PHYSIO-HISTOLOGICAL STUDIES ON THE PHYSIOLOGICAL OBESITY OF THE MEAT PIGS (REPORT IV), ESPECIALLY ON THE PRODUCTION SYSTEM OF CORTICOSTEROIDS STAINED WITH NAKAO'S Hcl-SbCl_3-REACTION WITHIN THE CYTOPLASMS OF THE ZONA RETICULARIS IN THE ADRENALS

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PHYSIO-HISTOLOGICAL STUDIES ON THE PHYSIOLOGICAL OBESITY OF THE MEAT PIGS (REPORT IV), ESPECIALLY ON THE PRODUCTION SYSTEM OF CORTICOSTEROIDS STAINED WITH NAKAO'S Hcl-$SbCl_3$-REACTION WITHIN THE CYTOPLASMS OF THE ZONA RETICULARIS IN THE ADRENALS

By

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Introduction

Mechanism of adipositas and the effect of fattening have been investigated histochemically in various organs of Yorkshire pigs used for the Pig Feeding Standard revising test in pork production (1). During these investigations, the occurrence of glycogen was found in the nuclei and cytoplasms of the zona reticularis in the adrenals of the pigs. No one has found glycogen there.

According to the previous report (2, 3, 4, 5) the authors described that to the effect that the pigs were considered as the physiological adipositas, it might be important to observe the relationship between the occurrence of glycogen in the nuclei and cytoplasms of the adrenal cells and the deposition of fat in the cytoplasms of the adrenal cells and that between these fat-containing cells corresponding to the ketosteroid-producing cells and the certification of ketosteroid. The authors also stated that those features will be described at another occasion.

By the present study there was found the occurrence of the ketosteroid-bearing cells stained with NAKAO's Hcl-$SbCl_3$-reaction and Bennett's ketosteroid staining in the adrenal of the pigs, and it was planned to examine the mode of ketosteroid-bearing cells, and the relationships between glycogen, fat and ketosteroid deposition.

Recently it has been shown that the working hypothesis as the regulation of the intracellular reaction by hormone (SUTHERLAND (6) 1960 and KONDO (7) 1963) might be established. According to SUTHERLAND's opinion (6), there were found the following systems: glycolysis from glycogen to glucose-6-P by the active phosphorylase, transformation of NADP to NADP·H_2 accelerated by the changes of glucose-6-P to 6-P-G, and the production system of NADP to NADP·H_2, accordingly these relations are closely connected with glycolysis, coenzymes and steroid hormone. According to ITIKAWA *et al* (2 and 3) in the animals besides pigs the glycogenolysis was done in the liver and the production of corticosteroids in the adrenals, so the presence of glycogen in the nuclei and cytoplasms of the swine adrenals found recently by the authors, might be effective to produce the corticosteroids. According to WILLIAMS (8) (1962), as the food intake increased, the beta cells of the pancreas was stimulated, producing more insulin; and when insulin was present in excess it promoted an excess deposit of fat, particularly with excess food ingestion. Insulin produced an increase in lipids and it was highly important in lipogenesis. Insulin was known to increase the output of glycosteroids, which in turn played an important role in stimulating the increased production of insulin antagonists. Obesity tended to produce hyperinsulinism and hyperadrenocorticism, which could in turn significantly further the obesity.

Accordingly, it has been clarified on the mechanism of obesity that hyperadrenocorticism accelerated hyperinsulinism to increase the fat metabolism. To the effect that the pigs were considered as the physiological adipositas, it might be important to observe the relationship between the occurrence of glycogen in the nuclei and cytoplasms of the adrenal cells, the deposition of fat in the cytoplasms of the adrenal cells, and the production of corticosteroid. These fat-containing cells corresponded to the ketosteroid-producing cells described at this occasion.

According to WILLIAMS (8) (1962), phosphorylase accelerated glycogenolysis, thereby increasing glucose-6-phosphate; and the amount of glucose-6-phosphate increased in the adrenal, with the result that more TPNH was produced. This co-factor accelerated splitting of the cholesterol side-chain and reduction of certain steroids, leading to increased synthesis of corticosteroids. These accelerations of the co-factor will be described at another occasion.

Materials and Methods for Studies

Seven sows and six hogs of Yorkshire were used. Boars were castrated at the age of 28 days after birth. These hogs and sows born from the same father and two different mothers, were bought at the age of 55 days after birth. During the age of 55 to 74 days after birth they were administered with the artificial milk, and then used for experiment for a 126 days from September, 1963 to January, 1964. The animals were studied on the Feeding Standard revising test in the case of pork production by the administration with various feeds such as H′-EC′ group (DCP 13.5, TDN 69.9), EC′-H′ group (DCP 12.1, TDN 69.8), F′-EC′ group (DCP 10.7, TDN 69.8) and EC′-F′ (DCP 12.1, TDN 69.9) in the Miyagi

Prefectural Agricultural Experimental Station at Sendai in 1963-1964. The rations of the feed are shown in Table 1.

Table 1. Rations used for experiment.

Feeding \ Exp. Groups \ Period	Former period				Later period			
	H′-EC′	EC′-H′	F′-EC′	EC′-F′	H′-EC′	EC′-H′	F′-EC′	EC′-F′
Concentrated/Barley	65 : 35	65 : 35	65 : 35	65 : 35	65 : 35	65 : 35	65 : 35	65 : 35
Combined feed (No.)	(No. 131)	(No. 132)	(No. 133)	(No. 132)	(No. 134)	(No. 135)	(No. 134)	(No. 136)
Yellow corn	26.0	28.0	30.0	28.0	32.0	29.0	32.0	28.0
Bran	15.0	16.0	18.0	16.0	17.0	16.0	17.0	12.0
Defattend rice-bran	6.0	7.0	7.0	7.0	7.0	7.0	7.0	5.0
Soybean oil meal	8.0	5.0	2.0	5.0	2.0	5.0	2.0	1.0
Fish meal	4.0	3.0	2.0	3.0	1.0	2.0	2.0	1.0
Starch meal	—	—	—	—	—	—	—	12.0
Others	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Barley	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0
Alphalpha meal	—	—	—	—	—	—	—	—

Pigs in each lot were fed with the ration given above in Table 1. Rations were administered under the dry lot-feeding conditions and the pigs were given suitable exercise for about two hours each day on the concrete floor. At first no stall was littered with straw, but the litter was used in winter. Experimental pigs were kept two by two of the same sex in the stalls of 15 square meters in

Table 2. Body weight and feed intake of the pigs according to sex and experimental periods.

Lots	Sex (Cases)	Ages (days)			Body weight (kg)				Feed intake (kg) during all periods			Remarks
		Initial	Middle period	Final period	Initial	Former period	Middle period	Final period	Former period	Final period	Whole period	
H′-EC′	♀ (2)	74	144	200	19.3	33.7	53.0	85.2	231.1	271.1	502.7	DCP 13.5
	♂ (2)	74	144	200	18.6	33.5	52.1	86.4	220.0	308.5	528.5	TDN 69.9
EC′-H′	♀ (2)	74	144	200	17.7	34.8	54.8	90.8	100.3	152.2	252.2	DCP 12.1
	♂ (2)	74	144	200	19.3	38.0	57.3	95.5	228.8	318.8	547.6	TDN 69.8
F′-EC′	♀ (2)	74	144	200	19.0	21.1	40.1	64.9	170.6	213.4	384.0	DCP 10.7
	♀ (2)	74	144	200	19.3	27.6	46.8	81.8	202.0	282.6	484.6	TDN 69.8
EC′-F′	♀ (2)	74	144	200	19.5	27.6	47.0	84.4	201.9	315.7	517.6	DCP 12.1
	♀ (2)	74	144	200	19.2	37.3	56.4	89.5	240.7	305.5	546.2	TDN 69.9

size. All the pigs showed an increase of body weight and rapid growth and good apetite. Autopsy was done at the age of 200 days after birth, and the body weight and feed intake according to sex and experimental periods are shown in Table 2.

The total adrenals from these sows and hogs were fixed in buffered formol or in CARNOY's fluid, embedded in paraffine and cut into 6 μ sections, and cut with frozen microtome for fat staining. The stains employed were: PAS-hematoxylin stain with or without saliva digestion for the glycogen; and NAKAO's Hcl-$SbCl_3$-reaction for steroid hormone.

Results

1. The relationships between the fat-stored cells and ketosteroid-bearing cells in zona reticularis

With regards to the histochemistry of ketosteroids of the adrenals many papers have been published, but they seemed to be not valuable because of coloring under the unpurified states of corticosteroids as the tissue sections. According to the many reports that demonstrated the presence or localization of steroids up to now, the nature of the products in the staining reactions consisted of the following substances: plasmal substance LISON (9) 1953), pseudo-plasmal substance (CHU (10) 1950), peroxide (CAIN (11) 1949), unsaturated fatty acids (BAYLEY (12) 1945), enzyme (GOMORI (13) 1950), unknown unsaponified substance (UI (14) 1957) and cholesterol (SCHULTZ (15) 1924).

MAYEDA (16) (1962) described on some problems in the corticosteroids staining of the adrenals as follows: it is indirectly possible to presume the presence of steroids in the sudanophilic granules by means of the experiments.

According to YOSHIMURA (17) (1962) who mentioned doubt as to the ketosteroid-staining from the side of cytology, he asserted that it is dangerous to consider the barrier of steroids in the fat granules, and it is important to observe the hormonal barrier in the protein granules with the so-called masked fat as the precursoir. YOSHIMURA recognized the transformation of the iron-hematoxylin-stainable granules to fat granules from the side of light microscopy and observed the development of the mitochondria to the fat granules (storage form) of the proteinic granule with masked fat from electron microscopy. He explained the developmental stage in the secretion of the cortical cells such as vesicle formation of the swollen mitochondria in the hyperfunction of steroid hormone-production; and degenerative mitochondria with the long-term fat preserve in the hypofunctional development; and normal development of the mitochondria to both fat granule and proteinic granules with masked fat. Accordingly, he considered that the increase in the decrease of the stainable granules and those of vesicles in the mitochondria might be become a marker to determine the hormonal function of the cortical cells.

Table 3. Relationships between the fat deposition and ketosteroid production in the cortical cells of swine adrenals

Feeding		H′-EC′ Group								E′C-H′ Group				F′-E′C Group						E′C-F′ Group							
Sex		♀				♂				♀		♂		♀				♂		♀				♂			
Name		1		8		11		18		2		12		3		6		16		4		5		14		1	
Localization	Fat and Keto-steroid	Ketosteroid	Fat	Ketosteroid	Fat	Ketosteroid	Fat	Ketosteroid	Fat	Ketosteroid	Fat	Ketosteroid	Fat	Ketosteroid	Fat	Ketostreoid	Fat	Ketosteroid	Fat	Ketosteroid	Fat	Ketosteroid	Fat	Ketosteroid	Fat	Ketosteroid	Fat
Cortex	Z. glome-rularis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
Cortex	Z. fasci-culata	−	+	−	+	−	⧺	+	⧺	+	⧺	+	⧺	+	⧺	−	+	−	⧺	−	+	−	+	+	⧺	+	⧺
Cortex	Z. reticul-aris	−	+	+	⧻	+	⧺	⧺	⧺	+	⧻	⧺	⧺	⧺	⧺	−	+	⧺	⧺	⧺	⧻	⧺	⧻	+	⧻	⧻	⧻
Medull-ary	Invasion of cortex to medullary	−	+	−	⧻	+	⧺	⧺	⧺	−	⧻	⧺	⧺	+	⧺	−	+	⧺	⧺	+	⧻	+	⧻	+	⧺	+	+

Remarks: Ketosteroid indicated by Nakao's HCl-$SbCl_3$ reaction, fat was stained with Sudan III-stain.

NAKAO (18, 19, 20, 21) (1962) found a new method which he called $SbCl_3$-reaction to demonstrate corticosteroid, and he stated on the antimony-granules in this histochemical regions. However, owing to the correspondence to the sudanophilic part and Hcl-$SbCl_3$-reactive part, they were considered to be sudanophilic cells which contained ketosteroid, and indicated the significance and localization of the fat-stored cells shown in his report.

In the present investigation the relationships between the sudanophilic granules, Hcl-$SbCl_3$-granules, and glycogen-laden nuclei and glycogen-bearing cytoplasms, are discussed. These results are shown in the tables (Fig. 1). The following opinions are summarized from these tables:

The relationships between the fat deposition and ketosteroid production in the cortical cells of the swine adrenals are indicated in the Table 3.

It was noticed that a large amount of the cytoplasms in the zona reticularis of the swine adrenals showed a strong positive reaction to NAKAO's HCl-$SbCl_3$ reaction for the demonstration for ketosteroids; seven sows and six hogs contained the ketosteroid-laden cytoplasms of the cells in the zona reticularis (in 85%) or zona fasciculata (in 44%). And also in the zona glomerularis the ketosteroid-laden cells were found in only 8 percent of all cases.

Seven sows and six hogs contained the fat-stored cells in the zona fasciculata (in 100%) and in the zona reticularis (in 100%). According to previous report (1, 2, 3,) the authors consider the sudanophilic cells as a ketosteroid-bearing cells because of the correspondence to the stainability of sudanophilia and antimony granules (NAKAO) in the cells of zona reticularis.

They were divided into two types of rich fat-stored cells (in 85%) and poor fat-stored cells (in 25%) in the zona fasciculata et reticularis.

The combination of the fat-stored cells and ketosteroid-bearing cells are shown in Table 4.

Table 4. Various types of the cells indicating the relation between the fat-stored cells and ketosteroid-bearing cells in the zona reticularis et fasciculata of the swine adrenals.

		Fat-stored cells		
		Rich	Poor or None	Total
Ketosteroid-bearing-cells	Rich	6 (46%)		6 (46%)
	Poor	5 (39%)		5 (39%)
	None		2 (15%)	2 (15%)
	Total	11 (85%)	2 (15%)	13 (100%)

Table 5. Relationships between the glycogen deposition within the nuclei and cytoplasms of the cortical cells and ketosteroid production in the cortical cells of swine adrenals.

Group	H′-EC′ Group								EC′-H′ Group				F′-EC′ Group						E′C-F′ Group							
Sex	♀				⚥				♀		⚥		♀				⚥		♀				⚥			
Name	1		8		11		18		2		12		3		6		16		4		5		14		15	
Localization / Glycogen, Ketosteroid	Nucl. (Cytopl.) glycogen	Ketosteroids	Nucl. (Cytopl.) glycogen	Ketosteroids	Nucl. (Cytopl.) glycogen	Ketosteroids	Nucl. (Cytopl.) glycogen	Ketosteroids	Nucl. (Cytopl.) glycogen	Ketosteroids	Nucl. (Cytopl.) glycogen	Ketosteroids	Nucl. (Cytopl.) glycogen	Ketosteroids	Nucl. (Cytopl.) glycogen	Ketosteroids	Nucl. (Cytopl.) glycogen	Ketosteroids	Nucl. (Cytopl.) glycogen	Ketosteroids	Nucl. (Cytopl.) glycogen	Ketosteroids	Nucl. (Cytopl.) glycogen	Ketosteroids	Nucl. (Cytopl.) glycogen	Ketosteroids
Cortex: Z. glomerularis	− (−)	−	− (−)	−	− (−)	−	− (−)	−	− (−)	−	− (−)	−	− ($+_s$)	−	− (−)	−	− (−)	−	− ($+_s$)	−	− (−)	−	− ($+_s$)	−	− (−)	+
Cortex: Z. fascularis.	− ($+_s$)	−	− (−)	−	− (−)	−	− (−)	+	− (−)	$+_s$	− ($+_s$)	$+_s$	− (+)	+	− (+)	−	− (−)	−	− (+)	−	− (+)	−	− (++)	+	− (−)	+
Cortex: Z. reticularis	+++ (+++)	−	+ (+)	+	++ (+++)	+	+++ (++)	++	+ (++)	+	+++ (++)	++	+++ (++)	++	+++ (++)	−	+++ (++)	++	+ (+)	++	+++ (++)	++	++ (++)	+	+++ (+++)	+++
Invasion of cortex to medullary	− (−)	−	− (−)	−	− (−)	+	− (−)	++	− (−)	−	− (−)	++	− (−)	+	− (−)	−	− (−)	++	− (−)	+	+ (+++)	+	++ (+++)	+	− (−)	+

From the point of views in Table 4, there were observed three types such as rich fat-stored cells—rich ketosteroid-bearing cells, rich fat-stored cells—poor ketosteroid-bearing cells and poor fat-stored cells—none ketosteroid cells. There may exist the remarkable ketosteroid producing stage, poor ketosteroid producing stage and resting ketosteroid-producing stage.

2. The relationships between the steroid-bearing cells, and nuclear and cytoplasmic glycogen deposition.

Seven sows and six hogs contained the intranuclear glycogen-(0%)-and intracytoplasmic glycogen (23%)-bearing cells in the zona glomerularis; intranuclear glycogen (0%)- and intracytoplasmic (54%) bearing cells in the zona fasciculata; and intranuclear glycogen (100%) -and intracytoplasmic glycogen (100%)-bearing cells in the zona reticularis. Accordingly there were found glycogen in both cytoplasms and nuclei of the cells in the zona reticularis in 100 percent.

On the contrary the ketosteroid-bearing cells were shown in 8 percent of all zona glomerularis, in 46 percent of all zona fasciculata and in 85 percent of all zona reticularis.

The combination of the nuclear-cytoplasmic glycogen-bearing cells and ketosteroid-bearing cells are shown in Table 6.

Table 6. Various types of cells indicate the relation between the glycogen-bearing cells and ketosteroid cells in the zona reticularis of swine adrenals.

Ketosteroid \ Glycogen		Glycogen-bearing cells: Rich ⧻ , ⧺	Glycogen-bearing cells: Poor, None +, −	Total
Ketosteroid-bearing cells	Rich	6 (46%)	1 (8%)	7 (54%)
	Poor, None +, −	5 (38%)	1 (8%)	6 (46%)
	Total	11 (84%)	2 (16%)	13 (100%)

From the Table 4, there were observed four types such as rich glycogen-rich ketosteroid in 46 percent, rich glycogen-poor ketosteroid in 38 percent, poor glycogen-rich ketosteroid in 8 percent and poor glycogen-poor ketosteroid in 8 percent of all cases. Accordingly, those might indicate the various types of the metabolic stages.

3. On the localization of glycogen, fat and ketosteroids in the zona reticularis.

According to previous reports (1, 2, 3, 4, and 5), the presence of glycogen in the nuclei of the swine adrenals were found to be remarkably stronger or

Table 7. Relationships between the glycogen, fat and ketosteroid in the various types of the zona reticularis.

Group		H′-EC′ Group												E′C-H′ Gr.					
Sex		♀						♂						♀			♂		
Name		1			8			11			18			2			12		
Localization in Z. reticularis	Glycogen / Ketosteroid / Fat	Nuc. (Cyt.) glycogen	Ketosteroid	Fat	Nuc. (Cyt.) glycogen	Ketosteroid	Fat	Nuc. (Cyt.) glycogen	Ketosteroid	Fat	Nuc. (Cyt.) glycogen	Ketosteroid	Fat	Nuc. (Cyt.) glycogen	Ketosteroid	Fat	Nuc. (Cyt.) glycogen	Ketosteroid	Fat
I	Invasion to medullary	− (+)	−	+	− (−)	−	+++	− (−)	+	++	− (−)	++	++	− (−)	−	+++	− (−)	++	++
II	Medullary zone	+++ (+++)	−	+	+ (++)	+	+++	++ (+++)	+	+++	+++ (++)	++	+++	+ (+++)	+	+++	+++ (−)	+++	+++
III	Boundary zone	+ (+)	−	+	− (+)s	+	+++	− (−)	−	++	++ (−)	−	++	− (−)	+	++	+ (−)	+	+
IV	Evaginated new zone	+++ (+++)			+ +			++ (+++)			+++ (+++)			− (+)			− (−)		

Group		F′-E′C Group									E′C-F′ Group											
Sex		♀			♀			♂			♀						♂					
Name		3			6			16			4			5			14					
Localization in Z. reticularis	Glycogen / Ketosteroid / Fat	Nuc. (Cyt.) glycogen	Ketosteroid	Fat	Nuc. (Cyt.) glycogen	Ketosteroid	Fat	Nuc. (Cyt.) glycogen	Ketosteroid	Fat	Nuc. (Cyt.) glycogen	Ketosteroid	Fat	Nuc. (Cyt.) glycogen	Ketosteroid	Fat	Nuc. (Cyt.) glycogen	Ketosteroid	Fat	Nuc. (Cyt.) glycogen	Ketosteroid	Fat
I	Invasion to medullary	+ (+)	+	++	− (−)	−	+	− (−)	++	++	− (−)	+	+++	+ (+++)	+	+++	++ (+++)	+	+++	− (−)	+	+
II	Medullary zone	+++ (++)	++	+++	++ (+++)	−	+	+++ (++)	+++	+++	++ (−)	+++	+++	+++ (++)	++	+++	++ (++)	−	++	+++ (+++)	+	++
III	Boundary zone	+ (+)	+	++	+ (+)	−	+	− (+)s	−	++	− (−)	−	+	− (+)	−	+	− (+)	−	++	− (+)s	+++	+++
IV	Evaginated new zone	+++ (+)			+++ (+++)			+++ −			+ (++)			− (−)			− (−)			+++ (+++)		

more intensive in the hogs than in the sows, and almost all of the intranuclear glycogen present in the juxta medullary zone of zona reticularis ajoins the medullary, and also a large amount of glycogen in the nuclei existed in the boundary zone (temporary cortex or central body). The present study described the existence of various types of the localization of glycogen, fat and ketosteroids. They were divided into four types such as the deposition in the juxta medullary zone (Type II), that in the boundary zone (Type III), that in the imigrated cortical portion to the medullary (Type I), and that around the process-like development of the medullary (Type IV).

In the present investigation the glycogen-, fat- and ketosteroid-containing cells are shown in the Tables 5 and 6.

Table 8. Localization of the intranuclear and intracytoplasmic glycogen- intracytoplasmic fat-, and intracytoplasmic ketosteroid-deposition in the zona reticularis of 13 pigs.

Type	Appeared substances, cases and percentage / Localization	Intra-nuclear	Intracytoplasmic deposition			
		Glycogen	Glycogen	Ketosteroid	Fat	Examined cases
I	Invasion to medullary	3 (23%)	3 (23%)	9 (70%)	13 (100%)	13
II	Juxta medullary zone	13 (100%)	13 (100%)	10 (80%)	13 (100%)	13
III	Boundary zone	5 (40%)	10 (80%)	5 (40%)	13 (100%)	13
IV	Evaginated new zone	9 (70%)	10 (80%)			13
Cases examined		13	13	13	13	13

The opinions summarized from these tables are the following:

a) Usually the intranuclear glycogen deposition was shown in the juxta medullary zone at 100 percent and in the evaginated new zone at 70 percent, but in the boundary zone at 40 percent and the imigrated cortical portion to the medullary at 23 percent.

b) The intracytoplasmic glycogen deposition existed in the order to juxta medullary zone (100%)>boundary zone (80%), evaginated new zone (80%)> imigrated cortical portion to the medullary (23%).

c) Ketosteroid deposited in the cells of the juxta medullary zone (80%) and the imigrated cortical portion to medullary (70%), and that of the boundary zone (40%). Ketosteroids appeared in the nearest portion of the zona reticularis around the medullary.

d) In all cases fat deposited in the juxta medullary zone (100%), the boundary zone (100%) and the imigrated cortical portion to the medullary (100%).

Accordingly from this results there might have occurred more intensive cases of glycogen-, fat- and ketosteroid-deposition in the juxta medullary zone than in the other types. On the contrary, glycogen-, fat- and ketosteroid-deposition in the imigrated cortical portion to the medullary and in the boundary zone were various. This might be considered to be due to the metabolic difference in the portion of the zona reticularis.

4. The shape of the intracytoplasmic ketosteroid deposition.

The presence of ketosteroid in the cytoplasms of the swine adrenals were found to be remarkably stronger or more intensive in the juxta medullary zone near the medullary and in the imigrated portion of the zona reticularis within the medullary than in the boundary zone of zona reticularis ajoined to the zona fasciculata.

In severe cases there occurred a large amount of ketosteroid in the cytoplasms, and this extended to the deep zone of the zona fasciculata, and also to the spongy zone. The cells indicated a network of round-like appearance in the juxta medullary zone, and that of strand-like appearance in the boundary zone.

Ketosteroid-laden cytoplasms contained a large amount of short rod-like or ovoid granules that stained reddish brown with NAKAO's HCl-$SbCl_3$ reaction. This coloring raction disappeared at 30 minutes or 1 hour after the reaction, so it is important to observe it instantly.

These ketosteroid-granules consisted of various types such as the entire type filled with the voluminous granules, diffuse type and the intralipidic type in the fat-droplets. The ketosteroid-granules lost stainability after a day and were seen to be blackish brown crystaloids within the fat-droplets in the cytoplasm. The cells contained a large amount of the small fat-droplets. There were observed the ketosteroid-granules within them, but in the cells with no fat-droplets the ketosteroid-granules existed in the cytoplasms. It is necessary to observe whether these ketosteroid-laden cells contained no fat droplets correspondent to the glycogen-rich cells. But in the portion with the glycogen rich cells there were found the ketosteroid-laden cells without fat droplets.

5. Relationships between sex and ketosteroid-laden cells.

According to the previous report, the appearance of intranuclear glycogen did not seen to be related to the feeding, but it was clear that it is related to sex. The intranuclear glycogen tended to appear more intensive in the hogs than in the sows. By the present study it was found that the ketosteroid-laden cells tended to appear more intensive in the hogs than in the sows as shown in Table 9.

Table 9. Relationships between glycogen, fat and ketosteroid deposition in the swine adrenals of the hogs and sows.

Relation / Sex / Combination / Zone	Relation between ketosteroid (K) and fat (F)								Relation between glycogen (G) and ketosteroid (K)							
	Hogs				Sows				Hogs				Sows			
	K+F+	K+F−	K−F+	K−F−	K+F+	K+F−	K−F+	K−F−	G+K+	G+K−	G−K+	G−K−	G+K+	G+K−	G−K+	G−K−
Z. glomerularis	0	1	0	5	0	0	0	7	0	1	1	4	0	1	1	5
Z. fascicularis	4	0	2	0	2	0	5	0	2	0	2	2	1	4	1	1
Z. reticularis	6	0	0	0	5	0	2	0	6	0	0	0	5	2	0	0
Invasion to medullary	6	0	0	0	3	4	0	0	1	0	5	0	2	0	1	4

Table 10. Various types by means of observing the combination of glycogen-, ketosteroid-, and fat-laden cells in the zona reticularis of swine adrenals.

Sex / Substance / Type	Hogs (6 cases)					Sows (7 cases)				
	Nuclear glycogen	Cytoplasmic glycogen	Ketosteroid	Fat	Total cases	Nuclear glycogen	Cytoplasmic glycogen	Ketosteroid	Fat	Total cases
Type 1.	+	+	+	+	1	+	+	+	+	2
	+	+	−	+		+	+	−	+	
	+	−	+	+		+	−	+	+	
Invasion to medullary	−	+	+	+		−	+	+	+	
	−	+	−	+		−	+	−	+	1
	−	−	+	+	5	−	−	+	+	1
	−	−	−	+		−	−	−	+	3
Type 2.	+	+	+	+	5	+	+	+	+	5
	+	+	−	+	1	+	+	−	+	2
	+	−	+	+		+	−	+	+	
Medullary zone	−	+	+	+		−	+	+	+	
	−	+	−	+		−	+	−	+	
	−	−	+	+		−	−	+	+	
	−	−	−	+		−	−	−	+	
Type 3.	+	+	+	+	1	+	+	+	+	1
	+	+	−	+	1	+	+	−	+	2
	+	−	+	+		+	−	+	+	
Boundary zone of Z. reticularis	−	+	+	+	1	−	+	+	+	1
	−	+	−	+	2	−	+	−	+	2
	−	−	+	+		−	−	+	+	1
	−	−	−	+	1	−	−	−	+	
Total cases					6					7

The ketosteroid-laden cells with fat deposition tended to appear more intensive in the zona fasciculata et reticularis and medullary of the hogs than in the sows.

The variation of the intranuclear or intracytoplasmic deposition, ketosteroid- and fat-laden cells, was divided into the undermentioned types by means of observing the combination of glycogen-, ketosteroid-and fat- deposition. These results are shown in Table 10.

Summary and Conclusion

Physio-histological studies on the mechanism of adipositas and the effect of fattening have been investigated histochemically in various organs of the Yorkshire pigs used for the experiment on the feeding standard of the meat pigs. During these investigations, the occurrence of glycogen was noticed in the nuclei and cytoplasms of the zona reticularis in the adrenals of the pigs.

The present study described the occurrence of ketosteroid-bearing cells stained with NAKAO's Hcl-$SbCl_3$-reaction in the cytoplasms of the zona reticularis of the swine adrenals. It was planned to examine the mode of the ketosteroid-bearing cells, the relationships between glycogen, fat and ketosteroid deposition. The results are summarized as follows:

1) It was noticed that a large amount of the cytoplasms in the zona reticularis of the swine adrenals showed a strong positive reaction to NAKAO's HCl-$SbCl_3$ reaction for the demonstration of the ketosteroids. Seven sows and six hogs contained the ketosteroid-laden cytoplasms in the zona reticularis in 85 percent and that in the zona fasciculata in 44 percent, and also in the zona glomerularis in only 8 percent.

2) The rich type of the fat-stored cells tended to have the rich ketosteroid-laden cells, and in opposition the rich type of the fat-stored cells to have the poor ketosteroid-laden cells, and also the poor type of the fat-stored cells to have the poor ketosteroid-laden cells. There might exist a remarkable ketosteroid-production stage, poor ketosteroid production stage and resting ketosteroid-production stage.

3) Observing the combination of the nuclear-cytoplasmic glycogen-bearing cells and the ketosteroid-bearing cells, they were divided into four types such as rich glycogen-rich ketosteroid in 46 percent, rich glycogen-poor ketosteroid in 38 percent, poor glycogen-rich ketosteroid in 8 percent, and poor glycogen-poor ketosteroid in 8 percent of both sows and hogs. Those might be expected to indicate the various types of the metabolic stages.

4) The modes of the ketosteroid deposition were various, as follows; Type I of the deposition in the imigrated cortical portion to the medullary; Type II of that in the juxta medullary zone, Type III of that in the boundary zone, and Type IV of that in the process-like development of the medullary (evaginated new reticular zone in the zona fasciculata). Usually the intranuclear- and

intracytoplasmic glycogen deposition, fat deposition and ketosteroid deposition were found in the juxta medullary zone of the zona reticularis in 100 percent. On the contrary, the rich ketosteroid and fat deposition were found in 70~100 percent in spite of the poor glycogen deposition in the Type I of the invaded zona reticularis into the medullary. These changes seemed to be the various phases of the metabolic cycle for the corticosteroid production of the swine adrenals.

5) The presence of ketosteroid in the cytoplasms of the swine adrenals were found to be remarkably stronger or more intensive in the juxta medullary zone near the medullary and in the imigrated portion of the zona reticularis within the medullary than in the boundary zone of the zona reticularis ajoined to the zona fasciculata. In severe cases there occurred a large amount of ketosteroid in the cytoplasms, and this extended to the deep zone of the zona fasciculata, and also to the spongy zone. The ketosteroid-laden cytoplasms contained a large amount of short rod-like or ovoid-like granules stained with NAKAO's $HCl\text{-}SbCl_3$ reaction.

6) The ketosteroid-laden cells tended to appear more intensive in the hogs than in the sows.

According to SUTHERLAND (6), KONDO (7) and ITIKAWA (2, 3), there were found the following hypothetic systems: glycogenolysis from glycogen to G-6-P by the active phosphorylase, transformation of NADP to $NADP \cdot H_2$ accelerated by the changes of G-6-P to 6-P-G, and the production system of NADP to $NADP \cdot H_2$, accordingly these reaction relations are closely connected with glycolysis, glycogenolysis, coenzymes and steroid hormone. ITIKAWA *et al* (2, 3) described that the presence of glycogen in the nuclei and cytoplasms of the swine adrenals found recently by them, might be effective to produce the corticosteroids. It has been clarified on the mechanism of obesity that hyperadrenocorticoticism accelerated hyperinsulinism to increase the fat metabolism.

In the cases of seven sows and six hogs, the presence of corticosteroid in the cytoplasms was observed and studies were made on the relationships between the glycogen, fat and ketosteroid deposition; so it seemed to be closely related to the physiological obesity. If the obesity should develop by the hyperinsulinism and hyperadrenocorticism, the glycogen deposition and ketosteroid production might play an important role in hyperadrenocorticism. It seemed important to have ketosteroid-deposition in the cytoplasms of the cells in the zona reticularis and zona fasciculata of the swine adrenals. It is very important to solve histochemically the problems of the co-factor and corticosteroid, and this may become the subject for future investigation.

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Plate 1

Explanation of Figures

Fig. 1. Ketosteroid deposition called Type II in the cytoplasms of the cells in the juxta medullary zone of the zona reticularis of the swine adrenal of No. 4. ×200, Nakao's HCl-$SbCl_3$ reaction for the demonstration of ketosteroid.

Fig. 2. Ketosteroid deposition called Type II in the cytoplasms of the cortical cells in the juxta medullary zone of the zona reticularis of the swine adrenals of No. 4. ×400, Nakao's HCl-$SbCl_3$ reaction for ketosteroid.

Fig. 3. Ketosteroid-laden cells called Type III in the boundary zone of the swine adrenal of No. 15. ×200, Nakao's HCl-$SbCl_3$ reaction for ketosteroid.

Fig. 4. Ketosteroid-laden cells called Type III in the boundary zone of the swine adrenal of No. 15. ×400, Nakao's HCl-$SbCl_3$ reaction for ketosteroid.

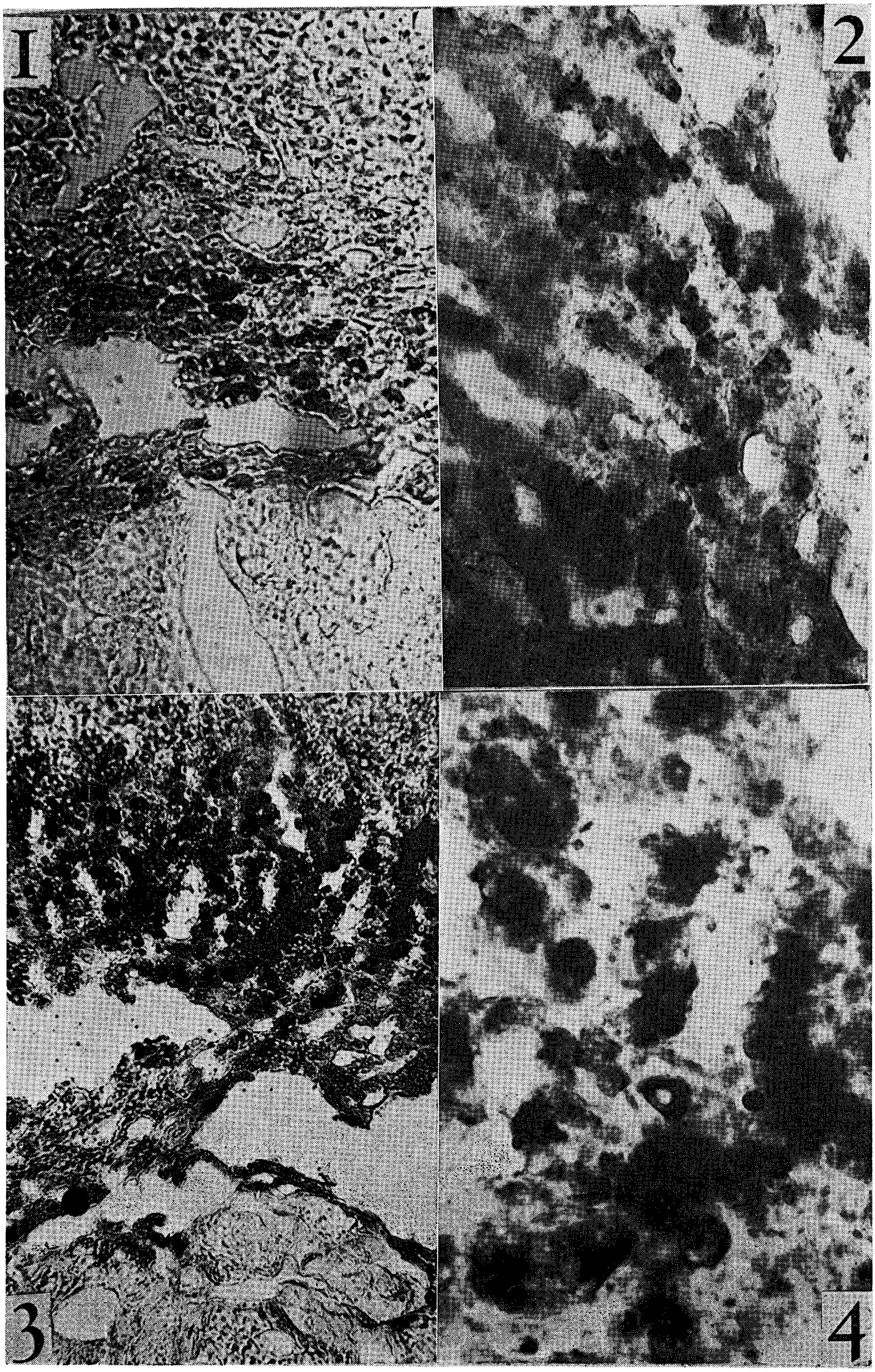

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Plate 2

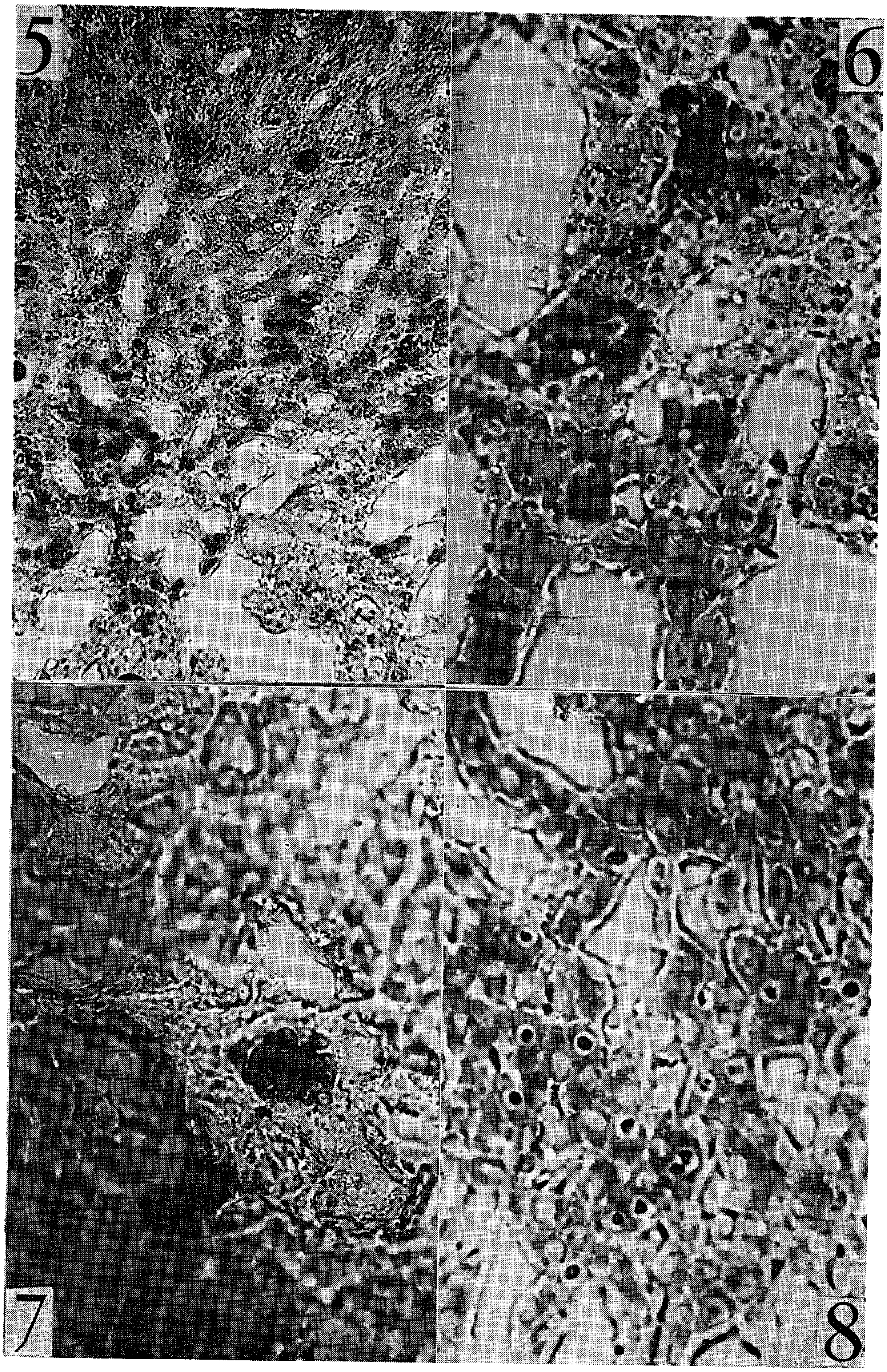
Explanation of Figures

Fig. 5. Ketosteroid deposition called Type II in the cytoplasms of the cortical cells in the juxta medullary zone of the zona reticularis and in the medullary of the swine adrenal of No. 12 invading zona reticularis. $\times 200$, Nakao's HCl-$SbCl_3$ reaction for the demonstration of ketosteroid.

Fig. 6. Ketosteroid deposition called Type II in the cytoplasms of the cortical cells in the juxta medullary zone of the zona reticularis in the swine adrenal of No. 12. $\times 400$, and stained with Nakao's HCl-$SbCl_3$ reaction for the demonstration of ketosteroid.

Fig. 7. Ketosteroid deposition called Type I in the medullary of the swine adrenal of No. 14 invading zona reticularis. $\times 400$, and stained with Nakao's HCl-$SbCl_3$ reaction for ketosteroid.

Fig. 8. Crystal formation in the Nakao's HCl-$SbCl_3$ reaction at one day after staining in the cortical cells of the swine adrenal. $\times 400$, These crystals appeared in the fat droplets of the cytoplasm in the shape of irregular blackish brown masses.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