PHYSIO-HISTOLOGICAL STUDIES ON THE PHYSIOLOGICAL OBESITY OF THE MEAT PIGS (REPORT III), ESPECIALLY ON THE DIMINUTION OF THE OCCURRENCE OF GLYCOGEN WITHIN THE NUCLEI AND CYTOPLASMS OF THE ZONA RETICULARIS. AND ON THE APPEARANCE OF THE GLOBOID BODIES OF EPINEPHRINE WITHIN THE MEDULLARY CELLS IN THE ADRENALS OF THE PIGS IMMUNIZED WITH HOG-CHOLERA VIRUS

著者	ITIKAWA Osamu, HOSHINO Tadahiko, ISHIDA Kazuo, YONEYA Sadamitsu
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PHYSIO-HISTOLOGICAL STUDIES ON THE PHYSIOLOGICAL OBESITY OF THE MEAT PIGS (REPORT III), ESPECIALLY ON THE DIMINUTION OF THE OCCURRENCE OF GLYCOGEN WITHIN THE NUCLEI AND CYTOPLASMS OF THE ZONA RETICULARIS, AND ON THE APPEARANCE OF THE GLOBOID BODIES OF EPINEPHRINE WITHIN THE MEDULLARY CELLS IN THE ADRENALS OF THE PIGS IMMUNIZED WITH HOG-CHOLERA VIRUS

By

Osamu ITIKAWA, Tadahiko HOSHINO, Kazuo ISHIDA
and Sadamitsu YONEYA

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

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Introduction

Previous reports (1—4) described that there existed the occurrence of glycogen within the nuclei and cytoplasms of the zona reticularis in the swine adrenals. These were found during the studies on the mechanism of adipositus and the effect of fattening has been done histochemically on the various organs of Yorkshire pigs used for the experiment of the feeding standard on the meat pigs. In the cases of 11 sows and 9 hogs there were observed the presence of glycogen in both cytoplasms and nuclei, glycogenolysis to glucose-6-phosphate, and the increase of fat related to the ketosteroids, so it seemed to be closely related to the physiological obesity. The production of insulin was accelerated by the production of ketosteroid. If the obesity would develope by the hyper-insulinism and hyperadrenocorticism, the glycogen deposition in the cytoplasms and nuclei might play an important role to hyperadrenocorticism. Though we once (4) reported on the absence of diabetetes mellitus in the meat pigs, the authors believed that it seemed to be important to have the glycogen-deposition in the cytoplasms and nuclei of the cells in the zona reticularis of the swine adrenals.

It was planned to examine the occurrence of the glycogen-bearing nuclei of the zona reticularis of the adrenals in the pigs immunized with hog-cholera virus. Moreover, by the present study, there were found a remarkable diminution of the occurrence of glycogen within the nuclei and cytoplasms of the zona reticularis, and the appearance of the globoid bodies of epinephrine within the medullary cells in the adrenals of the pigs immunized with hog-cholera virus. No one has found the histochemical views of hyperepinephrinism in the adrenal medullary cells of the hog-cholera immunized pigs. In the present investigation, special attention was also paid to the relationships between the presence of glycogen in the cortical cells (both nuclei and cytoplasms) and the absence of hyperepinephrinism in the medullary cells of the swine obesity and that between the absence of glycogen in the cortical cells and the presence of hyperepinephrinism in the medullary cells of the hog-cholera immunized pigs.

With regard to this meaning, it is important to refer to the following works: LUDFORD's regulation of epinephrine production, storage and discharge, CRAMER's mechanism of self control, LILLIE's explanation of PAS-stained epinephrine, and CLEMENTI's explanation (8) of osminophilic epinephrine. At the present time, there were found globoid-bodies stained with PAS in the swine medullary cells by the authors, but LUDFORD (5) and CRAMER (6) (1928) observed the osminophilic globoid-bodies in the above-described cells as the proof of hyperepinephrinism. Accordingly, it seemes to be necessary to consider that both PAS-positive and osminophylic globoid-bodies are similar to the reconstructed epinephrine (LUDFORD) (5).

According to WILLIAMS (9) (1962), the adrenal medulla consists of networks of anastomosing cords of polyhedral cells which secrete the hormonally active catecholamines, epinephrine and norepinephrine, dopamine, and other biologically inert precursors. These chromaffine cells as they are called stain brown with chromic acid. In addition, the medulla contains numerous sympathetic ganglion cells. These cells and the chromaffin cells are supplied by preganglionic fibers of the splanchnics. The fetal adrenal contains only norepinephrine, epinephrine appearing sometimes after birth. Epinephrine and norepinephrine, together with dopamine, are secreted by the chromaffin cells when these are stimulated directly by the acetylcholine-secreting preganglion fibers of the splanchnic nerves.

Isotopic experiments on the conversion of labeled phenylalanine to epinephrine (GURN & DELLUVA 1947) (10), that of phenylalanine and tyrosine to norepinephrine and epinephrine (UDENFRIEND, 1953 (11), 1956 (12), and that of C^{14}-dopa to radioactive epinephrine and norepinephrine (UDENFRIEND, 1956) (12) were performed.

According to ENGEL (13), CAHILL (14), FINE (15) (1960), since the effect of epinephrine, the increased liberation of glucose from the liver, is known to be mediated through the enzyme phosphorylase, the possibility exists that the effect of epinephrine on adipose tissue lipolysis is phosphorylase dependent. CAHILL,

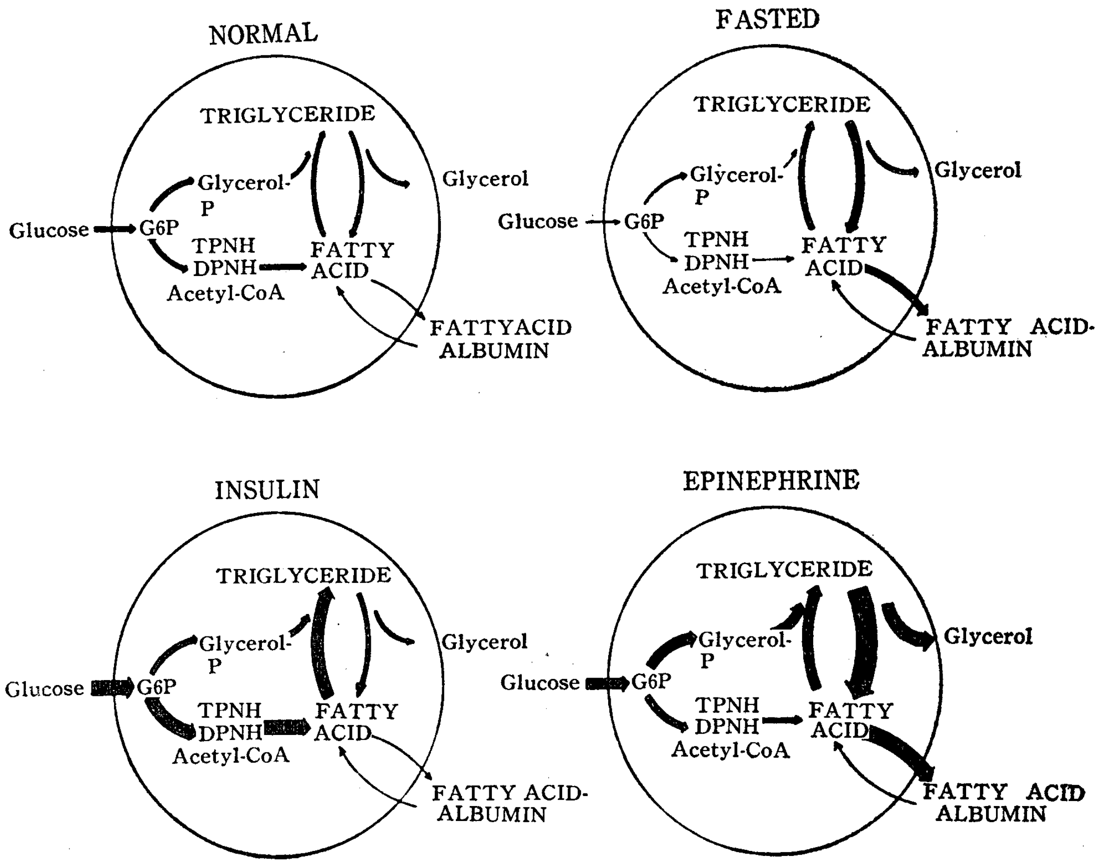

Fig. A. Tissue Regulation of Fatty Acid Metabolism
Cited from Cahill, G.F., Jr., Leboeuf, B., and Renold, A.E. : Am. J. Clin. Nutrition, 8 : 733, 1960.

LEBOEUF and RENOLD (14) (1960) described the factors concerned with the regulation of fatty acid metabolism in the adipose tissues by the fasting, insulin and epinephrine (see Fig. A). A speculative summary indicating that in fasted (or diabetic) rats, the decreased glucose uptake leads to an excess of lipolysis over triglyceride synthesis and excessive release of free fatty acids (FFA). With insulin, much more glucose is taken up than is needed to esterify the fatty acids released by lipolysis. The surplus glucose is converted to fatty acids, which are incorporated into triglyceride. Epinephrine stimulated marked lipolysis, and the high intracellular concentration of fatty acids stimulates increased glucose uptake, but much of the fatty acid is not used for re-esterification and is released from the cell.

LUDFORD (1928) (5) has prepared a pictographic summary of the changes which he believes to take place in the cells of medulla during secretion of epinephrine. On nerve stimulation the inclusions are expelled into the venous sinus where they can be directly observed, and chemical analysis shows that the amount of epinephrine in the adrenal veins is strikingly increased. Much has been written about the regulation of epinephrine production, storage and discharge, CRAMER (6) (1928) argues in favor of a special mechanism of "self control". He stresses the fact that the discharge of epinephrine stimulates the sympathetic and that sympathetic stimulation causes the pouring out of more

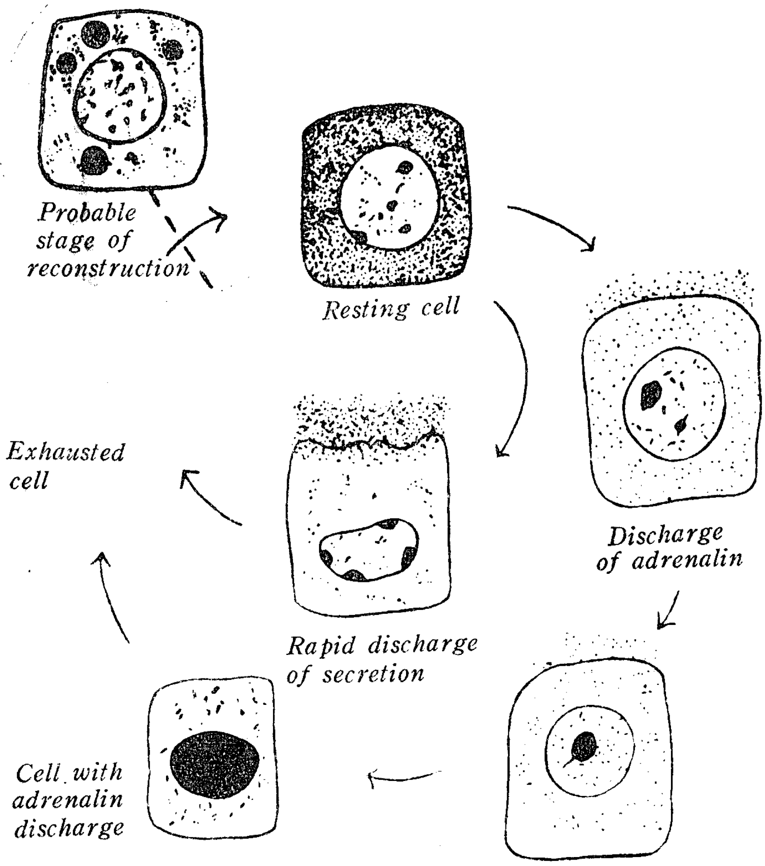

Fig. B. Pictographic Summary by Dr. R.J. Ludford of Cellular Changes during Secretory Cycle of Medullary Cells of Mouse's Adrenal
Cited from Cramer, Fever, Heat Regulation, Climate and the Thyroid-adrenal Apparatus, Longmans, Green & Co., Ltd. 1928

epinephrine. Obviously the individual would suffer from hyperepinephrinism, and the glands would rapidly become exhausted unless regulated. In the resting phase, according to CRAMER and LUDFORD, lipoid is concentrated in the outer two-thirds of the cortex and the medulla is charged with epinephrin. With extreme activity, cortical lipoid shifts toward the medulla, the capillaries of the medulla are dilated and epinephrin is expelled into the engorged central vein. Finally with self control and inhibition, cortical lipoid is reduced, the medulla is protected from the cortex by a band of cells in which there is no epinephrine and epinephrine disappears from the central vein, but continues to form in the medulla as the gland becomes recharged.

LILLIE (7) (1950) observed that a PAS-positive reaction is given by the material which gives a positive chromaffin reaction. He interprets this as being caused by the production of aldehyde from the hydroxy-methyl-amino group of epinephrine or one of its precursors, 3,4-dihydroxyphenylserine. LISON (1953) points out that the chromaffin reaction is the result of the oxidation of epinephrine mainly at the catechol group ($-C_6H_3(OH)_2$). If this is so, then the hydroxy-methyl-amino groups are not affected and epinephrine will still be able to react. Similarly no epinephrine would still be able to react after taking part in the chromaffin reaction.

CLEMENTI *et al* (8) (1963) described that the electron microscope had shed new light on the synthesis and storage of adrenal catecholamines. According to his opinion, as early as 1902, it was observed that the reaction between catecholamines and OsO_4 is rapid and that the product is stable; and this treatment has become one of the most common methods for the study of chromaffin cells. By this reaction, catecholamines can be indirectly revealed in granules possessing a typical density and appearance, when adrenomedullary tissue sections are examined under the electron microscopy.

Accordinlgy, it has been clarified that there existed the relationships between hyperadrenocorticism-hypoepinephrism in the swine obesity and hypoadrenocorticism-hyperepinephrism in the hog-cholera immunized pigs. To the effect that the pigs were considered as the physiological adipositus, it might be important to observe the relationship between the occurrence of glycogen in the nuclei and cytoplasms of the cortical cells and the under-secretion of epinephrine in the medullary cells of the swine adrenals because there were found contraries in the hog-cholera immunized pigs. These morphological changes of the regulation of epinephrine production in the immunized pigs and that of the meat pigs will be reported at another occasion.

Materials and Methods for Studies

Materials used for the studies were 11 sows and 9 hogs of Yorkshire, and 29 pigs (unknown as to their sex) immunized with hog-cholera virus. Eleven sows and nine hogs were studied on the feeding standard by the administration with various feeds mentioned in the previous report I in the Miyagi Prefectural Agricultural Experimental Station in Sendai in 1963. Twenty nine pigs immunized with hog-cholera virus were killed at the National Institute of Animal Health in Tokyo in 1963.

The total tissues from these pigs were fixed in buffered formol, embedded in paraffine and cut into 6μ sections. The stains employed were PAS-hematoxylin stain with or without digestion for glycogen and epinephrin.

Results

1. Diminution of the occurrence of glycogen within the nuclei and cytoplasms of the zona reticularis in the adrenals of the pigs immunized with hog-cholera virus.

According to the previous report, 11 sows and 9 hogs contained the glycogen-laden nuclei of the cells in the zona reticularis or zona fasciculata in 100 percentage. The presence of glycogen in the nuclei of swine adrenals were found to be remarkably stronger or more intensive in the hogs than in the sows. Glycogen deposition in the nuclei consisted of the one called the entire bodies with glycogen, and the other called the ring form which the glycogen demonstrated by PAS stain was limited in the largest vacuoles occupying most of the nuclear space. They consisted of the two forms called the large entire bodies and the small entire ones. A few glycogen granules, small in size, appeared in the periphery of the vacuoles close to the chromatin granules attached to the nuclear membranes or near the dispersed chromatin granules in the nuclei. They were called the multiple granular form in the glycogen-laden nuclei. The size of the glycogen granules remarkably increased, united with each other, and they formed internal bodies in the whole vacuolar cavity. When the whole vacuolar cavity was filled with glycogen, they are called the large and small entire bodies.

The occurrence of the glycogen-laden nuclei was examined in the zona reticularis (sometimes in zona fasciculata) of the adrenals. Variation of the nuclei containing glycogen and that of the glycogen-laden cytoplasms were shown in Table 1 of the previous report 1.

Table 1. Averaged values of the glycogen deposition in the zona reticularis of the meat pigs.

Sex	Glycogen deposition / Cell-count	Intranuclear glycogen										Cytoplasmic intranuclear			
		Glycogen in nucleus & no glycogen in cytoplasn					Glycogen in both nucleus and cytoplasm					Glycogen in cytoplasm		Vacuoles	
		Ring form	Large entire bodies	Small entire bodies	Inner bodies	Multiple granular bodies	Ring form	Large entire bodies	Small entire bodies	Inner bodies	Multiple granular bodies	Total deposition	Glycogen bodies	Large vacuole	Small vacuole
Sows	Total cells of 11 cases		6	1	12	16	9	21	18	325	334	1292	847	62	43
	Average cells per 1 case		0.5	0.1	1.1	1.4	0.8	1.9	1.6	29.5	30.4	117.4	77.0	5.6	4.0
Hogs	Total cells of 9 cases	7	71	40	26	17	35	69	107	382	271	3614	190	172	48
	Average cells per 1 case	0.8	8.0	4.4	3.0	4.2	4.0	7.6	12.0	41.8	30.0	402.7	21.1	19.1	5.3

Whole cells with the glycogen-bearing nuclei or cytoplasms were counted in one section of the swine adrenal stained with PAS-hematoxylin using the squaremeter. On the average there occurred 67 cells/1 section with glycogen in both nuclei and cytoplasms in sows and 113 cells/1 section with glycogen in them in hogs; and 194 cells/1 section with intracytoplasmic glycogen in sows and 424 cells/1 section with them in hogs. These results are summarized in Table 1.

Though it was planned to examine the occurrence of the glycogen-bearing nuclei and cytoplasms of the zona reticularis of the adrenals in the pigs immunized with hog-cholera virus, there were found remarkable diminution of the occurrence of glycogen in the adrenals as shown in Table 2.

Table 2. Diminution of the glycogen deposition in both nuclei and cytoplasms of the adrenal cells in the pigs immunized with hog-cholera virus

Glycogen / Name of pigs	Intranuclear glycogen										Intracyto-plasmic		Intra-nuclear	
	Glycogen in nucleus and no glycogen in cytoplasms					Glycogen in nucleus and cytoplasms					Glycogen in cytoplasms		Vacuoles	
	Ring form	Large entire bodies	Small entire bodies	Inner bodies	multiple granular bodies	Ring form	large entire bodies	Small entier boies	Inner bodies	Multiple granular bodies	Total deposition	Glycogen bodies	Large vacuole	Small vacuole
1											6	22	7	2
2											40	30		
3							1	1			14	2	12	2
4							2	2			16	64	3	
5								2			6			
6											5	4	12	25
7												5		
8											1	5	3	1
9											2	8		1
10											10	6	39	35
11											24	8	10	5
12											6	1	8	5
13		1	1	1	2		2			6	10	54	20	3
14						1			3	1	30	10	6	
15						1			2		25	10	3	
16										1	16	23	2	
17							1			1	12	10	2	
18		1	1				1	2			154	32	1	1
19								1	1		315	14	8	5
20											75	123	1	1
21						1		1		2	191	19	1	7
22											6	11		
23						1					3	40	3	1
24											36	25		9
25	1	1			3	3	2	2		4	143	37	5	
26			2	1	4		1	3	1	3	90	14	11	2
27				1				1			25	12	8	3
28					1			1			15	20	2	
29								2			33	28	2	4
Total	1	3	4	3	11	6	11	13	7	22	1309	637	169	112
Average	0.03	0.10	0.14	0.10	0.30	0.20	0.4	0.45	0.24	0.76	45.1	22.0	58.3	3.9

The recent biochemical theories of the regulation of cellular reaction by the hormone according to SUTHERLAND (1960), WILLIAMS (1962) and KONDO (1963) have been published. They state that the production of ketosteroid and glycogenolysis in the adrenals are becoming an interesting problem to study the mechanism of fattening or physiological obesity.

To compare the investigative results of both meat pigs and hog cholera-immunized pigs, there were remarkable differences as shown in Table 3.

Table 3. Comparison with the glycogen deposition in the adrenals of the meat pigs and immunized pigs. (cell counts/1section)

Pigs use for the studies \ Glycogen		Glycogen deposition in the cortical cells				Total
		Glycogen in only nuclei	Glycogen in both nuclei and cytoplasms	Glycogen in only cytoplasms	Intra-nuclear vacuoles with no glycogen	
Meat pigs (11 sows and 9 hogs)	Total cells of 20 pigs	196	1571	5943	325	8035
	Average cells per 1 section	9.8	79.6	297.2	16.3	401.8
Pigs immunized with hog-cholera virus (29 pigs)	Total cells of 29 pigs	22	57	1946	281	2306
	Average cells per 1 section	0.8	2.0	67.1	9.6	79.5

The fact of intranuclear glycogen deposition in the pigs immunized with the hog-cholera virus was developed to remarkable diminution in comparison with that in the meat pigs. The total numbers of the cells with intranuclear glycogen deposition in the meat pigs were more than 40 times of that in the pigs immunized with hog-cholera virus. Accordingly, it seemed to be inhibited by the intranuclear glycogen deposition in the process of the immunization.

2. The appearance of the globoid bodies of epinephrine within the medullary cells in the adrenals of the pigs immunized with the hog-cholera virus.

LUDFORD (1928) presented a pictographic summary of the gross changes during the secretory cycle of the medullary cells. The variety of the medullary cells during the secretory cycle was divided into five types such as the resting cells, epinephrine discharged cells, rapid discharged cells, exhausted cells and reconstructed cells (contained the globoid bodies in the cytoplasms). He stated that epinephrine is easily oxidized and therefore readily changes osmic acid to the black lower oxides or, perhaps, even to the metal itself, and stains with HEIDENHAIN's iron-hematoxylin. The medulla appears as even sheets of cells arranged in groups of round alveoli. The capillary spaces which separate these alveoli are almost all closed in the resting gland. Two types of cells can be distinguished. One type is filled with exceedingly fine granules stained dull black with osmic acid, and is called the blackened cells. The other type of cells has a clear, grayish, turgid appearance and contains one or more globoid bodies

(LUDFORD) of varying size stained with osmic acid, and is called the light cells. In a normal resting gland, the black type of cell preponderates. There are represented two different stages of functional activity. The black globoid bodies are not lipid, since they do not stain with Sudan, and are not removed by treatment with the organic solvents. Owing to his study, these globoid bodies are either epinephrine or an immediate precursor of epinephrine. Under certain conditions the adrenal medulla becomes the scene of extraordinary changes at the climax of which an almost explosive activity is exhibited by the medullary cells. The capillary spaces open up and become widely dilated, separating the groups of alveoli; medullary cells become vacuolated and lose in varying degrees their content of epinephrine granules, and the epinephrine granules are secreted in the lumen of blood stream. According to LUDFORD, a very active secretion of epinephrine can also be induced by the injection of certain bacterial vaccines.

The amount of epinephrine present in the medulla at a given moment—the load of epinephrine—is therefore, as STEWART and ROGOFF were the first to point out, only the difference between the rate at which epinephrine is secreted and the rate at which it is formed. An active gland may therefore be associated with a high or with a low load of epinephrine, and conversely an inactive gland may have a high load, or when the inactivity is due to exhaustion of the medulla may have a low load.

LUDFORD also presented a pictographic summary of the changes in the different parts of the adrenals in the condition of extreme secretory activity and in the conditions described as self-control and as inhibition. He concluded therefore that activity of the gland is accompanied by a spreading of the cortical lipoid over the whole cortex, inhibition of the gland by a disappearance of the cortical lipoid. Many bacterial infections stimulate the activity of the gland. Only if the infections is of sufficient virulence does the inhibitory effect manifest itself on the cortex, thus preventing it from carrying out its normal function assisting the active medulla in recharging itself with epinephrine.

In the present investigation there were observed one medullary cells filled with exceedingly fine granules stained with PAS, and other medullary cells had the clear appearance and contained the globoid bodies stained with PAS in the similality of LUDFORD's osminophilic adrenalin. Because these PAS-positive substance corresponded to adrenalin according to LUDFORD (1928) and CLEMENTI (1963).

It is necessary to observe the histochemical views of epinephrine of the cells in the resting, discharge, rapid discharge, exhaustion and reconstruction periods. The results from the investigation were as shown in Table 4a and 4b.

Table 4-a. Relationships between glycogen deposition in the reticular zone and epinephrine production in the medullary cells of the adrenals of the meat sows.

Alterations	Name of pigs	Sows 22	24	11	27	21	7	2	14	25	1	8	Total 11 cases	Average
Glycogen-deposition (cell-numbers/1 section)	Intranuclear glycogenic bodies	–	1	–	1	–	–	1	6	7	19	–	35	3.2
	Intranuclear glycogen bodies and cytoplasmic glycogen deposition	5	9	14	3	35	12	3	542	26	52	6	707	64.3
	Intracytoplasmic glycogen deposition	467	792	65	76	84	92	134	63	72	69	225	2139	194.5
	Intranuclear vacuolization with no glycogen	–	–	6	–	2	–	14	47	16	16	4	105	9.5
Epinephrine-production	Resting Period	+++	+++	++	+++	++	++	++	++	+++	+++	+++	Positive 100%	Negative 0%
	Discharged Period	++++	+++	++++	++	+++	+++	+++	+++	++	++	++	100%	0%
	Rapid discharged Period	–	–	–	+	++	+	–	–	+	+	++	55%	35%
	Exhaustion Period	–	–	–	–	–	–	–	–	+ S	+ S	+	9%	91%
	Reconstruction Period (Globoid bodies of epinephrines)	–	–	–	–	–	–	–	–	+ S	+ S	–	0%	100%
	Lipoidic globoid bodies	–	–	–	–	–	–	–	–	–	–	–	0%	100%

Remarks: S shows very slightly positive.

Table 4-b. Relationships between glycogen deposition in the reticular zone and epinephrine production in the medullary cells of the adrenals of the meat hogs.

Alterations	Name of pigs	Hogs. 28	29	4	5	12	13	3	26	8	Total (9 cases)	Average
Glycogen-deposition (cell-numbers/1section)	Intranuclear glycogen bodies	–	–	–	4	8	8	1	140	–	161	18.0
	Intranuclear glycogen bodies and cytoplasmic glycogen deposition	14	22	478	29	99	63	–	118	34	864	96.0
	Intracytoplasmic glycogen deposition	468	28	21	1872	206	689	53	87	397	3804	422.6
	Intranuclear vacuolization with no glycogen	12	1	62	1	7	12	3	115	7	220	24.4
Epinephrine-production	Resting period	+++	+++	+++	++++	++++	++++	++++	++++	+++	Positive 100%	Negative 0%
	Discharged period	+++	+++	+++	+++	+++	+++	+++	+++	+++	100%	0%
	Rapid discharge period	++	+	–	–	++	–	–	++	+	56%	34%
	Exhaustion period	–	–	–	–	–	–	–	–	+	11%	89%
	Reconstruction period (Epinephrine globoid bodies)	–	–	+S	+S	–	–	–	–	–	0%	100%
	Lipidic globoid bodies	–	–	–	–	–	–	–	–	–	0%	100%

Remarks: S shows very slightly positive.

3. Epinephrine production in the medullary cells of the adrenals in the meat sows and hogs, especially on the views of hypoepinephrinism.

Almost all of the sows indicated the resting period and discharge period at 100 per cent of them, and there were found no appearance of the epinephrine globoid bodies and lipoidic globoid bodies as described by RUDFORD and CRAMER. The medullary cells in both sows and hogs consisted of two types, one were deep reddish, being closely packed with the PAS-positive epinephrine granules, and the others were a light pink with few granules of epinephrine. The former was called the medullary cells in the resting period, and the latter that in the discharged period. The degree of the appearance of the resting cells in the hogs was indicated more intensively than in the sows. Few cases (♀ no. 25, 1, 8, and ♂ no. 4, 5) showed slight appearance of globoid bodies in the restructed periods, but it is very scant in the field. It is interesting that the intranuclear and intracytoplasmic deposition of glycogen in the cortex was parallel to the resting period of epinephrine production in the medullary. Accordingly, the hyperadrenocorticism and hypoepinephrinism seemed to coexist in the swine adrenals. The severe cases of the glycogen deposition in both nuclei and cytoplasms might be seen with very slight changes such as the abnormality of epinephrine production in the exhaustion or reconstruction periods (globule bodies). But it is not possible to observe the lipidic globoid bodies as the latter mentioned in the hog-cholera immunized pigs.

4. Epinephrine production in the medullary cells of the adrenals in the pigs immunized with the hog-cholera virus, especially on the epinephrine globoid bodies.

Both the first group and second group of the hog-cholera immunized pigs, indicated the absence or disappearance of the resting period, and the cellular changes in the rapid discharged period, exhaustion period and reconstruction period. Especially there were found characteristic changes of the globoid bodies, and lipidic globule-bodies in the medullary cells. In the rapid discharge period, laked cells extensively vacuolated with the lake appearance were shown, and the presence of epinephrine in the central vein or in the alveoli were observed at indifferent stages. However, the groups of light cells which, while giving no indication of secretory activity, do not contain epinephrine granules occupied the greater part of the field. In the exhaustion period, the light cells have no epinephrine granules and no nucleus, and the mixture of epinephrine and plasms existed in the lumen of vein. The globoid bodies as called by RUDFORD and CRAMER were the intracellular inclusions stained with PAS stain and has been undisolved by the organic solvent and saliva digestion. And also the lipidic globoid bodies as called by RUDFORD and CRAMER, were the bulk of the reticulated globules expelled into the alveolar lumen. Their bulk was dissolved by the organic solvent during embedding and staining, and consisted therefore

Table 5-a. Relationships between glycogen in the reticular zone and epinephrine production in the medulla of the adrenals of the first group of pigs immunized with hog-cholera virus.

Alterations \ Name		Hog-cholera-immunized Pigs (First Group)														Total	Average
		1	2	3	4	5	6	7	8	9	10	11	12	13	14		
Glycogen-deposition (cell-counts/1 section)	Intranuclear glycogenic bodies	−	−	−	−	−	−	−	−	−	−	−	−	5	−	5	0.4
	Intranuclear glycogen bodies and cytoplasmic glycogen deposition	−	−	2	4	2	−	−	−	−	−	−	−	8	5	21	1.5
	Intracytoplasmic glycogen deposition	28	70	16	80	6	9	5	6	10	16	32	7	64	40	389	27.8
	Intranuclear vacuolization with no glycogen	9	−	14	3	−	37	−	4	1	74	15	13	23	6	199	14.2
																Positive	Negative
Epinephrine-production (intensity or appearance per 1 section)	Resting period	−	++	−	+	+	+	−	−	−	++	−	−	−	−	36%	64%
	Discharged period	++	+++	+	+++	+++	+++	−	−	+	++	++	−	+++	+++	78%	22%
	Rapid discharge period	+++	++	+	++	+++	+	+	+	+	+	+	+	+	++	100%	0%
	Exhaustion period	+++	++	+++	+	++	++	+++	+++	++	+	+	++	+++	+++	100%	0%
	Reconstruction period (adrenalin globoid bodies)	+++	+	+++	++++	++	+	+++	++	+++	+	+	++	++	+++	100%	0%
	Lipoidic globoid bodies	++	−	+	+	+	−	++	−	+	+	−	++	−	+++	74%	26%

Table 5-b. Relationships between glycogen in the reticular zone and epinephrine production in the medulla of the adrenals of the second group of pigs immunized with hog-cholera virus.

Alterations \ Name		Hog-cholera-immunized pigs (Second Group)															Total	Average
		15	16	17	18	19	20	21	22	23	24	25	26	27	28	29		
Glycogen-deposition (cell counts/1 section)	Intranuclear glycogenic bodies	–	–	–	2	–	–	1	–	–	–	5	7	1	1	–	17	1.2
	Intranuclear glycogen bodies and cytoplasmic glycogen deposition	3	1	2	3	2	–	3	–	1	–	11	8	1	1	2	38	2.7
	Cytoplasmic glycogen depostition	35	39	22	186	329	198	210	17	43	61	180	104	37	35	61	1557	111.2
	Intranuclear vacuolization with no glycogen	3	2	2	2	13	2	8	–	4	9	5	13	11	2	6	82	5.9
																	Positive	Nagative
Epinephrine-production (intensity of appearance per 1 section)	Resting period	–	–	–	–	++	–	–	–	–	–	–	–	–	–	–	7%	93%
	Discharge period	+++	+++	+	+	++	++	–	+	+	++	++	++	+	+	+	93%	7%
	Rapid discharge period	++	+	++	+	+	++	+	+++	++	+++	+++	++	+++	++	+	100%	0%
	Exhaustion period	++	+++	+++	+++	+	+++	+++	+++	+++	++	++	+++	+++	++	+	100%	0%
	Reconstruction period (adrenalin globoid bodies)	++	+	++	++	–	+++	++	++	+++	+++	+++	++	++	++	++	93%	7%
	Lipidic globoid bodies	++	+	++	+	–	++	+	++	++	+++	++	+++	+++	+	++	93%	7%

of lipoid material. Some portions of these globules have resisted the action of organic solvent and therefor are not lipoid in nature and stained with PAS. In the medulla of the adrenals of the pigs immunized with the hog-cholera virus these lipidic globoid bodies were found in very many cases.

It is very interesting that the absence of the intranuclear and intracytoplasmic deposition of glycogen in the zona reticularis was parallel to the abundance of the exhaustion, and the appearance of the globoid-bodies and lipidic globoid-bodies in the medulla. Accordingly, the hypoadrenocorticism and hyperepinephrniism seemed to coexist in the swine adrenals from the points of the aglycogenesis in the cortex and the epinephrine-production in the medulla.

To compare the results of both meat pigs and hog-cholera immunized pigs, remarkable differences were indicated as shown in Table 6.

Table 6. Comparison with the epinephrine production in the adrenals of the meat pigs and immunized pigs.

Epinephrine-production \ Degrees of intensity	Meat pigs						
	+ 4	+ 3	+ 2	+ 1	+ S	–	total
Resting period	5(25%)	13(65%)	2(10%)	–	–	–	20(100%)
Discharge period	3(15%)	15(75%)	2(10%)	–	–	–	20(100%)
Rapid discharge period	–	–	10(50%)	5(25%)	–	5(25%)	20(100%)
Exhaustion period	–	–	1(5%)	2(10%)	2(10%)	15(75%)	20(100%)
Reconstruction period (Epinephrine globoid bodies)	–	–	–	–	6(30%)	14(70%)	20(100%)
Lipidic globoid boides	–	–	–	–	–	20(100%)	20(100%)
Epinephrine-production \ Degrees of intensity	**Pigs immunized with hog-cholera virus**						
	+ 4	+ 3	+ 2	+ 1	+ S	–	total
Resting period	–	3(10%)	2(7%)	1(3%)	–	20(80%)	29(100%)
Discharge period	–	8(28%)	8(28%)	9(31%)	–	4(13%)	29(100%)
Rapid discharge period	–	6(21%)	9(31%)	–	–	4(48%)	29(100%)
Exhaustion period	–	15(52%)	10(35%)	4(13%)	–	–	29(100%)
Reconstruction period (Epinephrine globoid bodies)	1(3%)	10(35%)	11(41%)	4(12%)	2(6%)	1(3%)	29(100%)
Lipidic globoid bodies	–	4(12%)	10(33%)	8(24%)	1(3%)	6(18%)	29(100%)

Summary and Conclusion

Previously the occurrence of the glycogen deposition within both nuclei and cytoplasms of the zona reticularis in the swine adrenals, and the relationships between the lesions of diabetes mellitus and characteristic alterations of the swine adipositus were reported.

In the present study they were compared concerning the diminution of the occurrence of glycogen within the nuclei and cytoplasms of the zona reticularis, and on the appearance of the globoid bodies of epinephrine within the medullary cells in the adrenals of the pigs immunized with the hog-cholera virus. These investigations were done histochemically on the adrenals of Yorkshire pigs used for the experiment of the feeding standard on the meat pigs, and that of the immunized pigs with the hog-cholera virus.

It may be concluded from the results of this investigation as follows:

1) It was noticed that the intranuclear glycogen deposition showed a great difference between the swine obesity and the immunized pigs. Whole cells with the glycogen-bearing nuclei or cytoplasms were counted in one section of the swine adrenal stained with PAS-hematoxylin using the square-meter. On the average of swine adrenals of the meat pigs (both 11 sows and 9 hogs), there occurred 79 cells/1 section with glycogen in both nuclei and cytoplasms; and 10 cells/1 section with glycogen in only nuclei; and 297 cells/1 section with glycogen in only cytoplasms, and 16 cells/1 section with intranuclear vacuoles of no glycogen; nevertheless on the average of 29 immunized pigs, there were observed, 2 cells/1 section with glycogen in both nuclei and cytoplasms and 1 cell/1 section with glycogen in only nuclei, 7 cells with glycogen in only cytoplasms, and 10 cells/1 section with no glycogen within the giant nuclelar vacuoles.

2) To compare the results of meat pigs and hog-cholera immunized pigs, remarkable differences were indicated such as the changes of hypoepinephrinism or hyperepinephrinism.

Frequency of positive results of epinephrine production occurred in 100 per cent of the resting period, 100 per cent of the discharge period in the medulla of the meat pigs; and 20 per cent of the resting period and 87 per cent of the dischage period in the immunized pigs. On the contrary, there were 15 per cent of the exhaustion period, 0 per cent of the restruction period (with both epinephrine globoid bodies and lipidic globoid bodies) in the medulla of the meat pigs, and 100 per cent of the exhaustion period and restruction period (with both epinephrine globoid bodies and lipidic globoid bodies) in the immunized pigs.

3) On the basis of LILLIE's opinion, it seemed reasonable to assume that the PAS-positive reaction gives a positive chromaffine reaction, and that this reaction causes the production of aldehyde from the hydroxy-methyl-amino group of epinephrine or one of its precursors. By the investigation of the PAS-positive epinephrine in the medulla there seemed to be the views of resting, discharge, exhaustion and reconstruction periods in the epinephrine production in reference to LUDFORD and CRAMER's work on the morphological varieties of osminophilic epinephrine.

4) From the above-mentioned results, the diminution of the occurrence of glycogen within the nuclei and cytoplasms of the zona reticularis, and the histochemical views of the hyperepinephrinism such as the globoid bodies of the epinephrine reconstruction and the fine granules of the epinephrine-discharge in the cytoplasms of the medulla were found in the hog-cholera immunized pigs.

5) On the contrary of the hog-cholera immunized pigs, the remarkable occurrence of glycogen within the nuclei and cytoplasms of the zona reticularis, and the histochemical views of the hypoepinephrinism such as the resting storage of epinephrine granules in the cytoplasms of the medulla were indicated in the meat pigs.

In view of the above facts the most reasonable conclusion to be drawn from available data is that the nuclear and cytoplasmic glycogen deposition was accelerated by the under-secretion of epinephrin in the swine obesity, and was inhibited by the oversecretion of epinephrine in the hog-cholera immunization. Accordingly, it has been clarified that ther were found histochemical facts of hyperadrenocorticism-hypoepinephrinism in the physiological obesity of the meat pigs, and that of hypoadrenocorticism-hyperepinephrinism in the pigs immunized with the hog-cholera. The results of the author's histochemical studies on the epinephrine-production, lend some support to RUDFORD and CRAMER's pictorical hypothesis about the epinephrin-production.

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Plate 1

Explanation of Figures

Fig. 1. Alvelolar cells of medulla in the resting period of epinephrine production, stained with PAS-hematoxylin stain, and enlarged to 400 ×. Epinephrine granules filled in the cells and reacted strongly on PAS reaction, and indicated as the dark cells.

Fig. 2. Alveolar cells of medulla in the discharge period of epinephrine production, stained with PAS-hematoxylin stain, and enlarged to 400 ×. Epinephrine granules diminished in the cells and reacted weakly on PAS stain, and indicated as the light cells.

Fig. 3. So-called lake cells in the medullary alveoli were extensively vacuolated, and exhibited a laked appearance of the cytoplasm. The figure represented a condition of intense activity of the gland (Mark: L). Stained with PAS-hematoxylin stain, and enlarged to 400 ×.

Fig. 4. A large amount of the globoid bodies (Mark: GB) reacted strongly on PAS stain appeared in the reconstructed period of the alveolar cells of the medulla. Enlarged to 400 ×.

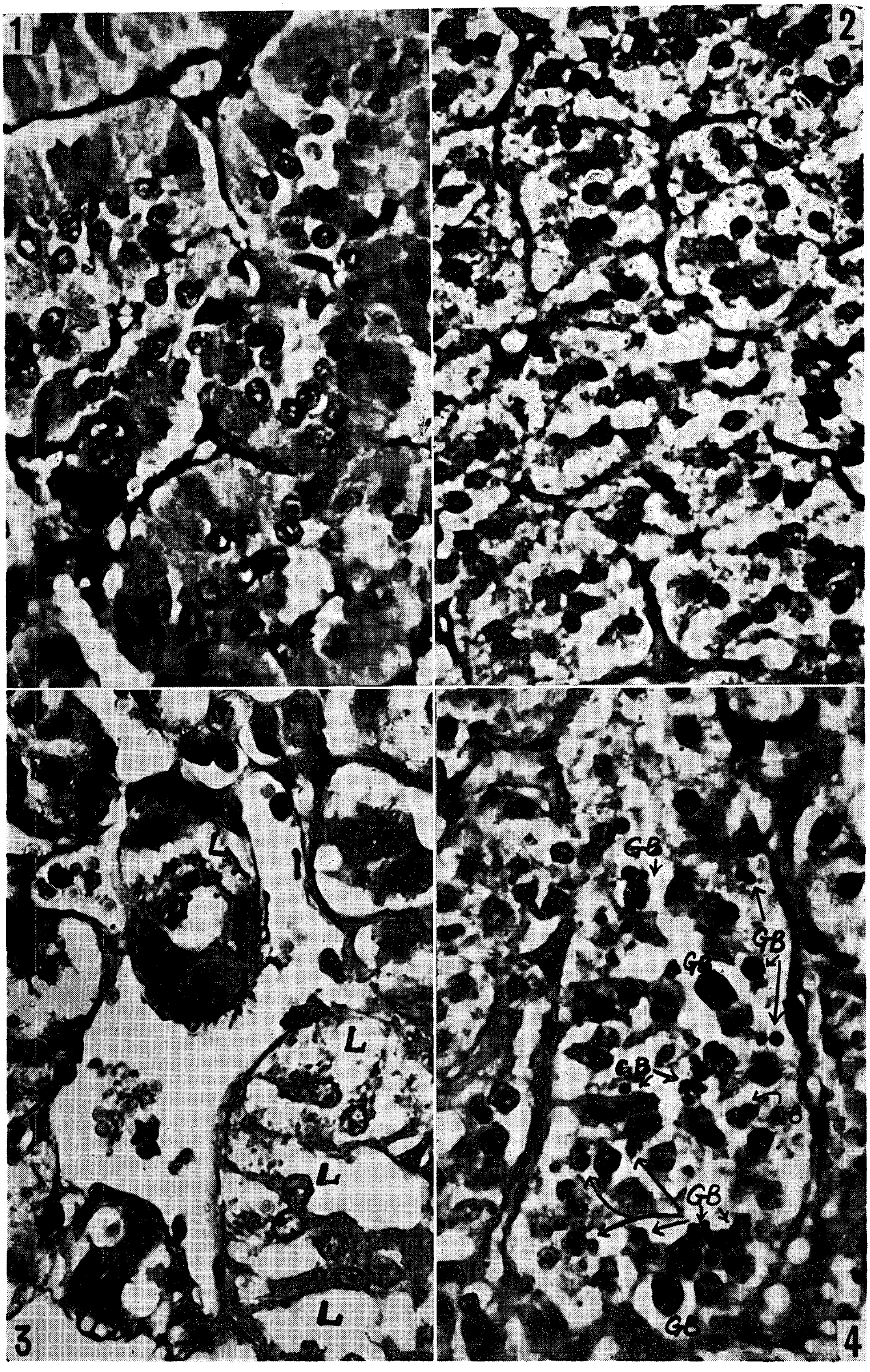
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Plate 2

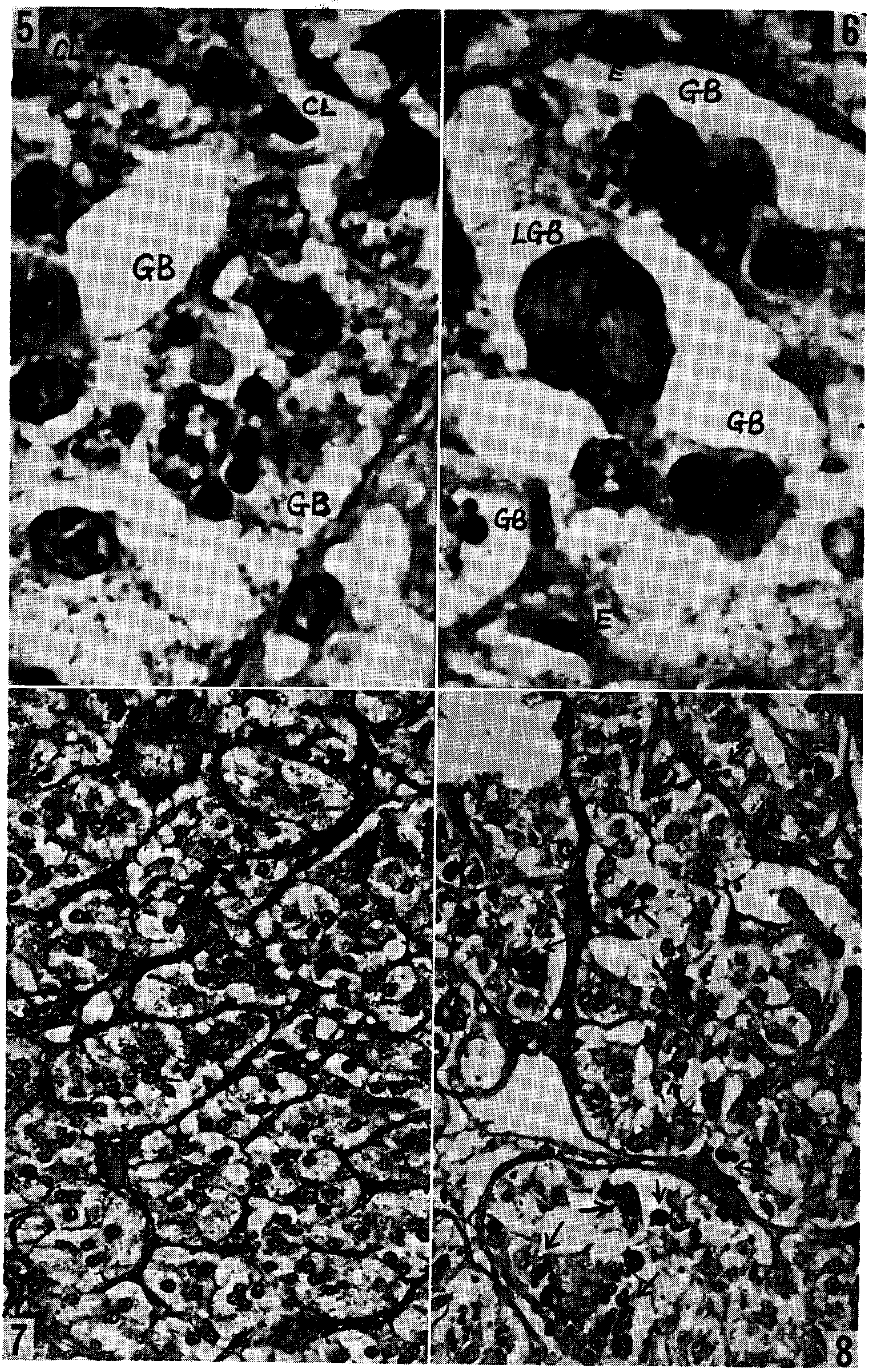
Explanation of Figures

Fig. 5. High-power view of adrenal medulla showing island of "light cells" with red "globoid bodies". The cells were swollen and contained a number of large globoid bodies which were not fat or lipoid, but probably epinephrine or a precursor of epinephrine. Those appeared in the reconstructed periods of epinephrine production (Mark : GB). Exhausted cell showed different stage in the collapse of nucleus accompanying the discharge of epinephrine (Mark : CL). Stained with PAS-hematoxylin stain and enlarged to 1000 ×.

Fig. 6. High-power view of adrenal medulla showing island of "light cells" with red "globoid bodies" and "lipidic globoid bodies". These bodies were expelled into the lumen of the medullary alveoli. Lipidic globoid bodies (Mark : LGB) showed that though the blulk of the reticulated globules has been dissolved by organic solvents, and consisted therefore of lipoid materials, some portions of these granules have resisted the action of organic solvent and were therefore not lipoid in nature. Globoid bodies (Mark : GB) were localized in the reconstructed period. The nuclei of exhausted cells were pyknotic and atrophy (Mark : E). Stained with PAS, and enlarged to 1000 ×.

Fig. 7. Weak-power view of the adrenal medulla showing island of "light cells" in the discharge period of the meat pigs. Stained with PAS-stain and enlarged to 200 ×. This view was characteristic in swine obesity and followed on the resting period of epinephrine production.

Fig. 8. Weak-power view of the adrenal medulla showing island of "light cells" with "globoid bodies" in the reconstructed period of the pigs immunized with hog-cholera virus. Stained with PAS-stain and enlarged to 200 ×. This view was characteristic in immunized pigs and followed on the absence of the resting period of epinephrine production (Compare on Fig. 7).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