.7(21.7-20.5)	0.4(0.7-0.3)
	4th	12	2151	7.7(8.9- 6.6)	69.5(71.1-69.5)	22.0(24.6-21.6)	0.8(1.2-0.5)
	9th	14	4257	10.0(10.0- 9.3)	64.5(65.0-64.0)	24.5(24.7-24.0)	1.0(1.4-0.9)
	13th	9	2120	9.0(9.9- 7.6)	63.4(65.0-63.3)	27.5(29.1-25.7)	1.1(1.5-0.7)
	18th	8	1405	14.5(16.3-13.2)	54.5(55.7-52.4)	29.0(32.2-27.9)	2.0(2.6-1.7)
Post-weaning	2nd	6	1568	15.1(16.5-13.6)	59.1(61.0-57.4)	23.0(25.0-21.4)	2.7(3.5-2.1)
	5th	6	1171	16.2(18.3-14.7)	62.9(65.0-60.0)	17.8(19.7-16.1)	3.1(4.2-2.5)

Note: The animals were weaned at the 22nd day post-partum.
* : Confidence interval ($\alpha = 0.05$).

increase at the middle stage of pregnancy and a remarkable increase at the post-weaning stage. Lymphocyte counts showed a slight decrease at the later stage of pregnancy and lactation. On the contrary, however, neutrophile counts showed a slight increase at the later stage of pregnancy and a remarkable increase at the later stage of lactation. Eosinophile counts showed a definite decrease with advanced pregnancy. This decrease was maintained during the active lactation period.

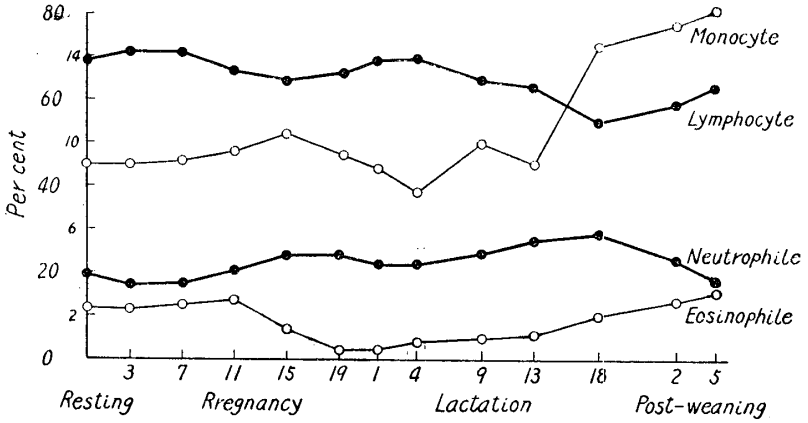


Fig. 1. Differential leucocyte counts in circulating blood.

2. *The wandering cells in the mammary tissues at the farrowing, mid-lactation and post-weaning stage.*

Most of the wandering cells in the mammary tissues were lymphoid cells composed of small and large lymphocytes, plasmacytes, monocytes and histiocytes. The remaining wandering cells were composed of neutrophiles, eosinophiles and mast cells. The number of these wandering cells is presented in Table 2.

As shown in Table 2, the wandering cells in the mammary tissues were more at the farrowing and weaning stage (Figs. 2 and 3) than at the mid-lactation. The increase of the wandering cells was somewhat more pronounced at the post-weaning stage. It was noticed that in the mammary tissues at this stage a great number of neutrophiles migrated into the intralobular connective tissue and then penetrated into the glandular epithelia and lumina (Fig. 4). Eosinophiles and mast cells were found in the interstitium alone. They showed no penetration into the glandular epithelia and lumina at any stage.

The number of the lymphoid cells that penetrated into the glandular epithelia showed no difference according to the stage, but the number of the lymphoid cells that penetrated into the glandular lumina showed an increase at the farrowing and post-weaning stage. No penetration of neutrophiles into the glandular epithelia was found at the farrowing stage and mid-lactation.

Table 2. Number of wandering cells in the mammary tissues.

	Immediate post-partum	Mid-lactation	2nd day of post-weaning
Number of animals used	5	6	6
Interstitium			
Lymphoid cells**	209 ±36.4*	126 ± 4.7*	226 ±43.6*
Neutrophiles**	3.0 ± 2.0	0.7 ± 0.7	8.0 ± 2.8
Eosinophiles	2.6 ± 1.1	1.0 ± 0.9	2.0 ± 1.2
Mast cells	1.5 ± 1.0	2.5 ± 1.5	3.0 ± 1.9
Glandular epithelia			
Lymphoid cells	26 ± 7.5	21 ± 5.0	22 ±11.4
Neutrophiles**	0	0	1.5 ± 1.2
Eosinophiles	0	0	0
Mast cells	0	0	0
Glandular lumina			
Lymphoid cells**	8.0 ± 1.5	2.6 ± 1.9	13.7 ± 3.0
Neutrophiles**	0.2 ± 0.4	0	7.3 ± 1.5
Eosinophiles	0	0	0
Mast cells	0	0	0

Note: Each cellular element is counted from 50 alveoli. Lymphoid cells are composed of normal and degenerative lymphocytes, plasmacytes, monocytes and histiocytes. Interstitium indicates the intralobular connective tissue area at the distance of 10μ from the alveolus. *: Confidence interval ($\alpha = 0.05$). **: Differences among three stages are statistically significant ($\alpha = 0.05$).

3. *Comparative cytochemistry of the lymphoid cells that appeared in the mammary tissues at the farrowing and post-weaning stage.*

The lymphoid cells which appeared in the intralobular connective tissue area of the mammary glands at the farrowing and post-weaning stage were composed of small and large lymphocytes, plasmacytes, monocytes and histiocytes. The small lymphocytes were characterized by their small round nuclei stained dark blue with basic dyes and hematoxylin and had a slight amount of cytoplasm. The typical plasmacytes were distinguished by whell-like nuclei and the surrounding pale area. Sometimes binucleate plasmacytes were found. Two kinds of plasmacytes were found; one was rich in RNA and the other was poor in it. Large lymphocytes, monocytes and histiocytes could not be distinguished from one another. These cellular elements are described as large lymphoid cells. Some of them had large round, oval or kidney shaped nuclei and relatively a large amount of cytoplasm stained with hematoxylin and toluidine blue. The remaining had large irregular, oval or kidney shaped nuclei stained from dark blue to pale blue with basic dyes and had flat or elongated cytoplasm unstained with basic dyes such as Azure II or toluidine blue. The basic substance in the cytoplasm of the large lymphoid cells was

RNA. It was also found that RNA rich lymphoid cells appeared abundantly in the mammary tissues at the farrowing stage (Fig. 5) but RNA poor lymphoid cells appeared abundantly in the mammary tissues at the post-weaning stage.

The lymphoid cells that stained weakly or moderately by the PAS method were found frequently in the mammary tissues. Some of them had variable amounts of small granular inclusions stained sharply by the PAS method (Fig. 6). Since the PAS positive substance in the lymphoid cells was not digested by saliva (37°C, 2 hours) and unstained metachromatically with toluidine blue, it was found to be neutral mucopolysaccharides. These PAS positive lymphoid cells were rarely found in the mammary tissues at the farrowing stage but abundantly in the mammary tissues at the post-weaning stage.

Discussion

As already mentioned, the majority of the large lymphoid cells which appeared in the intralobular connective tissue area of the mammary glands at the farrowing stage had a considerable amount of RNA. It is known that the lymphocytes, especially the monocytes and plasmacytes in younger stage contain RNA. Thus the lymphoid cells just stated were obviously ordinary types of young lymphocytes, monocytes and plasmacytes. On the contrary, however, since the lymphoid wandering cells which appeared in the mammary tissues at the post-weaning stage were composed of many PAS positive large lymphoid cells and RNA poor large lymphoid cells, they may be considered as the cells in the older or degenerative stage accompanied with the regression of the mammary glands.

The neutrophiles which migrated into the mammary tissues at the farrowing stage were very few in number as in the mid-lactation. At the post-weaning stage, however, active penetration of the neutrophiles into the intralobular connective tissue, the glandular epithelia and lumina was found. At this stage, the mammary glands began to take a regressive change, and accordingly, the active penetration of the cells mentioned above into the mammary tissues may be considered as an inflammatory process due to the regression of the mammary glands.

Jeffers (7) stated that the cellular elements which appeared within the mammary alveoli at the regression stage were a picture of degeneration of the wandering leucocytes, especially of the lymphoid wandering cells and of the neutrophiles, attending to the regression and involution of the mammary glands. If the relation is accepted in the present investigation, it becomes highly probable that an increase of the neutrophiles and monocytes in the circulating blood at the later stage of lactation and weaning stage is attended to the regression of the mammary glands.

Merril and Smith (9), in cow, and Luke (8), in swine, reported that

eosinopenia, lymphopenia and neutrophilia occurred at the farrowing time. Halberg and Bock (4) observed eosinopenia at the last-third pregnancy and immediate post-partum in mice. In the present investigation, as already mentioned, the eosinophile counts in the circulating blood showed a remarkable decrease at the pre- and post-partum, they began to increase with lactation and finally reached the normal counts at the post-weaning stage. The results just stated seem to indicate that the farrowing stage differs physiologically from the weaning stage according to the following relations; first, the wandering cells, such as lymphoid cells containing RNA appeared abundantly in the mammary tissues at the farrowing stage, while neutrophiles and PAS positive large lymphoid cells containing no RNA appeared abundantly at the post-weaning stage; second, the eosinophiles in the circulating blood decreased in the former stage, but increased in the later stage as mentioned above.

Dougherty and White (2), White and Dougherty (13) and Jakobson and Hortling (5, 6) proved that the regulation of circulating lymphocytes and eosinophiles was under the control of the pituitary-adrenal system. Poulton and Reece (11), Andersen and Sperry (1) and Schultze (12) reported that a sharp increase in the activity of the pituitary-adrenal system occurred during parturition. Merril and Smith (9) indicated that the decrease of lymphocytes and eosinophiles at the farrowing time was due to an increased secretion from the adrenal cortex in resposne to the stress of parturition. It is suggested that there is a close correlation between the increased migration of the lymphoid wandering cells containing much RNA in the mammary tissues at the pre- and post-partum as already mentioned and an increased activity of the pituitary-adrenal system with advanced pregnancy and parturition as shown by Merril and Smith (9). This will be fully discussed in another paper.

Summary

The scope of the present investigation was to determine whether the changes of the differential leucocyte counts in the circulating blood at the farrowing and post-weaning stage are correlated with the increase of the wandering cells in the mammary tissues at the same stage, and also whether the cytological characters differ in different cellular elements which appeared in the mammary tissues. The results are summarized as follows:

1. At the farrowing time, the eosinophile counts in the circulating blood showed a definite decrease, and the lymphoid cells containing RNA appeared abundantly in the mammary tissues.

2. At the post-weaning stage, the monocyte, neutrophile and eosinophile counts in the circulating blood showed an increase. At this stage the migration and penetration of neutrophiles, lymphoid cells containing no RNA and PAS positive lymphoid cells into the mammary tissues increased abundantly,

suggesting that the inflammatory process due to the regression of the mammary glands is occurring.

Acknowledgments: The author expresses his hearty thanks to Prof. Y. Toryu for his valuable suggestions throughout the course of this work, and to Asst. Prof. H. Tamate for his kindness during this work.

References

1) Andersen, D. H. and W.M. Sperry (1937). J. Physiol., **90**, 296.
2) Dougherty, T. F. and A. White (1944). Proc. Soc. Exp. Biol. and Med., **57**, 295.
3) Emmel, V. E., H. C. Weatherford and M. H. Streicher (1927). Am. J. Anat., **38**, 1.
4) Halberg, F. and F. Bock (1953). Proc. Soc. Exp. Biol. and Med., **83**, 339.
5) Jakobson, T. and H. Hortling (1953). Acta Endocrinol., **15**, 265.
6) Jakobson, T. and H. Hortling (1953). Acta Endocrinol., **15**, 368.
7) Jeffers, K. R. (1935). Am. J. Anat., **56**, 257.
8) Luke, D. (1953). Brit. Vet. J., **109**, 241.
9) Merril, W. G. and V. R. Smith (1954). J. Dairy Sci., **37**, 546.
10) Okada, M. (1956). Tohoku J. Agr. Res., **7**, 35.
11) Poulton, B. R. and R. P. Reece (1957). Endocrinol., **61**, 217.
12) Schultze, A. B. (1955). J. Dairy Sci., **38**, 611.
13) White, A. and T. F. Dougherty (1945). Proc. Soc. Exp. Biol. and Med., **58**, 135.

Explanation of Figures

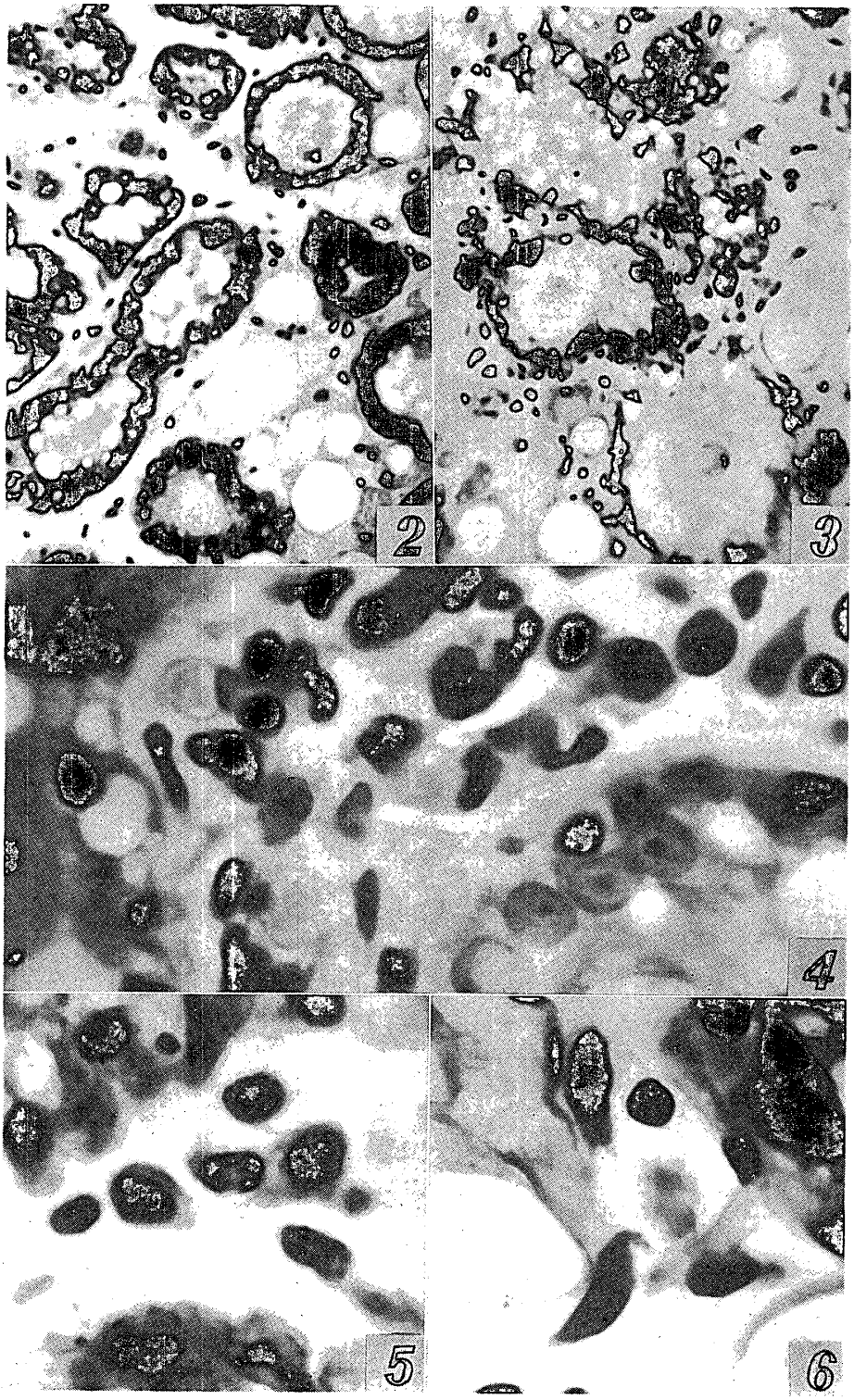
Fig. 2. Mouse mammary tissues at the farrowing time. Increased migration of lymphoid wandering cells in the intralobular connective tissue. ×450. Hematoxylin-Azure II-eosin stain.

Fig. 3. Mouse mammary tissues at the post-weaning stage. The regression of the alveoli and the migration of wandering cells in the intralobular connective tissue. ×450. Hematoxylin-Azure II-eosin stain.

Fig. 4. Mouse mammary tissue at the regression stage. Increased migration of neutrophiles in the intralobular connective tissue with regression of the glands. ×1800. Hematoxylin-Azure II-eosin stain.

Fig. 5. Mouse mammary tissue at the farrowing time. Large lymphoid cells containing RNA in the intralobular connective tissue. ×2000. Toluidine blue stain.

Fig. 6. Mouse mammary tissue at the regression stage. PAS positive large elongated lymphoid cells in the intralobular connective tissue. ×2000. PAS stain.


2
3
4
5
6