HISTOLOGY OF THE MAMMARY GLAND V. EFFECT OF ACTH ON THE LYMPHOID CELL COUNTS IN THE MAMMARY GLANDS OF LACTATING MICE AND RATS

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By

Mitsuo OKADA

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

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Introduction

In spite of vast literatures on the colostrum bodies(4, 9, 10, 17, 18, 19, 24), the physiological significance of their occurrence is obscure. Early investigators(4, 9, 10) reported that the primary significance of the colostrum bodies is an absorption of milk fat in the inactive and stagnant mammary alveoli. Recently Okada(17) concluded, from his experimental study, that the occurrence of the colostrum bodies in the intact rats was not due to the stagnant milk. Further, he(Okada, 19) reported, in the mice, that the majority of the colostrum bodies of lymphoid cell origin in the mammary alveoli at the immediate post-partum contained much RNA and differed from those in the glands at the regression stage.

It is known that the lymphoid cell counts in the mammary glands increase during advanced pregnancy and immediate post-partum and that this increased migration of the lymphoid cells induced an occurrence of many colostrum bodies (17). Recently, Halberg and Bock (11), and Okada (18) observed in the mice that a remarkable decrease of the circulating eosinophiles resulted during the later pregnancy and immediate post-partum. Merril and Smith(15) observed in the cow a decrease of the circulating eosinophiles and lymphocytes at the time of parturition and stated that their decrease at this stage was due to an increased activity of the adrenal cortex with advanced pregnancy and parturition. Further, Okada (19) observed that the dengenerative lymphoid cell counts in the lymphoid organs and gut increased significantly at the time of parturiton and suggested that this was also due to an increased activity of the pituitary-adrenal system.

The above mentioned papers indicated that at the later pregnancy and immediate post-partum there may be a close correlation between the increased

migration of the lymphoid cells into the mammary glands and the increased activity of the pituitary-adrenal system. Therefore, the present investigation was designed to clarify the effect of ACTH (adrenocorticotropic hormone) on the behavior of the lymphoid cells in the circulating blood and in the mammary glands of the active nursing mice and rats.

Materials and Methods

Albino female mice, 4 to 5 months old, and rats, 8 months old, were used in this study. Each animal was kept under the same feeding and management.

Eight active nursing mice received a single subcutaneous injection of ACTH (Armour's) at the 8th or 10th day after parturition. Three active nursing rats received twice subcutaneous injection of ACTH (N. V. Organon's) at the 10th and 11th days after parturition. The dose in the mice was 2 mg (2 I. U.) dissolved in 0.5 *ml* of water, and that in the rats, 3.3 mg (3.3 I. U.) dissolved in 1.0 *ml* of water. At one hour before the injection, and at 6, 12 and 24 hours after the injection, blood and small pieces of the inguinal and abdominal mammary glands were taken for investigation. The animals were sacrified at 24 hours after the injection and pieces of the mammary glands, thymus, spleen, ileum and duodenum were taken for study.

Four active nursing mice were adrenalectomized under light ether anesthesia by the dorsal approach. The operation was performed in two stages; the left adrenal was removed first and then the right one was removed after two days. Immediately after the second operation, the animals received a single subcutaneous injection of 2 mg of ACTH (Armour's) dissolved in 0.5 *ml* of water. The physiological saline was administered through the course of the experiment. At 6, 12 and 24 hours after the injection, the blood and small pieces of the inguinal and abdominal mammary glands were taken for observation. The animals were sacrified at 24 hours and pieces of the thymus, spleen, ileum and duodenum were taken for examination.

Seven mice and four rats at the resting stage were used as controls.

Blood was obtained by lancing the tail veins. The first flow of the blood was avoided and the second flow was used for the differential leucocyte counts. Blood smears were stained by the Mäy-Giemsa method. The organs obtained were fixed in Zenker-formol solution and embedded in paraffin and cut serially at the thickness of 6 μ.

The staining methods were as follows: For the wandering cells, Maximow's hematoxylin-Azure II-eosin 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 RNA, toluidine blue stain; RNA was confirmed by the ribonuclease treatment (37°C, 1 hour).

Results

1. *Effect of ACTH on the differential leucocyte counts in the circulating blood.*

Alterations in the differential cell counts of the circulating blood following the injection of ACTH are given in Table 1.

Table 1. Effect of ACTH on the differential leucocyte counts in the circulating blood.

		Leucocytes counted	Differential cell counts in per cent		
			Lymphoid cells	Neutrophiles	Eosinophiles
Mice					
Pre-injection 1 hour		3937	74.5 (74.5*—74.5)	24.9 (24.9*—24.9)	0.6 (0.9—0.5*)
Post-injection	6 hours	1952	65.4 (65.4—65.4)	34.3 (34.3—34.3)	0.3 (0.6—0.1)
	12 hours	1874	50.7 (50.7—50.7)	48.9 (48.9—48.9)	0.4 (0.7—0.2)
	24 hours	3315	74.2 (74.2—74.2)	24.8 (24.8—24.8)	1.0 (1.1—0.8)
Rats					
Pre-injection 1 hour		1585	64.8 (64.8—64.8)	34.4 (34.4—34.4)	0.8 (1.1—0.5)
Post-injection	6 hours	913	49.5 (53.9—44.9)	39.9 (54.5—45.3)	0.6 (1.2—0.3)
	12 hours	1269	50.1 (50.1—50.1)	49.4 (49.4—49.4)	0.5 (1.0—0.3)
	24 hours	838	61.1 (63.3—56.7)	37.9 (42.3—35.4)	1.0 (1.7—0.5)
Adrenalectomized mice					
Post-injection	6 hours	1834	64.4 (64.4—64.4)	34.3 (34.3—34.3)	1.3 (1.8—0.8)
	12 hours	3528	70.4 (70.4—70.4)	28.3 (28.3—28.3)	1.3 (1.8—0.9)
	24 hours	2217	76.0 (76.0—76.0)	22.1 (25.3—20.5)	1.9 (2.7—1.4)

* Confidince interval ($\alpha = 0.05$)

In the intact mice and rats that received the injection of ACTH, the lymphoid cell counts and eosinophile counts decreased at 6 and 12 hours following the injection. On the contrary, the neutrophile counts increased at 6 and 12 hours after the injection.

In the adrenalectomized mice that received the hormone injection, the lymphoid cell and neutrophile counts 6 hours after the injection showed the same level as those of the intact mice that received the hormone injection. However, the eosinophile counts at this stage were larger than those of the intact mice that received ACTH. At 12 and 24 hours following the injection, the lymphoid cell counts remarkably increased and the neutrophile counts decreased. The eosinophile counts at these stages persisted at higher levels than those of the intact mice that received ACTH.

2. *Effect of ACTH on the lymphoid cell counts in the mammary glands.*

The results are presented in Table 2, from which it is shown that the lymphoid cell counts in each region of the mammary glands of the intact mice and rats increased significantly after the injection of ACTH. In the mice, this increased cell counts reached the maximum at 6 hours following the injection and then decreased gradually. In the rats, the cell counts increased gradually with advanced periods following the injection and reached the maximum at 24 hours after the injection.

Table 2. Effect of ACTH on the lymphoid cell counts in the mammary glands.

		Interstitium	Glandular epithelia	Glandular lumina
Mice				
Pre-injection 1 hour		165±26.0*	28±5.7*	5.8±2.41*
Post-injection	6 hours	270±63.3	51±8.8	17.3±5.83
	12 hours	240±45.4	40±5.5	5.1±2.70
	24 hours	228±42.8	28±4.3	2.5±3.00
Rats				
Pre-injection 1 hour		133±16.9	11±4.1	1.8±1.41
Post-injection	6 hours	306±32.2	32±8.9	5.3±6.58
	12 hours	310±11.9	28±8.4	5.3±3.99
	24 hours	357±14.8	34±1.4	10.0±5.92
Adrenalectomized mice				
Post-injection 12 hours		251±31.5	24±3.1	8.3±1.40

Note: Each cellular element is counted from 50 alveoli. Interstitium indicates the intralobular connective tissue area at the distance of 10 μ from the alveolus.
* Confidince interval ($\alpha=0.05$).

The increased migration of the lymphoid cells into the mammary glands was noticeable in the peripheral region of the glands in both mice and rats (Figs. 1 and 2). The epithelial cells of the glands swelled and contained much RNA after the hormone injection.

In the mammary glands of the adrenalectomized mice that received the hormone injection, the lymphoid cell counts in each region of the glands 12 hours after the injection showed the same level as those at the same period of the intact mice that received ACTH. It was noticed, however, that the glands of the adrenalectomized mice 12 hours following the injection of ACTH showed slight regression change with the active migration and penetration of the

neutrophiles into the mammary glands (Fig. 3). In this glands the degenerative lymphoid cells were found frequently in the intralobular connective tissue area.

The lymphoid cells in the mammary alveoli were composed of the normal and degenerative lymphoid cells. In the intact mice and rats that received ACTH, the degenerative lymphoid cells were the typical colostrum bodies, that is, they had swollen cytoplasm and contained much RNA. However, in the adrenalectomized mice that received ACTH, the degenerative lymphoid cells in the mammary alveoli were mainly PAS positive large lymphoid cells containing no RNA.

3. *Effect of ACTH on the degenerative lymphoid cell counts in the lymphoid organs and gut.*

The degenerative lymphoid cells were noticed by the presence of the pycnotic nuclei or of the nuclear debris. The cell counts in the thymus, spleen, duodenum and ileum 24 hours following the injection of ACTH are given in Table 3.

Table 3. Effect of ACTH on the degenerative lymphoid cell counts in various organs.

	Thymus		Spleen		Lamina propria of gut	
	Cortex	Medulla	White pulp	Red pulp	Duodenum	Ileum
Mice						
Treated	$67 \pm 13.7^{*}$	$57 \pm 8.3^{*}$	$28 \pm 5.5^{*}$	$102 \pm 5.8^{*}$	$36 \pm 12.9^{*}$	$36 \pm 6.9^{*}$
Control	25 ± 4.2	31 ± 7.6	29 ± 4.9	69 ± 29.2	25 ± 4.7	21 ± 4.7
Rats						
Treated	43 ± 6.4	80 ± 31.7	54 ± 16.7	106 ± 11.1	33 ± 5.4	36 ± 9.0
Control	21 ± 12.5	18 ± 6.3	14 ± 8.8	72 ± 11.4	18 ± 4.1	14 ± 5.1
Adrenalectomized mice						
Treated	27 ± 6.5	35 ± 7.1	24 ± 9.6	56 ± 37.1	20 ± 3.7	21 ± 3.6

Note : Each cellular element is counted from 50 fields, one field is 36 μ^2.
* Confidence interval ($\alpha = 0.05$).

As shown in Table 3, the degenerative lymphoid cell counts in various organs of the intact mice and rats which received ACTH increased significantly except the cells in the white pulp of the spleen of the mice. However, the cell counts in any organ of the adrenalectomized mice which received ACTH showed no increase in comparison with those of the control animals.

The degenerative lymphoid cells which appeared in the thymus, the white pulp of the spleen and the lamina propria of the gut of the intact mice and rats which received ACTH were chiefly small and medium sized lymphocytes and plasmacytes. In the process of the degeneration, the nuclei of the small and medium sized lymphocytes showed no fragmentation but simple pycnosis and the cytoplasm were stained weakly or moderately with eosin. In the medulla near the sinus of the thymus and lamina propria of the gut especially of the duodenum, vacuolated degenerative plasmacytes were found frequently after the administration of ACTH (Figs. 4, 5 and 6).

Discussion

In the present investigation of the ACTH effect to the behavior of the lymphoid cells including the colostrum bodies in the mammary glands, it became evident that the increased activity of the adrenal cortex would induce the migration and penetration of the lymphoid cells into the mammary glands resulting in the appearance of the typical colostrum bodies. As already mentioned, the lymphoid cell counts in the mammary glands of the intact active nursing mice and rats increased significantly within 24 hours following the ACTH injection. These increased cell counts in the mammary glands occurred in correlation with or slightly later than the decrease of the lymphoid cell counts and eosinophile counts in the circulating blood. A lymphopenia or eosinopenia had been practically used as one of the indicators of the adrenocortical activity (3, 5, 6, 8, 13, 21). The lymphoid cells in the circulating blood of the mice and rats which received ACTH were chiefly lymphocytes. Therefore, it is obvious that the decrease of the lymphoid cell and eosinophile counts in the circulating blood of the intact active nursing mice and rats which received the ACTH injection was due to an increased adrenocortical activity induced by ACTH.

In the adrenalectomized mice, the lymphoid cell and eosinophile counts in the circulating blood 12 hours following the hormone injection did not show a marked decrease as those at the same period in the intact mice and rats which recived ACTH but rather showed an increase. On the other hand, the lymphoid cell counts in each region of the mammary glands of the adrenalectomized mice 12 hours after the ACTH injection showed an increase as those at the same period in the intact active nursing mice which received ACTH. However, this increase in the lymphoid cell counts might be due to a regression of the glands, because the glands at this period showed a regression change with migration and penetration of the neutrophiles into the mammary glands and many degenerative lymphoid cells were found in the intralobular connective tissue area. Thus, the increase of the lymphoid cells in the mammary glands of the intact active nursing mice and rats which received ACTH differed from that of the

adrenalectomized mice which received ACTH.

The finding in the present investigation that an increased adrenocortical activity in the mice and rats induced an increase dmigration and penetration of the lymphoid cells into the mammary glands will be accepted conveniently in the cases of the later pregnancy and immediate post-partum when the lymphoid cells in the mammary glands increased significantly, because a sharp increase in the adrenocortical activity has been found during these stages(1, 15, 20, 23).

Emmel *et al.* (9) studied the relation between the leucocytes and lactation in the active nursing albino rats and reported 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. In the present investigation, as already mentioned, the same relation was found in the intact active nursing mice and rats in the hyperadrenocortical state. However, there remains two questions: First, whether the decrease of the circulating lymphoid cells in the hyperadrenocortical state depended on the increased migration of the lymphoid cells into the mammary glands; Second, whether the migrated lymphoid cells into the mammary glands had an important role to the mammary function.

Concerning the first question, there are conflicting opinions. Dougherty and white(7) demonstrated in the mice and rabbits that within a few hours after the adrenocorticotropin treatment a general lymphocytolysis occurred in the lymphoid organs and thus, they stated that a lymphopenia in the hyperadrenocortical state depended on the lymphocytolysis in the lymphoid organs. Baker *et al.* (2) observed that, in the male rats treated with the pure adrenocorticotropin for long periods, a production of the lymphocytes in the lymphoid organs was impaired and, they thus stated that a lymphopenia in the hyperadrenocortical state resulted in the impaired production of the lymphocytes. Hungerford *et al.*(12) studied the effects of the pituitary and adrenal hormone on the number of the thoracic duct lymphocytes and suggested that the blood lymphopenia after ACTH treatment may be the result of the decreased delivery of the lymphocytes to the blood stream through the thoracic duct.

The data obtained in this investigation showed, in part, support to the conception of Dougherty and White (7), because, as already mentioned, the degenerative lymphoid cell counts in any organ increased after ACTH treatment.

Monden *et al.* (16) studied the effect of the adrenocortical hormone on a number of mitochondria in the circulating lymphocytes and claimed that the younger lymphocytes which contained more numerous mitochondria than the older forms had a higher sensitivity than the older forms to the adrenocortical hormone. Santisteban and Dougherty (22) reported that the adrenocortical hormone especially hydrocortisone tended to produce degenerative changes among the immature lymphocytes of the lymph nodes. In the present investigation, the typical colostrum bodies with much RNA appeared in the mammary

alveoli during 6 to 24 hours following the ACTH injection. As has already been studied in the previous papers(18, 19), the typical colostrum bodies were formed from the young large lymphoid cells with much RNA and they were nothing but a picture of the degeneration of the same cells within the mammary alveoli. On the other hand, the degenerative lymphoid cells which appeared in the thymus, the white pulp of the spleen and the lamina propria of the gut of the intact mice and rats which received the hormone injection were chiefly the younger forms of the lymphocytes and plasmacytes. Therefore, the findings of Monden *et al.*(16) may be also accepted in the case of the typical colostrum bodies as well as the degenerative lymphoid cells in the lymphoid organs and gut in the present investigation.

As mentioned above, the typical colostrum bodies in the mammary alveoli of the mice and rats resembled in their occurrences the degenerative lymphoid cells in the other organs. Accordingly, it may be assumed that the occurrence of the typical colostrum bodies as well as the degeneration of the young lymphoid cells in the lymphoid organs and gut is a series of phenomenon of the same nature.

As to the second question that whether the migrated lymphoid cells into the mammary glands had important role to the mammary function, Emmel *et al.*(9) suggested that the lymphoid cells which migrated into the mammary glands must contribute in products of the mammary-gland secretion. Johnson and Meites(14) reported that ACTH and adrenocortical hormone had an effect on the mammary growth in the rats. In the present investigation, it was noticed that the glandular epithelial cells were activated by the ACTH injection. The mammary development reported by Johnson and Meites(14) and the activation of the glandular cells in this investigation may be related with the blood supply to the mammary glands. This will be discussed in another paper.

Summary

The effect of ACTH on the differential cell counts in the circulating blood, the lymphoid cell counts in the mammary glands and the degenerative lymphoid cell counts in the thymus, spleen and gut were studied, using the active nursing mice and rats, with the following results.

The lymphoid cell and eosinophile counts in the circulating blood decreased during 6 to 12 hours following the hormone injection. The neutrophile counts increased during the same period.

The lymphoid cell counts in the mammary glands increased during 6 to 24 hours after the hormone injection and the typical colostrum bodies appeared in the mammary alveoli.

The degenerative lymphoid cell counts in any organ increased after the hormone injection.

On the basis of the data obtained, the cause of the occurrence of the typical colostrum bodies is discussed.

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Plate 1

Explanation of Figures

Fig. 1. Active nursing mouse mammary gland 12 hours after the single injection of 2 mg of ACTH. The increased migration and penetration of the lymphoid cells into the intralobular connective tissue and mammary alveoli are seen. ×1000. Maximow's stain.

Fig. 2. Active nursing rat mammary gland 12 hours after the twice injection of 3.3 mg of ACTH. The increased migration and penetration of the lymphoid cells into the intralobular connective tissue and mammary alveoli are seen. ×1000. Maximow's stain.

Plate 1

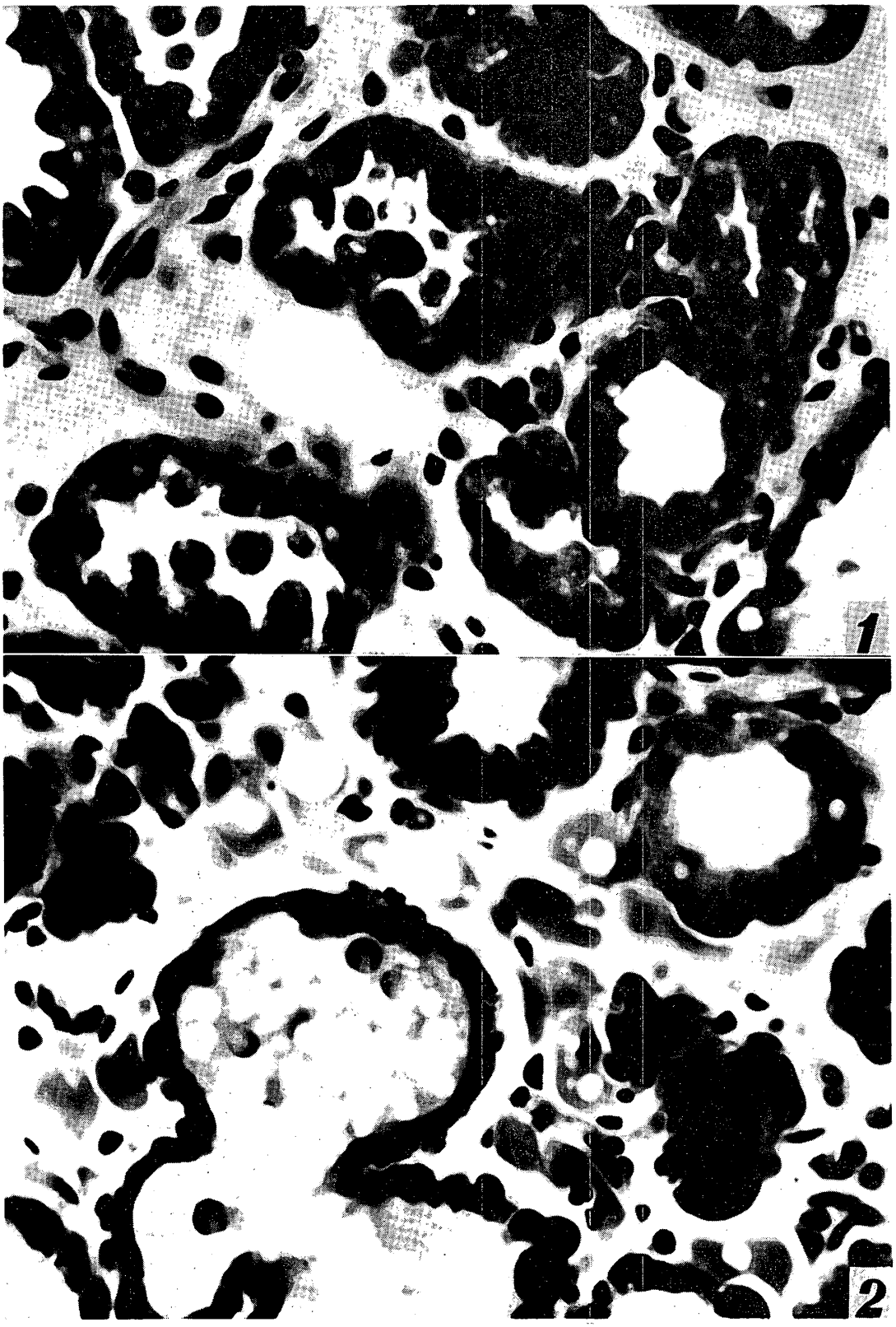

Plate 2

Explanation of Figures

Fig. 3. Mouse mammary gland 12 hours after the adrenalectomy and administration of 2.0 mg of ACTH. The operation and ACTH administration were made at the active nursing period. Regression change of the gland and degeneration of the lymphoid cells in the intralobular connective tissue and penetrated neutrophiles into the mammary alveoli are seen. ×1000. PAS stain.

Fig. 4. Medullar portion near the sinus of the rat thymus 24 hours after the twice injection of 3.3 mg of ACTH. A number of degenerative lymphocytes and vacuolated plasmacytes are seen. ×1800. Maximow's stain.

Fig. 5. Lamina propria of the mouse ileum 24 hours after the single injection of 2.0 mg of ACTH. Degenerative lymphocytes are seen. ×1800. Maximow's stain.

Fig. 6. Lamina propria of the mouse duodenum 24 hours after the single injection of 2.0 mg of ACTH. Degenerative plasmacytes are seen. ×1800. Maximow's stain.

Plate 2