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著者	OKADA Mitsuo
journal or publication title	Tohoku journal of agricultural research
volume	10
number	4
page range	369-381
year	1960-01-20
URL	<a href="http://hdl.handle.net/10097/29293">http://hdl.handle.net/10097/29293</a>

# HISTOLOGY OF THE MAMMARY GLAND

## VI. EFFECTS OF ACTH ON THE CELL COUNT IN MILK AND IN VARIOUS ORGANS OF MILKING GOATS

By

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*(Received October 7, 1959)*

### Introduction

In an earlier paper of this series on the behavior of the lymphoid cells including the colostrum bodies in the mammary glands (11), it was shown, in the mouse and the rat, that the increased activity of the adrenal cortex would induce a migration and penetration of the lymphoid cells into the mammary glands resulting in an appearance of the typical colostrum bodies.

It may be assumed from the fact above mentioned that in dairy animals, such as the cow and the goat, the pituitary-adrenocortical system is involved in the mechanism by which the cellular elements are released into the secreted milk. The present work was designed to investigate this assumption using goats.

### Materials and Methods

Three milking Saanens, two years old, were used in the study. Each animal was used for two experiments shown in Table 1 with fixed intervals following the operation.

**Table 1.** Experimental schedule.

No. of animals used	Initial body weight (kg)	First experiment	Second experiment
1	38	ACTH injection	Pituitary graft
2	59	ACTH injection	ACTH injection
3	70	Pituitary graft	Untreated

Armour's ACTH dissolved in 5.0 cc of water at the ratio of 2 mg (2 I.U.) per kilogram of the body weight was administered intramuscularly to the animals. The pituitary of two years old bullock of the Japanese-brown breed was used. It was transplanted subcutaneously to the intact milking goats.

The grafted pituitary in No. 3 goat was removed at six days after the graft and this animal was used as the control in the second experiment.

In the first and second experiments, milk and blood samples were drawn from each animal at one or two hours before the operation, and at one, two, three and four days after the graft, and at six, 12, 24 and 48 hours after the injection. The blood was obtained from the external jugular vein. The milk samples were obtained from the whole secreted fresh milk and examined.

Blood leucocytes were counted by the Thoma-Zeiss method, and differential leucocyte counts were made on the smear which were stained by the May-Giemsa method. Total and differential cell counts of the milk were observed by Breed's smear method. To differentiate the various cells in the milk, the smears were stained by Maximow's method.

At the close of the above experiments, the animals were sacrificed and pieces of the mammary glands, thymus, spleen, ileum, duodenum and adrenals were taken. The materials were fixed in Zenker-formol solution and embedded in paraffin and cut serially at the thickness of 6  $\mu$ , and were stained by Maximow's method for the wandering cells.

## Results

### 1. Total and differential leucocyte counts of the circulating blood.

Alterations in the total leucocyte count and in the percentages of lymphoid cells (lymphocytes and monocytes), neutrophiles, eosinophiles and basophiles in the circulating blood following the ACTH injection and the pituitary graft are given in Table 2.

**Table 2.** Effects of ACTH injection and pituitary graft on blood leucocyte count.

	Total count	Percentages				
		Lymphoid cells	Neutrophiles	Eosinophiles	Basophiles	
No. 3 (Pituitary graft)						
Pre-graft	8600	70.0	28.1	1.9	—	
Post-graft	1 day	9700	68.1	30.7	1.2	—
	2 days	10300	37.9	61.2	0.8	0.1
	3 "	10600	45.6	53.5	0.9	—
	4 "	9300	76.8	21.3	1.9	—
	5 "	11000	66.7	31.2	2.1	—
No. 1 (ACTH injection)						
Pre-injection	15000	25.6	73.3	1.1	—	
Post-injection	6 hours	16600	13.8	86.1	—	—
	12 "	13200	21.1	78.9	—	—
	24 "	18900	21.8	76.2	1.7	0.2
	48 "	15600	37.0	62.2	0.5	0.3

No. 2 (ACTH injection)

Pre-injection		13900	70.0	25.7	4.3	—
Post-injection	6 hours	23400	44.7	55.0	0.3	0.1
	12 "	22000	44.1	55.9	—	—
	24 "	14800	74.1	22.1	3.3	0.5
	48 "	14400	74.7	21.7	2.9	0.7

As shown in Table 2, both the ACTH injection and pituitary graft to the intact milking goats resulted in an increase of the total leucocyte count in the circulating blood. The increase in the total count was characterized by a marked increase of neutrophile count during two to three days after the pituitary graft and during six to 12 hours after the injection. On the other hand, however, lymphoid cell and eosinophile counts decreased significantly at the periods when the neutrophile count increased.

The alterations in various leucocytes of the circulating blood following the ACTH injection and the pituitary graft were almost of the same tendency, except that the former occurred during six to 12 hours following the injection and the latter, during two to three days following the graft.

### 2. Total and differential cell counts in the secreted milk.

An absolute number of the various cellular elements in the secreted whole milk were computed from the differential cell counts in the small amount of milk sample (0.01 ml) examined by the Breed's smear technique. Results obtained are given in Tables 3 and 4.

Table 3. Effect of pituitary graft on the cell count in the secreted whole milk.

	Milk quantity at sampling cc	Total count	Lymphoid cell count	Neutrophile count	Eosinophile count	Epithelial cell count	
No. 1							
Pre-graft	300	13.2±1.6*	3.5±0.4*	9.1±1.5*	—	0.6±0.4*	
Post-graft	1 day	550	103.3±1.1	5.3±0.5	97.0±1.4	—	1.0±0.6
	2 days	550	210.8±1.2	55.6±8.7	150.4±2.1	—	4.8±1.3
	3 "	650	92.7±2.6	40.8±7.1	49.8±4.6	—	2.1±2.6
No. 3							
Pre-graft	280	10.7±3.0	5.6±1.1	4.0±1.0	—	1.1±0.6	
Post-graft	1 day	450	15.5±3.3	6.1±1.2	8.3±0.3	—	1.1±0.3
	2 days	290	11.7±4.2	7.2±1.5	4.1±1.9	—	0.4±0.3
	3 "	280	18.7±2.0	11.5±0.8	6.1±1.8	—	1.1±0.7
	4 "	260	11.2±1.4	5.7±0.4	4.3±0.5	—	1.2±0.4
	5 "	300	12.1±2.3	7.0±0.7	4.1±1.1	—	1.0±0.9

Note: Unit of cell count is million. \* Confidence interval ( $\alpha=0.05$ ).

Table 4. Effect of ACTH injection on the cell count in the secreted whole milk.

	Milk quantity at sampling cc	Total count	Lymphoid cell count	Neutrophile count	Eosinophile count	Epithelial cell count	
No. 1							
Preinjection	500	9.9 ± 1.8*	2.4 ± 0.4*	6.0 ± 2.2*	—	1.5 ± 2.1*	
Post-injection	6 hours	400	35.9 ± 6.3	6.2 ± 0.7	19.6 ± 1.6	—	10.1 ± 5.6
	12 "	420	16.7 ± 5.2	3.5 ± 0.7	10.0 ± 3.6	—	3.2 ± 1.4
	24 "	400	151.3 ± 12.4	12.3 ± 1.3	114.3 ± 15.6	—	24.7 ± 6.7
	48 "	410	32.7 ± 10.1	8.9 ± 0.5	20.5 ± 8.1	—	3.3 ± 1.3
No. 2							
Preinjection	480	12.0 ± 1.5	5.7 ± 1.4	4.7 ± 1.0	—	1.6 ± 1.5	
Post-injection	6 hours	300	61.9 ± 3.1	26.1 ± 0	34.5 ± 4.4	0.1 ± 0.4	1.2 ± 1.3
	12 "	200	30.7 ± 5.4	11.4 ± 0.6	18.4 ± 6.2	0.1 ± 0.3	0.8 ± 0.2
	24 "	600	18.0 ± 3.4	4.8 ± 1.9	12.6 ± 8.2	0.1 ± 0.7	0.5 ± 0.2
	48 "	450	30.3 ± 9.3	8.4 ± 1.0	21.3 ± 3.6	0.1 ± 0.3	0.5 ± 0.2
No. 2							
Preinjection	450	18.4 ± 5.7	3.6 ± 0.6	13.2 ± 6.1	0.3 ± 0.7	1.3 ± 0.6	
Post-injection	6 hours	650	66.3 ± 3.4	10.4 ± 1.3	50.7 ± 4.7	0.4 ± 0.3	4.8 ± 0.3
	12 "	300	28.7 ± 2.9	7.1 ± 0.3	18.6 ± 1.0	—	3.0 ± 0.1

Note: Unit of cell count is million. \* Confidence interval ( $\alpha=0.05$ ).

The total cell counts in the whole milk of the intact milking goats ranged from 9.9 to 18.0 million. These total counts increased in various levels significantly after the pituitary graft or the ACTH injection. In the goats which received the pituitary graft, this increased cell count reached the maximum at two or three days following the graft, and in the goats which received the ACTH injection, at six or 24 hours following the injection. These increased cell counts in the secreted milk occurred almost concurrently with decreased lymphoid cell and eosinophile counts in the circulating blood.

The cellular elements which appeared in the secreted milk were mainly lymphoid cells and neutrophiles, and partly epithelial cells and eosinophiles. In general, the increase in the cell counts following the operations was due to the increase of the lymphoid cells and neutrophiles.

### 3. Wandering cell counts in the mammary glands.

No. 1 goat was sacrificed at three days after the pituitary graft, No. 2 goat, at 12 hours after the hormone injection and No. 3 goat, as the control, without treatment, and the mammary glands were examined histologically and the number of the lymphoid cells, neutrophiles in the intralobular interstitium, epithelial walls and glandular cavities were counted. The results are presented

in Table 5.

**Table 5.** Wandering cell counts in the mammary glands.

	Interstitialium		Epithelium		Lumina	
	Lymphoid	Neutro.	Lymphoid	Neutro.	Lymphoid	Neutro.
No. 1	1052	47	132	—	28	14
No. 2	910	39	224	4	20	11
No. 3	870	—	188	—	19	—

Note: Each cellular element is counted from 50 fields, one field of which is  $(100\mu)^2$ .

The glandular epithelial cells of Nos. 1 and 2 goats fully developed and expanded with more fat droplets as compared with those of No. 3 goat. The glandular acini were 80 to 120  $\mu$  in diameter and contained many decapitated cytoplasmic clusters (Fig. 1).

Many wandering cells were found in the intralobular interstitium, epithelial walls and glandular cavities. Most of the wandering cells in each region of the glands were composed of lymphocytes and partially of large lymphoid cells which may be classified as monocytes or histiocytes (Fig. 1). It was noticed, however, that a small number of the neutrophiles was found in the intralobular interstitium, epithelial walls and glandular cavities of the glands of Nos. 1 and 2 goats. In these animals, lymphoid cell counts in each region of the glands slightly increased than those of the gland of No. 3 goat which was used as the control.

#### 4. *Histological observations of adrenal, thymus, spleen and intestine.*

After the animals were sacrificed, the adrenals, thymus, spleen, duodenum and ileum were taken and examined histologically, and the number of the degenerative lymphoid cells in each organ was counted.

At first, the adrenal, thymus and spleen were weighed. The results obtained are given in Table 6.

**Table 6.** Weights of various organs.

	Body weight at sacrificed kg	Adrenal		Thymus g	Spleen g
		Left g	Right g		
No. 1	30	3.15	2.45	2.9	33.5
No. 2	56	3.60	3.80	23.1	65.6
No. 3	69	1.65	1.75	21.5	150.0

As shown in Table 6, the right and left adrenals of Nos. 1 and 2 goats were about twofold in weight as compared with those of No. 3 goat, and they were larger in volume than those of the latter. It was found that these increased weight and volume of the adrenals were due to the hypertrophy of

the glandular cells in the cortex (Figs. 2 to 5).

The thymus of each animal showed a fatty degeneration and was embedded in adipose tissue. Especially, the lobe of the thymus of No. 1 goat disappeared and the right lobe of it left only a trace. The thymus of Nos. 2 and 3 goats showed almost no difference in weight being 23.1 g and 21.5 g respectively. Under histological examination, it was noticed that the cortex of the thymus of No. 2 goat showed more advanced atrophy than that of No. 3 goat and the medullar portion was extremely expanded as well as that of No. 1 goat (Figs. 6 and 7).

In the cortex and medullar of the thymus, many degenerative lymphoid cells were found. Especially, in the medullar portion of the thymus of Nos. 1 and 2 goats, the degenerative lymphoid cells occurred abundantly as shown in Table 7. These degenerative lymphoid cells were mainly lymphocytes, and were noticed by the presence of pyknotic nuclei or nuclear debris. It was noticed that in these medullar portion of the thymus the aggregation of the migrated eosinophiles were seen everywhere and some of them were degenerated in these regions (Fig. 8).

**Table 7.** Degenerative lymphoid cell counts in various organs.

	Thymus		Spleen		Intestine	
	Cortex	Medulla	White pulp	Red pulp	Duodenum	Ileum
No. 1	76	101	34	132	143	163
No. 2	87	93	54	87	73	160
No. 3	73	23	3	42	70	31

Note: Each cellular element is counted from 50 fields, one field of which is  $(100\mu)^2$ .

The spleens of Nos. 1 and 2 goats were smaller than those of No. 3 goat and showed histologically a slight atrophy of the white pulp. In the white and red pulp of the spleens of Nos. 1 and 2 goats, the degenerative lymphoid cells increased significantly as well as in the medullar portion of the thymus. In the red pulp of the spleen of these goats, many migrated neutrophiles were found (Fig. 9).

The duodenum and ileum of each animal showed histologically almost the same picture as the spleen. The degenerative lymphoid cells were found abundantly in the lamina propria of these organs of Nos. 1 and 2 goats. The degenerative lymphoid cells in this region were mainly the cells which were classified as plasmacytes, and they took a pyknotic degeneration as well as the degenerative lymphoid cells which appeared in the thymus (Fig. 10).

### Discussion

It was noticed in this study that the pituitary graft or the ACTH injection

to the milking goats induced an alteration in the percentages of various leucocytes in the circular blood. These alterations in the circulating leucocytes were characterized by an increase of neutrophile count and a decrease of eosinophile and lymphoid cell counts. A lymphopenia or eosinopenia had been practically recognized as one of the indicators of the adrenocortical activity (1, 2, 3, 5, 6, 7 and 12). The lymphoid cells in the circulating blood of the milking goats were mainly lymphocytes. Therefore, it is obvious that the decrease of the eosinophile and lymphoid cell counts and the increase of the neutrophile count in the milking goats which received the operation were due to an increased adrenocortical activity induced by the graft or the injection. As a matter of fact, the glandular cells of the adrenal cortex of the goats which received the operation swelled largely, and the cortex were twofold in weight and in volume as compared with that of the control animal, showing an increase activation of the glandular cells. These facts showed that the influences of the grafted pituitary and of the administered hormone were mediated through the adrenal cortex.

As shown in Tables 3 and 4, in the goats which received the operation, the lymphoid cell and neutrophile counts in the secreted whole milk increased significantly and those in various regions of the mammary glands also increased. Okada (11) reported, in active nursing mice and rats, that the lymphoid cell counts in the intralobular interstitium, epithelial walls and glandular lumina of the mammary glands increased significantly during six to 12 hours following the injection of ACTH and many colostrum bodies occurred during the same period. In the milking goats as well as in the nursing mice and rats, an increased activity of the adrenal cortex induced an increased migration into the mammary glands and an increased penetration into the alveoli or secreted milk. In these goats it was observed that the neutrophile count in the secreted milk and also in various regions of the mammary glands increased as well as the lymphoid cell count. Such increase in the neutrophile count, however, was not observed in the mice and rats which received ACTH (11). The increase of the neutrophile counts in the mammary glands and in the secreted milk of the goats seemed to be related the large number of neutrophiles in the circulating blood.

In the previous paper (11), it was reported that the glandular cells of the mammary glands were activated by the ACTH injection. This finding was ascertained in the milking goats used in this study. Johnson and Meites (8) reported that ACTH and adrenocortical hormone had an effect on the mammary growth in the rats. Sinai (13) also reported that a large doses of ACTH produced a marked hypertrophy of the mammary tissue of mice. The mammary development reported by Johnson and Meites (8) and Sinai (13), the activation of the glandular cells in mice and rats reported by Okada (11) and also the



activation of mammary glands in the milking goats mentioned above may be related with increased blood supply to the mammary tissue. Still more, it is suggested that there is a close correlation between the increased blood supply to the mammary tissue mentioned above and the increased lymphoid cell and neutrophile counts in the mammary glands following the activation of the adrenal cortex mentioned already. Because, if it is true that the blood supply to the mammary glands increase, this increased blood should transfer many lymphoid cells and neutrophiles into the mammary glands. Thus, it was conceived that the wandering leucocytes in the blood stream transferred into the glandular alveoli and lactiferous ducts and finally appeared in the secreted milk.

Dougherty and White (3 and 4) reported that the lymphoid organs, such as thymus and spleen, showed a degenerative change following the injection of ACTH or adrenal cortical extracts and concluded that this change was characterized by pyknosis and shedding of the cytoplasm of the lymphocytes. As already mentioned, many degenerative lymphoid cells, most of which were lymphocytes, appeared in the thymus, spleen and in the lamina propria of the intestine of the goats which received the pituitary graft of the ACTH injection. Okada (9, 10 and 11), in mice and rats, suggested that an increased occurrence of the degenerative lymphoid cells in the lymphoid organs and in the lamina propria of the intestine, and an increased appearance of the colostrum bodies in the mammary glands were a series of phenomenon of the same nature under the hyperadrenocortical state. The present investigation indicates that the above mentioned facts in mice and rats was accepted in the milking goats. Thus the so-called colostrum bodies were nothing but a picture of the degenerative lymphoid cells and neutrophiles in the glandular alveoli or in the secreted milk (10). It may be concluded, therefore, that the occurrence of the colostrum bodies was controlled under the influence of the anterior lobe of the pituitary mediated by adrenal cortex.

### Summary

The results obtained in this study are summarized as follows:

1. The lymphoid cell and eosinophile counts in the circulating blood decreased during two to three days following the pituitary graft and during six to 12 hours following the ACTH injection, and neutrophile count increased during the same period.
2. The lymphoid cell and neutrophile counts in the secreted whole milk and in the mammary glands increased during the same period mentioned above.
3. The degenerative lymphoid cell counts in the thymus, spleen and lamina propria of the intestine increased at three days following the pituitary graft or at 12 hours following the ACTH injection.

The data suggest that in the normally milking goats the lymphoid cells and neutrophils are released into the secreted milk under the stimulation of the pituitary-adrenocortical system.

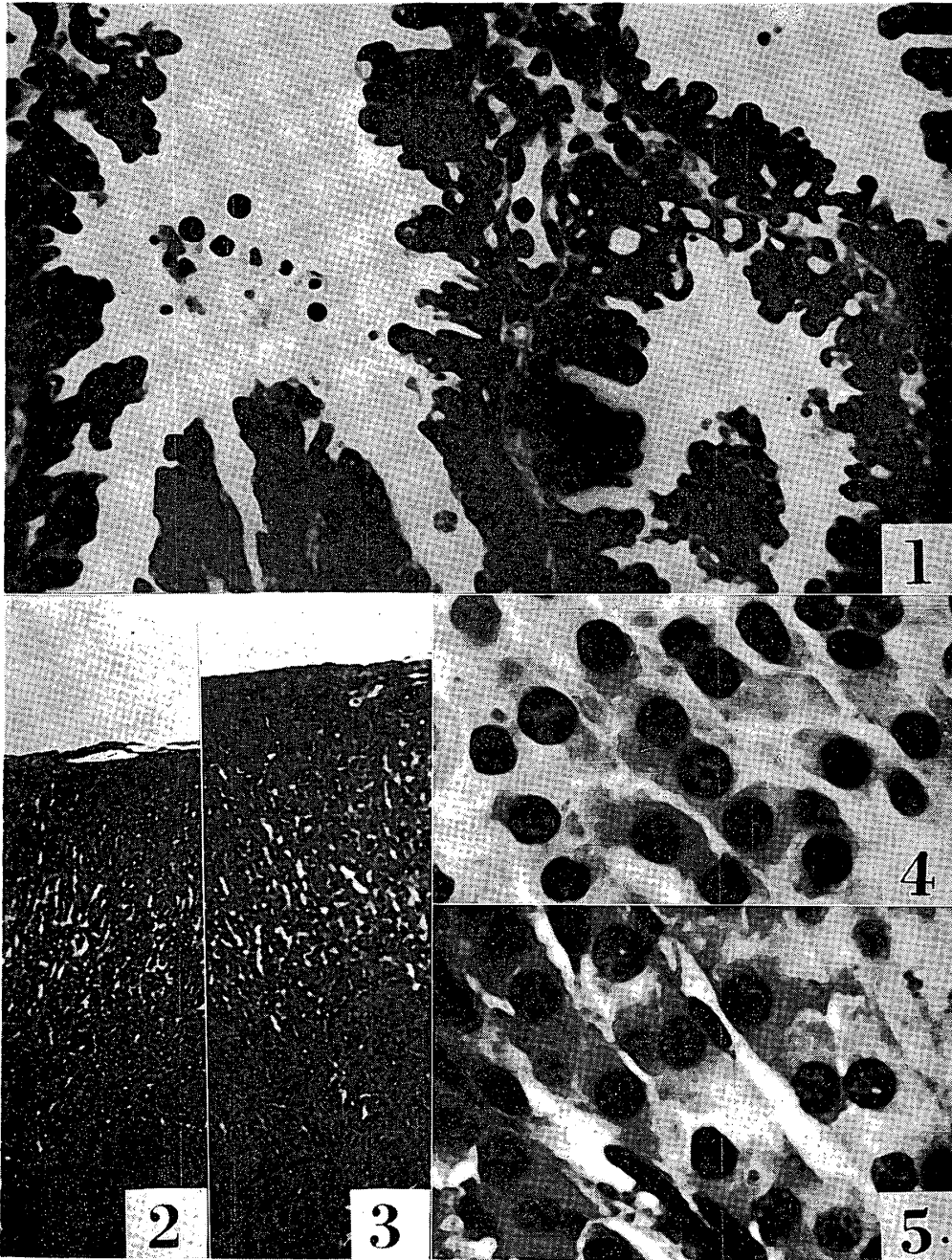
Acknowledgment: The author wishes to express his hearty thanks to Prof. Y. Toryu for his valuable suggestions and criticism throughout the course of this work, and to Assist. Prof. H. Tamate for his kindness during this work.

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**Plate 1****Explanation of Figures**

- Fig. 1. The mammary gland of No. 2 goat which received ACTH. Maximow's stain.  $\times 800$ .  
The glandular epithelial cells expand largely with many fat droplets. In the glandular cavities many cellular elements and decapitated cytoplasm appear.
- Figs. 2 and 3. The adrenal cortex of Nos. 2 and 3 goats. Maximow's stain.  $\times 30$ .  
Fig. 2 is of No. 3 goat which received no treatment, and Fig. 3 is of No. 2 goat which received ACTH. The adrenal cortex of Fig. 3 is more elongated than that of Fig. 2.
- Figs. 4 and 5. The glandular cells of the adrenal cortex of Nos. 2 and 3 goats. Maximow's stain.  $\times 1200$ .  
The cells of the cortex of No. 2 goat (Fig. 5) showed more hypertrophy than those of No. 3 goat (Fig. 4).



**Plate 2****Explanation of Figures**

- Figs. 6 and 7. The thymus of Nos. 1 and 3 goats. Maximow's stain.  $\times 200$ .  
Fig. 6 is of No. 3 goat which received no treatment, and Fig. 7 is of No. 1 goat which received the pituitary graft. The cortex of thymus of Fig. 7 atrophies and the medullar portion of it expands extremely as compared with that of Fig. 6.
- Fig. 8. The medulla of No. 1 goat which received the pituitary graft. Maximow's stain.  $\times 1800$ .  
In this portion many degenerative lymphoid cells and degenerative eosinophiles appear. These cells show pyknotic degeneration.
- Fig. 9. The red pulp of the spleen of No. 2 goat which received ACTH. Maximow's stain.  $\times 1800$ .  
In this portion many neutrophiles and degenerative lymphoid cells appear.
- Fig. 10. The lamina propria of the duodenum of No. 1 goat which received the pituitary graft. Maximow's stain.  $\times 1800$ .  
In this portion many degenerative lymphoid cells appear. These cells are mainly plasmacytes and their nuclei show pyknotic fragmentation.

