PHYSIO-HISTOLOGICAL STUDIES ON THE PHYSIOLOGICAL OBESITY OF THE MEAT PIGS, (REPORT VIII) ESPECIALLY ON THE HISTOCHEMICAL OBSERVATIONS OF THE SUTHERLAND'S HYPOTHESIS CONNECTED WITH GLYCOGENOLYSIS, COENZYMES AND STEROID HORMONE AS THE HORMONAL REGULATION OF THE INTRACELLULAR REACTION IN THE ADRENALS OF LANDRACE-F_1-MEAT PIGS

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By

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Introduction

Physio-histological studies on the mechanism of adipositas and the effect of fattening has been done histochemically in the various organs of Yorkshire pigs used for Pig-Feeding-Standard Revising Tests in the case of pork production (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10). Owing to the previous reports (1, 2, 3, and 4) there was found the occurrence of glycogen in the nuclei and cytoplasms of the zona reticularis (and also in the zona fasciculata) of the swine adrenals. Thus far no one has found glycogen in the nuclei of the adrenals. With regard to this physiological meaning, it is expected that the relationships between glycogen deposition, fat storage and ketosteroid production will become important to clarify the mechanism of adipositas or obesity, and that of fattening for the meat pigs. However, it has been shown that the working hypothesis as the regulation of the intracellular reaction by the hormone [Sutherland (11–12) 1960 and Kondo (13) 1963] might be established.

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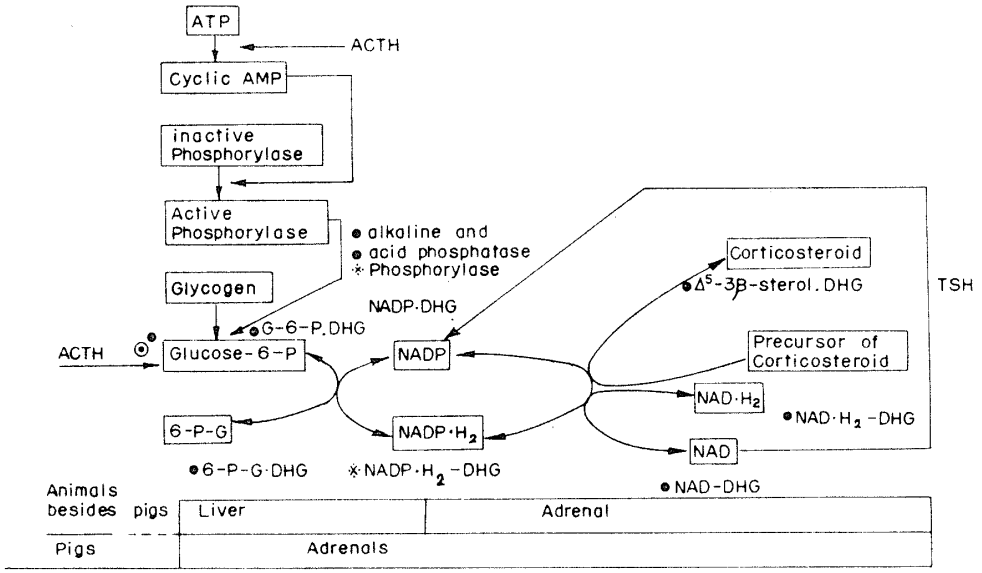
According to Sutherland's opinion (11–12), there were biochemically found the following metabolic systems: glycogenolysis from glycogen to glucose-6-P by the active phosphorylase, transformation of NADP to NADP·H_2 accelerated by the changes of glucose -6-P to 6-P-gluconolactone, and the production system of NADP to NADP·H_2, and the production of precursor of corticosteroid to corticosteroid; accordingly these relations are closely connected with glycolysis, coenzymes and steroid hormone (Table 1). The chemical reactions shown in Table 1 are related to each other. Owing to the author's opinion (2), in the animals besides pigs the glycogenolysis was done in the liver and the production of corticosteroids in the adrenals. The presence of glycogen in both nuclei and cytoplasms of the swine adrenals found recently by us, might be effective to produce the corticosteroids.

Table 1. Schematic diagram with regard to regulation cellular reactions by the hormone (Itikawa et al 1964)



Remarks: ◉ indicated the description in Tohoku J. Agr. R., 14(4), 1964, and ● in present paper, and * will be reported in other occasion.

By the present study there were observed the presence of alkaline and acid phosphatase for glycogenolysis, that of G–6–P dehydrogenase and 6–P–G dehydrogenase for the demonstration of the changes of glucose–6–P to 6–P–gluconolactone, that of NADP dehydrogenase and NADP·H_2 dehydrogenase for the transformation of NADP to NADP.H_2 to produce coenzyme, that of succinic dehydrogenase in the mitochondria for the cellular respiration, and that of Δ^5-3β–hydroxysterol dehydrogenase for the production of steroid in the adrenals of the Landrace-F_1 meat pigs.

According to Williams (14) (1962), recent investigations have helped to clarify the mechanisms involved in the synthesis and release of corticosteroids, as follows: the adrenal has a relatively high concentration of glycogen in the zona fasciculata

and zona reticularis, but very little in the zona glomerulosa. The following sequence of events seemed significant in synthesis and secretion of corticosteroids formed in the adrenals. ACTH stimulated formation of cyclic adenylic acid, which, with various cofactors, led to the activation of phosphorylase. Phosphorylase accelerated glycogenolysis, thereby increasing glucose–6–Phosphate. Since a very active hexosemonophosphate shunt was present in the adrenal tissue, the amount of glucose–6–phosphate metabolized via this way is increased, with the result that more $NADP \cdot H_2$ was produced. This cofactor accelerated splitting of the cholesterol side-chain and reduction of certain steroids, leading to increased synthesis of corticosteroids. As the food intake increased, the β-cells of the pancreas were stimulated, producing more insulin. This hormone was highly important in lipogenesis, and when it was present in excess it promoted excess deposit of fat, particularly with excess food ingestion. Insulin produced an increase in lipids in each and enlargement of each. Insulin was known to increase the output of glucosteroids, which in turn played an important role in stimulating 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 meat pigs were considered as the physiological adipositas, it might be important to observe the relationship between the occurrence of glycogen in both nuclei and cytoplasms of the adrenal cells, the deposition of fat in the cytoplasms of the adrenal cells, enzymatic activities of phosphatase for glycogenolysis, $DPNH_2 \cdot$dehydrogenase for coenzymes, and that of $\Delta 5$–3β–hydroxysteroid dehydrogenase for the synthesis of corticosteroid. Furthermore, the stimulation of the β-cells of the pancreas will be reported at another occasion to certify the relationships between hyperinsulinism and hyperadrenocorticism.

Materials and Methods for Studies

Materials used for the studies were 16 Landrace-F_1 pigs. All the animals were studied on the feeding standard by the administration with various feeds mentioned in Table 1. These experiment was done in the Miyagi Prefectural Agricultural Experimental Station in Sendai in 1964. Sixteen Landrace-F_1 piglets (sows and boars of the same number) born from two Yorkshire mother pigs and one Landrace father pig, were purchased at the age of 35 days after birth, and fed with artificial milk up to the age of 65 days. All boars were castrated at the age of 31 to 33 days.

All the pigs showed in increase of body weight, rapid growth and good appetite. Autopsey of Landrace-F1 pigs was done at the age of 185 days after birth, and the body-weight and feed intake according to sex and experimental periods were shown

Table 2. Ration used for the Eexperiment of Feeding Standard, and Body-weight and Feed Intake of the Landrace-F_1, Meat Pigs, according to sex and Experimental Periods.

Group	Results / Feed & periods / Sex	Ration used for experiment: Concentrates : Barley in Former or later Period	DCP in former or later Period	TDN in former or later Period	Age (days): Initial period	Middle period	Final period	Body weight (kg): Initial period	Former period	Middle period	Final period	Feed intake (kg) during all periods: Former period	Final period	Whole period
H′	♀ (2)	60:40	14.6	72.5	65	135	185	22.3	56.7	79.9	119.6	368.0	347.3	715.3
	♂ (2)	(60:40)	(12.5)	(72.5)	65	135	185	20.6	54.3	74.9	114.9	347.8	361.0	708.8
E′	♀ (2)	60:40	12.1	72.4	65	135	185	22.3	50.4	72.7	102.2	327.6	294.6	622.2
	♂ (2)	(60:40)	(9.9)	(72.6)	65	135	185	21.2	60.5	81.6	114.5	399.9	313.6	713.5
C′	♀ (2)	70:30	14.5	67.7	65	135	185	22.2	56.9	78.1	116.2	477.8	360.3	838.1
	♂ (2)	(70:30)	(12.3)	(67.9)	65	135	185	21.6	57.4	79.0	114.4	411.6	370.7	782.3
A′	♀ (2)	70:40	12.1	67.5	65	135	185	22.0	52.8	74.8	111.7	336.8	328.9	665.7
	♂ (2)	(70:30)	(9.9)	(67.5)	65	135	185	21.5	63.8	84.8	123.1	481.5	447.7	929.2

in Table 2.

The total adrenals from these Landrace-F_1 pigs were fixed either in buffered formol or in Carnoy's second solution, embedded in paraffine, and cut into 6μ sections, and stained with PAS for glycogen after saliva digestion, and with acrolein Schiff reaction for protein. The adrenals fixed in buffered formol, were cut with the frozen microtome, and then stained with Sudan III or Sudan black B for fat and lipoids. The fresh adrenals of Landrace-F_1 pigs were cut with the cryostat microtome at 25°C below zero, and stained with the various enzyme-histochemical procedures for the demonstration of succinic dehydrogenase, G–6–P dehydrogenase, 6–P–G dehydrogenase, DPN– and DPN·H_2-diaphorases, and alkaline and acid phosphatases, and Δ5–3β–hydroxysteroid dehydrogenase.

Results

1. The occrfrnce of glycogen within the nuclei and cytoplasms of the zona reticularis in the adrenals and epinephrine production in the medullary cells of the adrenals in the Landrace meat pigs.

According to the previous report (1, 2, 3, and 4), there were found the occurrence of the glycogen deposition within both nuclei and cytoplasms of the zona reticularis in the Yorkshire swine adrenals. In the present study, 7 sows and 6 hogs of the Landrace-F_1 contained the glycogne-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. The results were shown in Table 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 square-meter. On the average there occurred 2 cells/1 section with glycogen in the nuclei in sows and 73 cells in them in hogs; 3 cells/1 section with glycogen in both nuclei and cytoplasms in sows and 23 cells/1 section with glycogen with them in hogs; and 317 cells/1 section with intracytoplasmic glycogen in sows and 553 cells/1 section with them in hogs. These results are summarized in Table 4.

To compare the investigative results of both Yorkshire-and Landrace-F_1-meat meat pigs, there were remarkable differences as shown in Table 4. The fact of intranuclear glycogen deposition in the Landrace-F_1 meat pigs was developed to slight diminution in comparison with that in the Yorkshire meat pigs. The total

Table 3. Relationships between glycogen deposition in the adrenals cells and epinephrine production in the medullary cells of the adrenals of the Landorace-F_1 Meat Pigs.

	Sex	Sows								Hogs							
	Group	H′		E′		C′		A′		H′		E′		C′		A′	
Alterations	Animal No.	1	8	2	7	3	6	4	5	11	18	12	17	13	17	14	15
Glycogen-deposition (cell-counts/1 section)	Intranuclear glycogenic bodies	11	3	11		2	1		6	5	86	69	33	43	24	207	10
	Intranuclear glycogen bodies and intracytoplasmic glycogen deposition	6	8	5		1	2	1	1	3	18	11	18	35	11	58	10
	Intracytoplasmic glycogen deposition	556	350	430		357	118	131	274	280	370	710	255	798	468	577	397
	Intranuclear vacuolization with no glycogen	3	15	2		1				39	6	16	2	16	8	3	5
Epinephrine-production	Resting period	⫴	⫴	⫴	Missng	⫲	⫴	⫲	⫲	⫲	+	$+_s$	⫲	+	⫲	+	⫲
	Discharge period	−	−	−		⫲	⫲	⫲	⫲	⫲	⫴	⫴	⫲	⫲	⫲	⫲	⫲
	Rapid discharge period	+	+	+		⫲	⫲	⫲	⫲	⫲	⫴	⫴	⫲	⫲	⫲	⫲	⫲
	Exhaustion period	−	−	−		+	−	+	−	−	+	⫲	−	⫲	−	+	−
	Reconstruction period (Globoid bodies)	−	−	−		−	−	−	−	+	−	$+_s$	−	+	$+_s$	+	$+_s$
	Lipoidic globoid bodies	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−

Table 4. Comparison with the glycgoen deposition in the cortex and epinephrine production

Localization	Races / Sex / Degree	Yorkshire meat			
		Sows		Hogs	
		Total cells of 11 sows	Average per 1 section	Total cells of 9 hogs	Average per 1 section
Glycogen deposition (all numbers per 1 section)	Intranuclear glycogen bodies	35	3.1	161	17.9
	Intranuclear and cytoplasmic glycogen	707	64.2	864	96.0
	Intracytoplasmic glycogen	2144	194.4	3799	422.1
	Intranuclear vacuole with no glycogen	105	9.6	220	24.4
Epinephrine production (degree of intensity)	Resting period	Positive 100%	Negative –	Positive 100%	Negative –
	Discharge period	100%	–	100%	–
	Rapid discharge period	55%	45%	56%	44%
	Exhaustion period	9%	91%	11%	89%
	Epinephrine globoi dbodies	–	100%	–	100%
	Lipidic globoid bodies	–	100%	–	100%

Remarks : *This results were reported

numbers of the cells with intranuclear glycogen deposition in the Landrace were only half the ones in the Yorkshire. On the other hand, the total numbers of the cells with intracytoplasmic glycogen deposition in the Landrace-F_1 were about twice the numbers of the ones in the Yorkshire. According to previous report (5), 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. On the basis of Lillie's opinion, it seemed reasonable to assume that the PAS-positive reaction gives a possitive chromaffine reaction, and that this reaction causes the production of aldehyde from the hydroxymethyl-amino group of epinephrine or one of its precursors.

To compare the results of Landrace-F_1-and Yorkshire meat pigs, there were indicated no remarkable differences. Frequency of positive results of epinephrine production occurred in 100 per cent of the resting period and 100 per cent of the

in the medullary of the adrenals of the Yorkshire pigs and that of the Landreace-F_1 pigs.

pigs*		Landorais-F_1 meat pigs					
Total		Sows		Hogs		Total	
Total cells of 20 pigs	Average per 1 section	Total cells of 7 sows	Average per 1 section	Total cells of 6 hogs	Average per 1 section	Total cells of pigs	Average per 1 section
196	9.8	14	2.0	439	73.1	453	34.8
1571	79.6	24	3.4	135	22.5	159	12.3
5943	297.2	2216	316.6	3320	553.3	5536	425.9
325	16.3	21	3.0	54	9.0	75	5.8
Positive 100%	Negative –	Positive 100%	Negative –	Positive 100%	Negative –	Positive 100%	Negative –
100%	–	100%	–	100%	–	100%	–
75%	25%	100%	–	100%	–	100%	–
15%	85%	29%	71%	67%	23%	46%	54%
–	100%	–	100%	83%	17%	38%	62%
–	100%	–	100%	–	100%	–	100%

in the Tohoku J. Agr. Res., 15 (1): 61-81, 1964.

discharge period in the medulla of the Landrace-F_1 pigs and Yorkshire pigs. On the contrary, there were 46 per cent of the exhaution period and 38 per cent of the reconstruction period in the medulla of the Landrace-F_1 and 9 per cent of the exhaustion period and 0 per cent of the reconstruction period in the Yorkshire. However these epinephrine production in the Landrace-F_1 indicated more slight than that in the pigs immunized with hog-cholera virus (5), and these fact seemed different from the results of the Yorkshire.

2. Alkaline phosphatases occurred in the cytoplasms of the Landrace-F_1 adrenal cells.

The results in the present paper are shown in Table 5. Alkaline phosphatase was limited to the cortex of the adrenals. The zona glomerulosa was weakest in the sections stained with Seligman's azo-coupling method, but negative in the ones stained with Gomori's metalic method. Almost all of the cells in the zona fasciculata were strongest, but there were found the weakest reaction (No. 13). The cells of

Table 5. Relationships between alkaline and acid phosphatase in the adrenals of the Landrace-F_1 Meat Pigs

Enzymes	Localization		sex	Sows								Hogs							
			Group	H′		E′		C′		A′		H′		E′		C′		A′	
			Animal Name	1	8	2	7	3	6	4	5	11	18	12	17	13	16	14	15
Alkaline phosphatase	Cortex	Zona glomerulosa			MA −+	MA +s+	MA +s+	MA −+	MA −+	MA −+	MA ++				MA −+	MA −+	MA −+	MA −+	MA −+
		Zona fasciculata			⫼⫼	⫼⫼	++⫼	⫼⫼	⫼⫼	⫼⫼	++				⫼⫼	++	⫼⫼	++	⫼⫼
		Zona reticularis: Zona retiuclaris			−+	+s++	++⫼	−+	−++	+s++	−+				++	−−	+++	−−	−−
		Zona reticularis: Zona reticularis within medullary													−+	−−	−++	−+	−−
		Medullary			−−	−−	−−	−−	−−	−−	−−				−−	−−	−−	−−	−−
Acid phosphatase	Cortex	Zona glomerulosa		Missing	C ++	C −−	C −−	C −−	−−	C −−	C −−	Missing	Missing	Missing	C ++	C +−	C +−	C ++	C ++
		Zona fasciculata: Upper part			⫼−	⫼−	++−	⫼−	++	++	++				⫼−	++−	++−	⫼−	⫼−
		Zona fasciculata: Lower part							⫼−										
		Zona reticularis: Zona reticularis			⫼−	+−	⫼−	⫼−	+−	++					⫼−	⫼−	⫼−	⫼−	⫼−
		Zona reticularis: Zona reticularis within medullary			⫼−	⫼−	++−	⫼−	++	++					⫼−	⫼−	⫼−	⫼−	⫼−
		Medullary			−−	−−	−−	−−	−−	−−	−−				−−	−−	−−	−−	−−

Remarks : M indicated Gomori's metalic procedure, A: Ashbel and Seligman's Azo-coupling method, C showed control.

the zona reticularis were weak, while there were more reactive cells in the zona fasciculata.

Generally alkaline phosphatase activity in the sows was slightly stronger than that in the hogs. Owing to the previous report (8) described on the Yorkshire meat pigs about alkaline phosphatase activity in the three zones of the cortex stained with equal intensity, there were found negative or weakest reaction in the zona glomerulosa, and zona fasciculata of some cases. The cells of the zona reticularis indicated more reactive than that of the zona fasciculata. In comparison with alkaline phosphatase activity in the Yorkshire- and Landrace-F_1 meat pigs, there was a great difference between strong reaction in the zona reticularis of the Yorkshire pigs and that in the zona fasciculata of the Landrace-F_1 pigs. In addition to these changes, alkaline phosphatase activity in the medulla was negative in all Landrace pigs, while it was divided into two types such as activated form and inactivated in the Yorkshire pigs.

Owing to the previous report (8), there were indicated strong positive activity of alkaline phosphatase in the cortex and medullary of the adrenals of the sows; and strong positive one in the cortex and weak or negative one in the medullary of the hogs.

3. Acid phosphatase occurred in the cytoplasms of the Landrace-F_1 adrenal cells.

According to the previous report (8) our results confirmed that acid phosphatase activity was generally limited to the zona fasciculata and zona reticularis and medulla of the swine adrenals. No one has found the distribution of acid phosphatase in the adrenal cortex of the mammals. Only Fränko (16) reported on the histochemical evidence of the presence of acid phosphatase-positive and-negative cellular islets in the adrenal medulla of the rat.

The results in the present paper are shown in Table 5. Acid phosphatase activity was generally limited to the zona fasiculata and zona reticularis. In some adrenals (No. 8, 17, 13, 16, 14, and 15), the three zones of the cortex are stained with equal intensity. There existed the islets of the scattering zona reticularis throughout the medulla and the present authors called them "within medulla".

Generally acid phosphatase activity in the adrenals of the hogs was stronger than that of the sows as same as the previous report (8).

Owing to the previous report (8) described on the Yorkshire meat pigs, acid phosphatase was generally limited to the zona fasciculata, zona reticularis and medulla of the adrenals, but in some adrenals the three zones of the cortex were stained with equal intensity; and the cells of the zona fasciculata were negative or weakest, while there was more reactive cells in the zona reticularis.

In comparison with acid phosphatase activity in the Yorkshire-and Landrace-F_1 meat pigs, there were a great difference between strong reaction in the zona

glomerulosa and medullary of the Yorkshire pigs and negative reaction in those of the Landrace-F_1 pigs; there was a grat affinity between strong reaction in the zona fasciculata and zona reticularis of the adrenals of the Yorkshire pigs and those of the Landrace-F_1 pigs.

4. Succinic dehydrogenase activity occurred in the cytoplasms of the adrenal cells of the Landrace-F_1 meat pigs.

Succinic dehydrogenase activity was limited to the cortex of the swine adrenals. The results in the present paper are shown in Table 6. In some adrenals (No. 8, 7, 3, 6, 4, 13, and 15) succinic dehydrogenase of the three zones of the cortex are stained with equal intensity. Medulla was negative (No. 8, 7, 3, 6, 17, and 15) and weakest (No. 2, 4, 5, 13, 16, and 14). The cells of the zona fasciculata reacted weaker than that of the zona reticularis.

The zona reticularis was divided into three groups: type I) between zona fasciculata and medullary, type II) zona reticularis like islets extended to medullary, and type III) zona reticularis like cords extended to zona fasciculata. In these three regions, type I was stained with strongest intensity, and in some specimens type II and III were also strongest. Generally succinic dehydrogenase activity in the adrenals of hogs was all the same to that of sows.

In the previous report (8) described on the Yorkshire meat pigs, succinic dehydrogenase activity was generally limited to the cortex of the adrenals, and stained with equal intensity. In some specimens, the cells of the zona fasciculata were strongest, while there was more reactive cells in the zona reticularis; and also those were weakest, while they were strongest in the zona reticularis. Succinic dehydrogenase activity in the adrenals of the hogs was stronger than that of the sows.

In comparison with succinic dehydrogenase activity in the Yorkshire- and Landrace-meat pigs, there were a great difference between strong reaction in the zona fasciculata and zona reticularis of the Yorkshire pigs, especially on the hogs, and strong reaction in only zona reticularis of the Landrace-F_1 pigs. Accordingly the function in the Yorkshire hogs might be remarkably accelerated in comparison with that in the Landrace-F_1 hogs.

5. G-6-P dehydrogenase and 6-P-G dehydrogenase occurred in the cytoplasms of the adrenal cells of the Landrace-F_1 meat pigs.

According to Sutherland's opinion (12, 13), there were found the metabolic systems such as glycogenolysis of 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–gluconolactone, and the production system of NADP to NADP·H_2. Accordingly these reactions are closly connected with glycogenolysis, coenzymes and steroid hormone. Accordingly the certification of G–6–P dehydrogenase or 6–P–G dehydrogenase for the coenzyme production might be important in the

Table 6. Enzymatic activities of the glycogenolysis and that of the cellular respiration in the adrenals of the Landrace-F_1 Meat Pigs

Enzymes	Localization		Sex	Sows								Hogs							
			Groups	H′		E′		C′		A′		H′		E′		C′		A′	
			Animal name	1	8	2	7	3	6	4	5	11	18	12	17	13	16	14	15
Fat-storage (Sudan III or Black B)	Cortex	Zona glomerulosa			C	C		C		C						C	C	C	C
		zona fasciculata: upper part																	
		zona fasciculata: lower part																	
		zona reticularis: zona reticularis																	
		zona reticularis: zona reticularis within medullary																	
	Medullary																		
Ketosteroid, $SbCl_3$ HCl reaction	Cortex	Zona glomerulosa																	
		zona fasciculata: upper part																	
		zona fasciculata: lower part																	
		zona reticularis: zona reticularis																	
		zona reticularis: zona reticularis within medullary																	
	Medullary																		
4β-3β-hydroxysteroid dehydrogenase	Cortex	Zona glomerulosa		Missing								Missing	Missing	Missing					
		zona fasciculata: upper part																	
		zona fasciculata: lower part																	
		zona reticularis: zona reticularis																	
		zona reticularis: zona reticularis within medullary																	
	Medullary																		

Remarks : C indicated control without substrate.

study on the physiological obesity of the meat pigs.

The results in the present paper are shown in Table 5. G–6–P dehydrogenase activity was limited to the cortex and medulla of the swine adrenals. In some adrenals (No. 8, 4, 5, and 16), the three zones of the cortex were stained with equal intensity. The cells of the zona glomerulosa and zona fasciculata were negative (No. 2, 17, 13, 14 and 15) and weakest (No. 3 and 7). In some specimens, the cells of the zona fasciculata indicated more weak than that of the zona reticularis. Especially the island of ectopic cortical cells lying in the medulla were distinctly reactive and hense easily identifiable. Generally G–6–P dehydrogenase activity in the adrenals of the sows was slightly stronger than that in the hogs.

The results of 6–P–G dehydrogenase in the present paper are shown in Table 5. 6–P–G dehydrogenase activity was limited to the cortex and medulla of the swine adrenals. In some adrenals of hogs (No. 17, 16, and 14), the three zones of the cortex were stained with equal intensity. And also the cells of the zona glomerulosa and zona fasciculata were negative (No. 8, 7, 3, and 6) and weakest (No. 2 and 4) in the sows. Especially the island of the reticular zone lying in the medulla were distinctly reactive. Generally 6–P–G dehydrogenase activity in the adrenals of the hogs was stronger than that in the sows of Landrace–F_1 meat pigs.

6. DPN- and $DPNH_2$-dehydrogenase (or diaphorase) occurred in the cytoplasms of th adrenals of Landrace-F_1 meat pigs.

According to Rubin (16) (1963), a diphosphopyridine nucleotide (NAD)-depedndent enzyme system, $\Delta 5$–3β–hydroxysteroid dehydrogenase, can be demonstrated by both biochemical and histochemical means in the steroid-hormone producing organs of many vertebrates. As previously stated, phosphorylase accelerates glycogenolysis, and thereby G–6–P is increased. A very active G–6–P shunted via this pathway is increased, and as a result of that, more $NADP \cdot H_2$ is produced. According to Sutherland's opinion (12, 13), there were found the metabolic systems such as glycogenolysis of glycogen to G–6–P by the active phosphorylase, transformation of NADP to $NADP \cdot H_2$ accelerated by the change of G–6–P to 6–P–G, and the production system of NADP to $NADP \cdot H_2$. As previously stated in the previous report, the transformation of NAD to $NAD \cdot H_2$ accelerated by the change of NADP to $NADP \cdot H_2$, and the production of NAD to NADP by TSH were found. By the present paper there were found the occurrence of NAD (or DPN)-diaphorase and $NADH_2$ (or $DPNH_2$)diaphorase in the cells of the cortex and medullary in the swine adrenals. However the certification of NADP (or TPN)-diaphorase and $NADP \cdot H_2$ (or $TPNH_2$)-diaphorase for the coenzyme production will be reported at another occasion.

The results in this paper on NAD- and $NADH_2$-diaphorase are shown in Table 7. NAD (or DPN)-diaphorase activity was limited to the zona reticularis, zona glomerulosa and medullary in the only one case of hogs. Almost all of the sows and hogs reacted negatively in the three zones of the cortex, and in some specimens

Table 7. Enzymatic activities of Co-factors, such as DPN-, or $DPNH_2$-Diaphorases in the Adrenals of the Landrace-F_1 Meat Pigs.

Enzymes	Localization		Sex	Sows								Hogs							
			Group	H′		E′		C′		A′		H′		E′		C′		A′	
			Animal name	1	8	2	7	3	6	4	5	11	18	12	17	13	16	14	15
DPN-Diaphorase	Cortex	Zona glomerulosa			−	−	−	−	−	−	−				−	⫲	−	−	−
		Zona fasciculata			−	−	−	−	−	−	−				−	−	−	−	−
		Zona reticularis	Zona reticularis		−	−	−	−	−	−	−				−	⫲	−	−	−
			Zona reticularis within medullary													⫲			
	Medullary				+	−	−	−	−	$+_s$	+				−	⫲	−	−	−
$DPNH_2$ Diaphorase	Cortex	Zona glomerulosa		Missing	⫲	⫲	⫲	⫲	⫲	⫲	⫲	Missing	Missing	Missing	⫲	⫲	⫲	⫲	⫲
		Zona fasciculata	Upper part		−	−	+	−	−	+	+				⫲	⫲	⫲	+	⫲
			Lower part				−			−	−								
		Zona reticularis	Zona reticularis		⫲	+	⫲	⫲	⫲	+	+				⫲		⫲	⫲	⫲
			Zona reticularis within medullary		⫲	⫲	⫲	⫲	⫲	+	⫲				⫲	⫲	⫲	⫲	⫲
	Medullary				⫲	⫲	⫲	⫲	⫲	+	+				⫲	⫲	⫲	⫲	⫲

Rmearks : All cases were used the control without the substrate, and indicated no reaction in the control.

the medulla reacted slightly weak positive (No. 8, 4, and 5 in the sows).

On the other hand, $NADH_2$ (or $DPNH_2$)-diaphorase activity was limited to the cortex and medullary of the adrenals. In some adrenals (No. 17, 13, 16, 14 and 15), especially on the hogs, the three zones of the cortex were stained with equal intensity. In some specimens (No. 8, 2, 7, 3 and 6), especially on the sows, the cells of the fasciculata were negative while there were more strongly reactive cells in the zona glomerulosa and zona reticularis. Especially the island of ecrtopic cortical cells lying in the medulla were distinctly reactive and hence easily

Table 8. Relationships between fat-storage, ketosteroid-production and activity of

Enzyme	Localization			Sex / Groups / Animal names	Sows H′ 1	Sows H′ 8	Sows E′ 2	Sows E′ 7	Sows C′ 3	Sows C′ 6
Fat-storage (Sudan III or Black B)	Cortex	Zona glomerulosa			B III	B III	B III	B III	B III	B III
					⫴ ⫴	⫴ –	⫴ –	⫴	⫴ +	⫴ –
		Zona fasciculata	Upper part		⫴ ⫴	⫴ ⫴	⫴ +	⫴ ⫴	⫴ ⫴	⫴ ⫴
			Lower part		⫴ ⫴	⫴ +	⫴ +	⫴ +	⫴ ⫴	⫴ ⫴
		Zona reticularis	Zona reticularis		⫶ ⫶	⫶ ⫴	⫶ ⫴	⫴ ⫴	⫶ ⫶	⫶ ⫴
			Zona reticularis within medullary		⫴ ⫴	⫴ ⫴	⫴ +	⫴ ⫴	⫴ ⫴	⫴ +
		Medullary			⫴ –	⫴ –	⫴ –	⫴ –	⫴ –	⫴ –
Ketosteroid, $SbCl_3$-HCl reaction	Cortex	Zona glomerulosa			–	–	–		–	–
		Zona fasciculata	Upper part		+	+	+		+	–
			Lower part		⫴	+	+		⫴	+
		Zona reticularis	Zona reticularis		⫴	⫴	⫴		⫴	⫴
			Zona reticularis within medullary		⫴	⫴	⫴		⫴	⫴
		Medullary			–	–	–		–	–
Δ^5-3β-hydroxysteroid dehydrogenase	Cortex	Zona glomerulosa				C	C	C	C	C
						+ –	+ –	+ –	⫴ –	⫴ –
		Zona fasciculats	Upper part			⫴ –	⫴ –	⫴ –	⫴ –	⫴ –
			Lower part			⫴ –	⫴ –	⫴ –	⫴ –	⫴ –
		Zona reticularis	Zona reticularis			⫴ –	⫴ –	⫴ –	⫴ –	⫴ –
			Zona reticularis within medullary			⫴ –	⫴ –	⫴ –	⫴ –	⫴ –
		Medullary				– –	– –	– –	– –	– –

Rmearks : B indicated the intensity of stainability with

identifiable.

Generally $NADH_2$ (or $DPNH_2$)-diaphorase activity in the adrenals of the hogs was stronger than that in the sows. It is very characteristic to find no activity of the zona fasciculata in the sows, and hyperactivity of the three cortical zones in the hogs.

7. Δ5-3β-hydroxysteroid dehydrpgenase activity occurred in the cytoplasms of the adrenal cells of Landrace-F_1 meat pigs.

The Δ5–3β hydroxysteroid dehydrogenase activity in the adrenals of the

Δ^5-3β-hydroxysterol dehydrogenase in the adrenals of the Landrace-F_1 Meat Pigs

Hogs											
A′		H′		E′		C′		A′			
4	5	11	18	12	17	13	16	14	15		
B III	B III	B III	B III	B III	B III	B III	B III	B III	B III		
++ +	+ −	+	−	−	++ +	++ +	++ +	++ −	++ −		
### ++	### +	### ###	### ++	### ++	### ###	### ++	### ###	### +	### ++		
### +	++ −	### ++	### ++	### ++	### ++	### ++	### ###	### +	### +		
### ++	++ ++	### ###	### ###	### ++	### ###	### ###	### ###	### ###	### ###		
### ++	### +	### ###	### ++	### ++	### ###	### ++	### ###	### ###	### ###		
+ −	+ −	### −	### −	### −	### −	### −	### −	++ −	### −		
−	−	−	−	−	−	−	−	−	−		
−	+	++	++	+	++	++	++	++	++		
−	+	###	###	+	###	###	###	###	###		
+	++	###	###	###	###	###	###	###	###		
+	++	###	###	###	###	###	###	###	###		
−	−	−	−	−	−	−	−	−	−		
C	C				C	C	C	C			
+ −	+ −				++ −	++ −	++ −	++ −	++ −		
### −	### −				### −	### −	### −	### −	### −		
++ −	++ −				### −	++ −	### −	### −	### −		
++ −	### −				### −	### −	### −	### −	### −		
### −	### −				++ −	### −	### −	### −	### −		
− −	− −				− −	− −	− −	− −	− −		

Sudan Black B, and III, with Sudan III, and Cshowed control.

hogs and sows did not differ as listed in Table 8. According to Rubin's paper, most of the other animals besides rats were females, so no record was made of the sex of the animals from which the pooled ox adrenals were obtained. In all cases, reactivity appeared to be strictly limited to adrenocortical cells. There was no activity in capsular or medullary regions, except when adrenocortical tissue had penetrated these areas. Especially the island-like cortical tissue lying in the medulla was distinctly reactive.

The cells of the zona glomerulosa were less active than those of the inner zones of the cortex, but those of the zona glomerulosa in the hogs were slightly stronger than those in the sows.

It is noteworthy that the intensity of staining for steroid dehydrogenase did not parallel the degree of $NADH_2$-diaphorase (cf Table 7.) but did apralled the amount of lipid in the cortical cells (cf Table 7.). These findings were similar to Rubin and Deane's opinion in the Armadillo adrenal glands. Generally $NADH_2$-diaphorase activity in the adrenocortical cells of the hogs was slightly stronger than those in the sows. It is not so easy to differentiate the degree of the reactivity for the strong stainability in both hogs and sows.

8. Lipid-storage and steroid-hormone production occurred in the cytoplasms cells of Landrace-F_1 meat pigs.

In the present investigation the relationships between the sudanophilic granules, $Sbcl_3$-Hcl-reactive granules, and $\Delta 5$-3β—hydroxysteroid dehydrogenase activity are discussed. These results are shown in the Table 7. It was noticed that a large amount of the cytoplasms in the zona reticularis of the swine adrenals showed a strong positive reaction by Nakao's method for the demonstration of ketosteroids. Seven sows and eight hogs contained the ketosteroid-laden cytoplasms of the cells in the zona reticularis (in 100%) or zona fasciculata (in 87%), and also no ketosteroid-laden cells were found in the zona glomerularis.

Adrenal fats were stained with Sudan III and Sudan Black B. As the results investigated by us, all lipidic substances strongly stained with Sudan Black B were localized in the zona glomerulosa, zona fasciculata, zona reticularis and medulla, but Sudan III stained weakly fat substances localized in the zona fasciculata and zona reticularis. Williams described the lipid-laden adrenocortex as the follows. The cells of the adrenal cortex become filled with cholesterol and lipid, and, eventually reaching the zona reticularis, become compact after having secreted their active hormones on the way. In the inactive state vacuolated cells predominante; during periods of increased of the discharge of stored lipid and cholesterol, the latter having been changed into steroid hormones. Functional zonation of the adrenal cortex has been established beyond any doubt in most laboratory animals.

In all sections stained with Sudan III, sudanophilic substances appeared to be strictly limited to adrenocortical cells, and there were no stainability in capsular, glomerular or medullary regions, except when adrenocortical tissue had penetrated

these areas. On the contrary Sudan-Black-philic substances appeared in all regions contained both cortex and medulla.

It is noteworthy that the intensity of staining for $\Delta 5$–3β–hydroxysteroid dehydrogenase did not parallel the degree of the amount of lipid stained with Sudan III nor that of ketosteroid in the cortical cells. The degree of the amount of lipid stained with Sudan black paralleled the activity of this steroid dehydrogenase in the cortical cells, but the amount of Sudan Black-philic substances did not paralleled the steroid dehydrogenase activity and the amount of ketosteroid in the medullary.

Generally the amount of ketosteroid in the hogs was stronger than that in the sows, and the amount of sudan-III-philic lipids, and that of ketosteroid parallel the activity of $\Delta 5$-3β-hydroxysteroid dehydrogenase.

Discussion

The above results indicate that the activities of alkaline phosphatase, acid phosphatase, succinic dehydrogenase, G–6–P dehydrogenase, 6–P–G dehydrogenase, DPN–diaphorase, $DPNH_2$–diaphorase and $\Delta 5$–3β–hydroxysteroid dehydrogenase, lipid storage, glycogen deposition and steroid-hormone production in the zona reticularis and other zones of the adrenals of the Landrace-F_1 pigs used for the experiment of the Feeding Standard on the pork production. No one has found the occurrence of these enzymes in the adrenocortical cells of the pigs, and the significance of these results in the physiological obesity of the meat pigs, and the distribution of these enzymes in the swine adrenals.

1. Alkaline phosphatase in the adrenals of Landrace-F_1 meat pigs.

According to Takamatsu (17) (1939) alkaline phosphatase of the cortical zone indicated the weakest in the normal dog, rabbit, mouse, guinea pig and man, but that of the zona reticularis under the various pathological states was strongest. Thereafter Takamatsu (18) (1940) observed that the alkaline phosphatase activity was limited to the adrenal cortex in the intensity of weakest or none degree, but the cells of the zona reticularis and zona glomerulosa appeared more intense under the pathological states.

Zorzoli (19) showed briefly that the adrenals of the rat and man signified strong alkaline glycerophosphatase reaction and those of the mouse and rabbit showed an equal reaction. Our results confirm the distribution of alkaline phosphatase in the swine adrenals just the same as in Zorzoli's report on the adrenals of mouse and rabbit.

There are many reports on alkaline phosphatase in the adrenals, such as Takamatsu (17, 18), Zorzori (19), Leathem and Stauber (20) Knigge (21), Barraclaugh (22), Elftman (23), Gruft (24), Dempsey, Greep and Deane (25), Jones (26), Howard (27), Merklin (28), Nicander (29), Yen-Ling (30), Kar(31), Allen and Slater (32). No

other papers are known to us which deal with the effect of adipositas upon adrenal akaline phosphatase in the sows and hogs. However to study the mechanism of adi positas it is important to clarify the relations between the effect of sex hormones upon adrenal alkaline phosphatase and metabolic activity during steroidogenesis. In this meaning Elftman (23), Yen-Ling (30), and Allen (32)'s reports are useful for the present studies on the swine adipositas. Our present results confirm that generally the alkaline phosphatase activity in the adrenals of the sows was slightly stronger than that of the hogs. According to the previous reports (8), the alkaline phosphatase activity in the adrenals of the sows was stronger than that of the hogs. According to Elftman (23) castration abolished the alkaline phosphatase activity in the cortex. Allen (32) confirmed the response of alkaline phosphatase activity in the cells of the outer fasciculata of the castrate, and Yen-Ling (30) found the depletion of adrenal cortical akaline phosphatase with estrogen administration to male mice.

According to the previous and present reports, in comparison with alkaline phosphatase activity in the Yorkshire- and Landrace-F_1 meat pigs, there was a great difference between strong reaction in the zona reticularis of the Yorkshire pigs and that in the zona fasciculata of the Landrace-F_1 pigs. There were indicated strong positive activity of alkaline phosphatase in the cortex and medulla of the Yorkshire-sow's adrenals, and strong positive one in the cortex and weak or negative one in the medulla of the Yorkshire-hog's adrenals; on the other hand there were shown strong alkaline phosphatase in the cortex, especially on the zona fasciculata, and negative one in the medullary of the adrenals in both sows and hogs of the Landrace-F_1. According to Allen (32), in the elevated production of adrenal steroids, increased phosphate turnover and oxidative metabolism during steroidogenesis, the role of alkaline phosphatase in glucose transport suggested that an increase of this enzyme would be expected under the conditions of ACTH stimulation. Our results seemed to be in accordance with those of Elftman (23) Allen (32) and Yen-Ling (30).

2. Acid phosphatase in the adrenals of Landrace-F_1 meat pigs.

Fränko (15) (1951) investigated the histochemical evidence of the presence of acid phosphatase-positive and -negative cell islets in the adrenal medulla of the rat. No other papers are known to us which deal with the distribution in the adrenocortical cells and the effect of adipositas upon adrenal acid phosphatase. Our results confirm that acid phosphatase activity was generally limited to the zona fasciculata and zona reticularis and medulla of the swine adrenals. No one has found the distribution of acid phosphatase in the adrenal cortex of the mammal. According to the previous and present reports, generally acid phosphatase activity in the adrenals of the hogs was stronger than that of the sows in both Yorkshire- and Landrace-meat pigs.

3. Succinic dehydgrogenase in the adrenals of Landrace-F_1 meat pigs.

The succinic dehydrogenase activity was limited to the cortex of the adrenal (Padykula (33), Dean and Greep (34), Rutenberg, Wolman and Seligman (35) Pearson (36), Nachlas (37), Cascarano and Zweifach (38)).

Our results confirm the distribution of the enzyme in the swine adrenals just the same as in Padykula's report on the rat. The enzymatic distribution in the various portions of the swine are also similar to that of Nachlas's findings.

No other papers are known to us which deal with succinic dehydrogenase on the course of corticosteroid production in the swine adrenals. Succinic dehydrogen ase activity in the adrenals of the hogs was stronger than that of the sows, and the enzymatic activity in the Yorkshire hogs might be remarkably accelerated in comparison with that in the Landrace-F_1 hogs, according to the previous and present reports. These results might be based upon the castration.

4. G-6-P dehydrogenase and 6-P-G dehydrogenase in the adrenals of Landrace-F_1 meat pigs.

No other papers are known to us which deal with G–6–P dehydrogenase and 6–P–G dehydrogenase on the course of glycogenolysis in the swine adrenals. In this present paper, the activities of G–6–P dehydrognease and 6–P–G dehydrogenase was limited to the cortex and medulla of the swine adrenals. Generally the G–6–P dehydrogenase activity in the adrenals of the sows was slightly stronger than that in the hogs, but 6–P–G dehydrognease activity in the adrenals of the hogs was stronger than that in the sows of Landrace-meat pigs.

5. DPN-(or NDA-) and $DPNH_2$-(or $NADH_2$-) dehydrogenase (or diaphorase) in the adrenals of Landrace-F_1 meat pigs.

According to Rubin, Deane and Hamilton (16) (1963), it is noteworthy that the intensity of staining for $\Delta 5$-3β–hydroxysteroid dehydrogenase did not parallel the degree of activity of $NADH_2$ diaphorase nor the amount of lipid in the cortical cells.

No one has reported the histochemical studies on DPN-and $DPNH_2$-diaphorase in the swine adrenals.

In this present report NAD (or DPN)- diaphorase activity was limited to the zona reticularis, zona glomerulosa and medulla in the only one hog, and almost of the sows and hogs reacted no activities in the cortex. On the other hand, $NADH_2$ (or $DPNH_2$)-diaphorase activity was limited to the cortex and medulla of the adrenals. The three cortical zones of the hogs were stained with equal intensity, but the cells of the zona fasciculata in the sows were negative while there were more strongly reactive cells of the zona glomerulosa and zona reticularis. Generally $NADH_2$-diaphorase activity in the adrenals of the hogs was stronger than that in the sows.

6. $\Delta 5$-3β-hydroxysteroid dehydrogenase in the adrenals of Landrace-F_1 meat pigs.

A diphosphopyridine nucleotide (NAD)-dependent enzyme system, $\Delta 5$–3β–

hydroxysteroid dehydrogenase, can be demonstrated by both biochemical and histochemical means in the steroid-hormone producing organs of many vertebrates (Rubin and Dorfman (39), Wattenberg (40), Levy (41), Rubin (42), Della Corte (43), Deane, Rubin (44), Hitzeman (45), Pesonen (46), Arvy (47), Bloch (48), Botte (49)). This enzyme system converts the Δ5–3β–hydroxysteroids, pregnenolone and dehydroepiandrosterone (DHA), to progesterone and Δ5–androstene-3, 17-dione, respectively. Recently, Berliner et al. (50) have obtained evidence indicating that the enzyme in the bovine adrenal gland is also able to oxidize Δ4–3β–hydroxyl compounds that are already hydroxylated at C–17 and C–21. According to Rubin, Deane and Hamilton (16) the histochemical studies of Δ5–3β–hydroxysteroid dehydrogenase activity in adrenals from animals of various mammalian orders lend support to the thesis of a pathway of steroid biosynthesis, that is, via oxidation of Δ5–3β–hydroxysteroids to Δ4–3–ketosteroids.

No one has reported the presence of Δ5–3β–hydroxysteroid dehydrogenase in the swine adrenals. According to Rubin, most of the other animal besides rats were females, and no record was made of the sex of the animals. In this present paper, there were found that the cells of the zona glomerulosa were less active than those of the zona fasciculata and zona reticularis, but those of the zona glomerulosa of the hogs were slightly stronger than those in the sows. Rubin and Deane stated that it is noteworthy that the intensity of staining for steroid dehydrogenase did not parallel the degree of $NADH_2$-diaphorase nor the amount of lipid in the cortical cells. However according to the present investigation, the occurrence of this steroid enzyme did not parallel the degree of $NADH_2$–dipahorase, but parallel to the amount of lipid in the cortical cells.

7. Lipid-storage and steroid-hormone production in the adrenals of the Landrace-F_1 meat pigs.

With regard to the histochemistry of ketosteroids in the adrenals may papers have been published, but they seemed to be not valuable because of coloring under the purified 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, 51), pseudo-plasmal substance (Chu, 52), peroxide (Cain, 53), unsaturated fatty acids (Bayley, 54), enzyme (Gomori, 55), unknown unsaponified substance (Ui, 56) and cholesterol (Schultz, 57).

Mayeda (58) 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 (59) 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 precursor.

Nakao (60, 61, 62 and 63) found a new method which he called $Sbcl_3$-Hcl-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 $Sbcl_3$-Hcl-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 previous report (7) there were observed three types such as rich fat stored cells/rich ketosteroid-cells, rich fat stored-cells/poor ketosteroid-cells, and poor fat-stored cells/none ketosteroid cells. Accordingly the present authors presumed that there may exist the remarkable ketosteroid producing stage, poor ketosteroid producing stage and resting ketosteroid producing stage.

In the present paper, generally the amount of ketosteroid in the hogs was stronger than that in the sows, and the amount of Sudan-III-philic lipids and ketosteroid paralleled the activity of $\Delta 5$–3β–hydroxysteroid dehydrogenase. From this point these findings were different from Rubin, Dean and Hamilton's ones. There seemed to be no marked difference between the amounts of sudanophilic lipids and the activity of $\Delta 5$–3β–hydroxysteroid dehydrogenase in both hogs and sows.

Summary and Conclusion

Physio-histological studies on the mechanism of adipositas and the effect of fattening has been investigated histochemically in various organs of the Yorkshire or Landrace meat pigs used for the Pig Feeding Standard Revising Test in case of pork production. During these investigations there has been found the occurrence of glycogen in the nuclei and cytoplasms of the zona reticularis in the adrenals of the pigs. No one has found glycogen in the nuclei of the adrenals. It seems to be important to show histochemically the Sutherland's hypothesis connected with glycogenolysis, coenzymes, and steroid hormone as the hormonal regulation of the intraceullar reaction for the establishment of the mechanism of adipositas.

By the present study there were found the occurrence of glycogen, alkaline phosphatase, acid phosphatase, succinic dehydrogenase, G–6–P dehydrogenase, 6–P–G dehydrogenase, DPN–diaphorase, $DPNH_2$–diaphorase, $\Delta 5$–3β–hydroxysteroid dehydrogenase, ketosteroid and lipid in the cells of the zona fasciculata and zona reticularis in the swine adrenals in the course of Sutherland's hormonal regulation.

Materials used for the studies were 16 Landrace-F_1 piglets: all the animals born from two Yorkshire mother pigs and one Landrace father pig, were studied on the feeding standard by the administration with various feeds, and autopsey of them was done at the age of 185 days after birth. The total fresh adrenals were cut with the Cryostat microtome at 25°C below zero, and stained

with the various enzyme-histochemical procedures.

The results investigated are summerized as follows:

1) In the present study the Landrace-F_1 and Yorkshire meat pigs, contained the glycogen-laden nuclei in the zona reticularis or zona fascicuclata. On the average there occurred 2 cells/1 section with glycogen in the nuclei, 3 cells/1 section with glycogen in both nuclei and cytoplasms, and 317 cells/1 section with intracytoplasmic glycogen in sows; and 73 cells in the nuclei, 23 cells in both nuclei and cytoplasms, and 553 cells in the cytoplasms in hogs. Generally 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.

In comparison with the occurrence of glycogen deposition within the nuclei and cytoplasms in the Yorkshire- and Landrace-F_1 meat pigs, the total numbers of the cells with intranulear glycogen deposition in the Landrace were only half the ones in the Yorkshire, and on the other hand those with intracytoplasmic glycogen deposition in the Landrace-F_1 were about twice the numbers of the ones in the Yorkshire.

Epinephrine production investigated by the PAS reaction in the medulla, indicated less than that in the pigs immunized with hog-cholera virus; and the results should no remarkable differences, except the slight appearance of the exhaution and reconstruction periods in comparison with those of Landrace-F_1 and Yorkshire meat pigs.

2) Generally alkaline phosphatase activity of the adrenocortical cells in the sows was slightly stronger than that in the hogs of Landrace F_1-meat pigs. In comparison with alkaline phosphatase activity in the Yorkshire- and Landrace- meat pigs, there were a great difference between strong reaction in the zona reticularis of the Yorkshire pigs and that in the zona fascicucllata of the Landrace-F_1 pigs. In addition to these changes, alkaline phosphatase activity in the medulla was negative in all Landrace pigs with regard to the presence of activated form in the Yorkshire pigs.

3) Generally, acid phosphatase activity of the adrenocortical cells in the hogs was stronger than that in the sows of the Landrace F_1 pigs the same as the Yorkshire pigs. In comparison with acid phosphatase activity in the Yorkshire- and Landrace F_1-meat pigs, there were a great difference between strong reaction in the zona glomerulosa and medullary of the Yorkshire pigs, and negative reaction in those of the Landrace pigs; there was a great affinity between the strong reaction in the zona fasciculata and the zona reticularis of both Yorkshire- and Landrace-F_1 pigs.

4) Generally the succinic dehydrogenase activity of the adrenocortical cells in the hogs was all the same as that of the sows of the Landrace-F_1 meat pigs. In comparison with succinic dehydrogenase activity in the Yorkshire- and

Landrace-F_1 meat pigs, there was a great difference between strong reaction in the zona fasiculata and zona reticularis of the Yorkshire pigs, especially on the hogs, and strong reaction in only the zona reticularis of the Landrace-F_1 pigs.

5) Accordingly, from the point of alkaline phosphatase, acid phosphatase and succinic dehydrognease, the metabolic function in the adrenals seemed to be accelerated more remarkable in the Yorkshire pigs than in the Landrace-F_1 pigs. Those function in the hogs seemed to be stronger than that in the sows.

6) Generally G–6–P dehydrogenase activity of the adrenocortical cells in the swos of Landrace-F_1 pigs was stronger than that in the hogs.

7) Generally 6–P–G dehydrogenase activity of the adrenocortical cells in the hogs of Landrace-F_1 pigs was stronger than that in the sows.

8) NAD-diaphorase activity was limited to the zona reticularis, zona glomerulosa and medullary in the only one hog. Almost all of both sows and hogs of Landrace-F_1 pigs reacted negatively in the three zones of the cortex.

9) $NADH_2$-diaphorase was limited to the cortex and medullary of the adrenals. Generally $NADH_2$-diaphorase activity in the adrenocortical cells of the hogs was stronger than that in the sows. It is very characteristic to find no activity of the zona fasciculata in the sows, but to observe hyperactivity in the three cortical zones in the hogs of the Landrace-F_1 pigs.

10) $\Delta 5$–3β–hydroxysteroid dehydrogenase activity was strictly limited to the three zones of the cortex, and generally that in the adrenocortical cells of the hogs was slightly stronger than that of the sows. The cells of the zona glomerulsoa were less active than those of the other zones of the cortex, but those in the hogs were slightly stronger than those in the sows.

11) The intensity of staining for steroid dehydrogenase did not parallel to the degree of $NADH_2$-diaphorase, but not parallel to the amount of lipid in the cortical cells.

12) It is noteworthy that the intensity of $\Delta 5$–3β-hydroxysteroid dehydrogenase did not parallel to the degree of the amount of ketosteroid in the cortical cells.

13) Generally the amount of ketosteroid in the hogs was stronger than that in the sows; the amount of lipid and ketosteroid in the hogs was stronger than that in the sows; the amount of lipid and ketosteroid paralleled to the activity of $\Delta 5$–3β–hydroxysteroid dehydrogenase.

In the case of sixteen Landrace pigs (8 sows and 8 hogs), there was observed the presence of glycogen within the nuclei and cytoplasms, glycogenolysis to glucose–6–P dehydrogenase or 6–P–G dehydrogenase in the zona fasciculata and zona reticularis. Since a very active hexosemonophosphate shunt was present in the adrenal tissue, the amount of glucose–6–P metabolized via this way was increased, with the result that $NADH_2$ was produced by the NAD-diaphorase or $NADH_2$-diaphorase. ' This co-factor accelerated splitting of the cholesterol side-chain and

the reduction of certain steroids, leading to increased synthesis of corticosteroids by the certification of the presence of lipids, ketosteroid and $\Delta 5$–3β–hydroxysteroid dehydrogenase in the zona fasciculata and zona reticularis.

Generally the occurrence of glycogen, the activities of acid phosphatase, succinic dehydrogenase, 6–P–G dehydrogenase, $NADH_2$-diaphorase, $\Delta 5$–3β–hydroxysteroid dehydrogenase, and the amount of ketosteroid were stronger than those in the sows. On the contrary, the activities of alkaline phosphatase and G–6–P dehydrogenease in the hogs were less than those in the hogs. The production of epinephrine and the amounts of lipids were indicated no difference between in almost of both the sows and hogs. NAD-diaphorase in almost all of both the sows and hogs reacted negatively in the three zones of the cortex. Accordingly these chemical pathways in the adrenocortical cells of the hogs seemed to be stronger than those of the sows in the Landrace-F_1 meat pigs.

As the food intake increased, the β-cells of the pancreas were stimulated, producing more insulin. This hormone was highly important in lipogenesis, and when it was present in excess it promoted an excess deposit of fat, particularly with the excess food ingestion. Insulin was known to increase the output of glucosteroids, which in turn played an important role in stimulating the increased production of insulin antagonists. The production of insulin was accelerated by the production of ketosteroid. Obesity tended to produce hyperinsulinism and hyperadrenocorticism, which can in turn significantly further the obesity.

Our present report described on the histochemical observations of the Sutherland's biochemical hypothesis connected with the glycogenolysis, coenzymes and steroid hormone as the hormonal regulation of the intracellular reaction in the adrenals of Landrace-F_1 meat pigs for the sake of the clarification of the mechanism of the obesity.

If the obesity developes by hyperinsulinism and hyperadrenocorticism, the glycogen deposition in the cytoplasms and nuclei might play an important role to hyperadrenocorticism. Moreover it is very important to solve histochemically the problems of the production of co-factor by NADP- and $NADP \cdot H_2$-dehydrogenase in the adrenals and that of the stimulation of β-cells in the pancreas. This will become the subject for future investigation.

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Plate 1

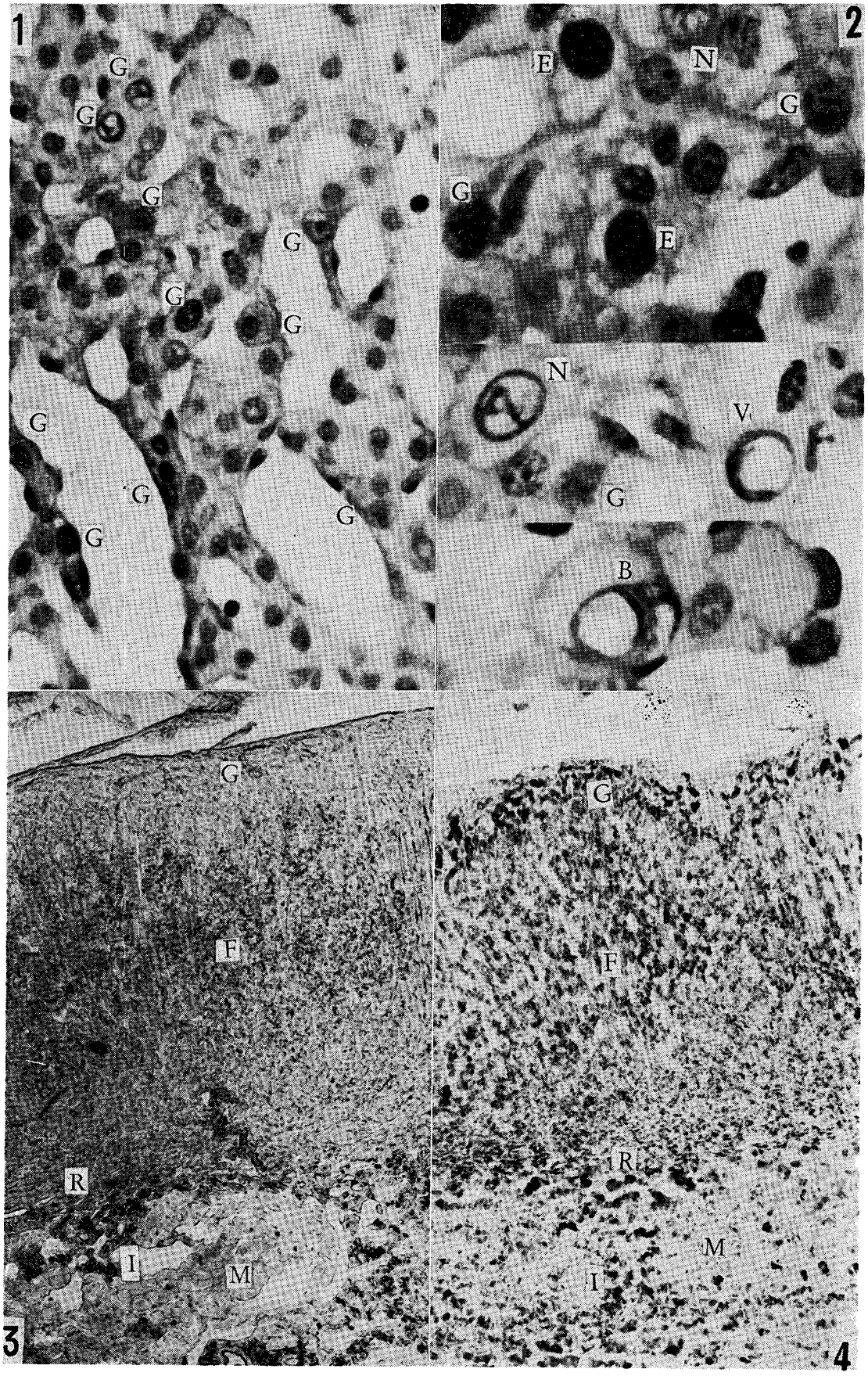
Explanation of Figures

Fig. 1. Intranuclear glycogen deposition in the zona reticularis of the adrenals of the Landrace-F_1 meat pig: No. 18., stained with PAS, and enlarged 160 X. (G indicated the intranuclear glycogen deposition within the nuclei)

Fig. 2. Various types of the intranuclear glycogen deposition in the zona reticularis of the adrenals of the Landrace-F_1, No. 14 and 18, stained with PAS, and enlarged 360 X. (E indicated the entire bodies, N: inner bodies, B: glycogen in both nuclei and cytoplasm, V: vacuolization with few or no glycogen, G: granular

Fig. 3. Fat storage in the zona fasciculata and zona reticularis of the adrenal of the Landrace-F_1, meat pigs, No. 11, stained with Sudan III, enlarged 16 X. (G : Zona glomerulosa, F : Zona fasciculata, R: zona reticularis and M: medullary)

Fig. 4. Fat storage in the zona glomerulosa, zona fasciculata, zona reticularis (R) and medullary (M) of the adrenal of the Landrace F_1 meat pigs, No. 13, stained with Sudan Black B, and enlarged 16 X.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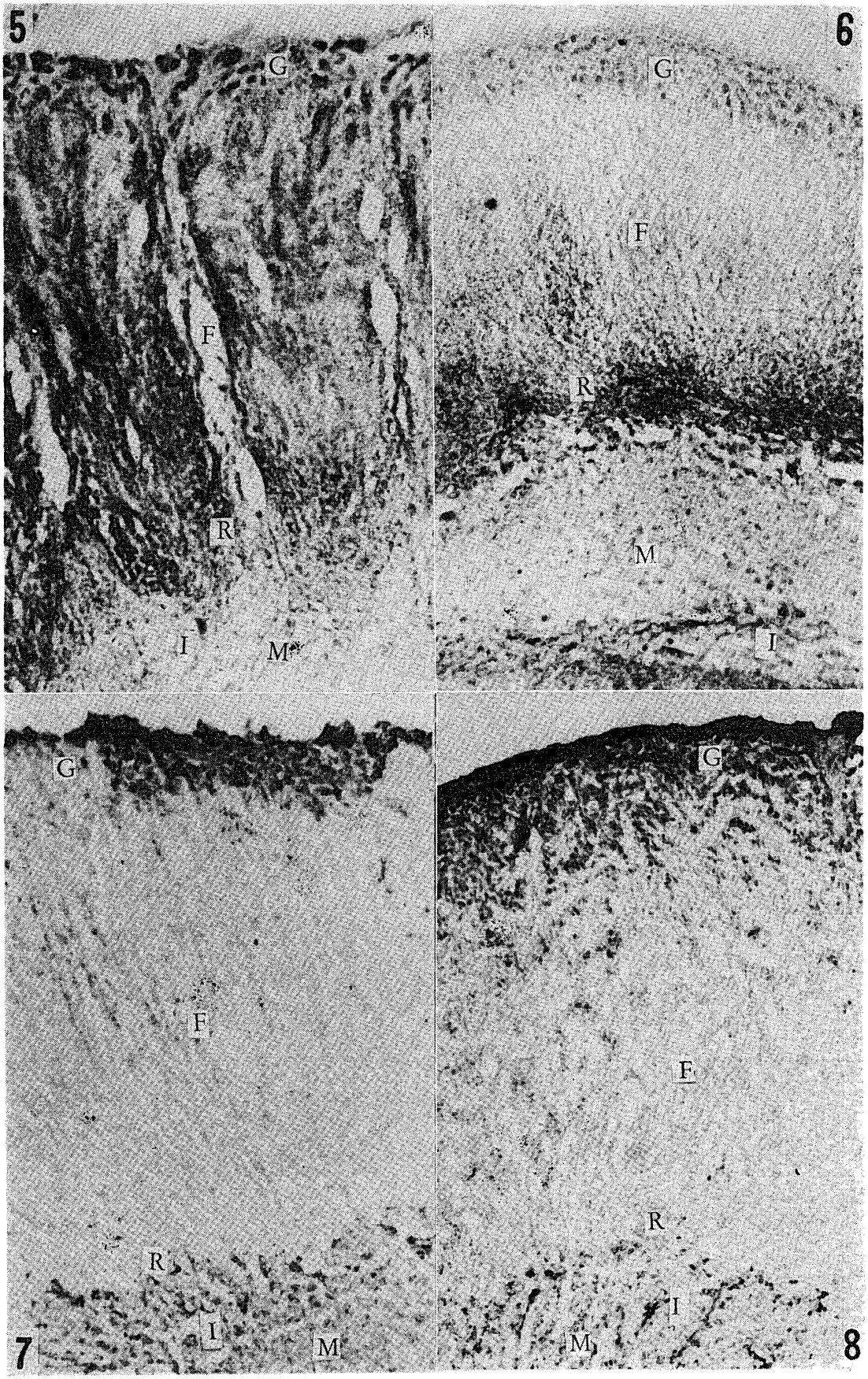
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Plate 2

Explanation of Figures

Fig. 5. G–6–P dehydrogenase activity in the zona fasciculata and zona reticularis of the adrenal of the Landrace-F_1 meat pig, No. 14, stained with Seligman's DHG-ase method, and enlarged 16 X (G: zona glomerulosa, F: zona fasciculata, R: zona reticularis, I: zona reticularis extended within the medullary, and M: medullary).

Fig. 6. 6–P–G dehydrogenase activity in the zona glomerulosa (G), zona fasciculata (F), zona reticularis (R), zona reticularis extended within the medullary (I), and medullary (M) of the adrenal of the Landrace-F_1 meat pig, No. 16, stained with Seligman's azo-coupling method, and enlarged 16 X.

Fig. 7. DPN-diaphorase activity in the capsule (C), zona glomerulosa (G), zona fasciulata (F), zona reticularis (R) and medullary (M) of the adrenal of the Landrace-F_1 meat pig, No. 13, stained with Seligman's azo-coupling method, and enlarged 16 X.

Fig. 8. $DPNH_2$-diaphorase activity in the zona glomerulosa (G), zona fasciculata (F), zona reticularis (R) and medullary (M) of the adrenal of the Landrace-F_1 meat pig, No. 5, stained with Seligman's azo-coupling method, and enlarged 16 X. This case indicated weak positive activity.

Plate 3

Explanation of Figures

Fig. 9. $DPNH_2$-diaphorase activity in the zona glomerulosa (G), zona fasciculata (F), zona reticularis (R), zona reticularis extended within the medullary (I) and medullary (M) of the adrenal of the Landrace-F_1 meat pig, No. 16, stained with Seligman's azo-coupling method, and enlarged 16 X. This case indicated strongly positive activity.

Fig. 10. Succinic dehydrogenase activity in the zona glomerulosa (G), zona fasciculata (F), zona reticularis (R), islet of zona reticularis extended with the medullary (I), and medullary (M) of the adrenal of the Landrace-F_1 meat pigs, No. 4., stained with Seligman's azo-coupling method, and enlarged 16 X.

Fig. 11. $\Delta 5$–3β–dehydrosteroid dehydrogenase activity in the zona glomerulosa (G), zona fasciculata (F), zona reticularis (R), and the islet of zona reticularis extended with the medullary (I), and medullary (M), of the adrenal of the Landrace F_1 meat pig, No. 5, stained ith Rubin's steroid hormone dehydrogenase method, and enlarged 16 X.

Fig. 12. $\Delta 5$–3β–dehydrosteroid dehydrogenase activity in the zona glomerulosa (G), zona fasciculata (F), zona reticularis (R), and adrenocortical islet within the medullary (I) and medullary (M), of the adrenal of the Landrace F_1–meat pig, No. 8, stained with Rubin's steroid hormone dehydrogenase method, and enlarged 16 X.


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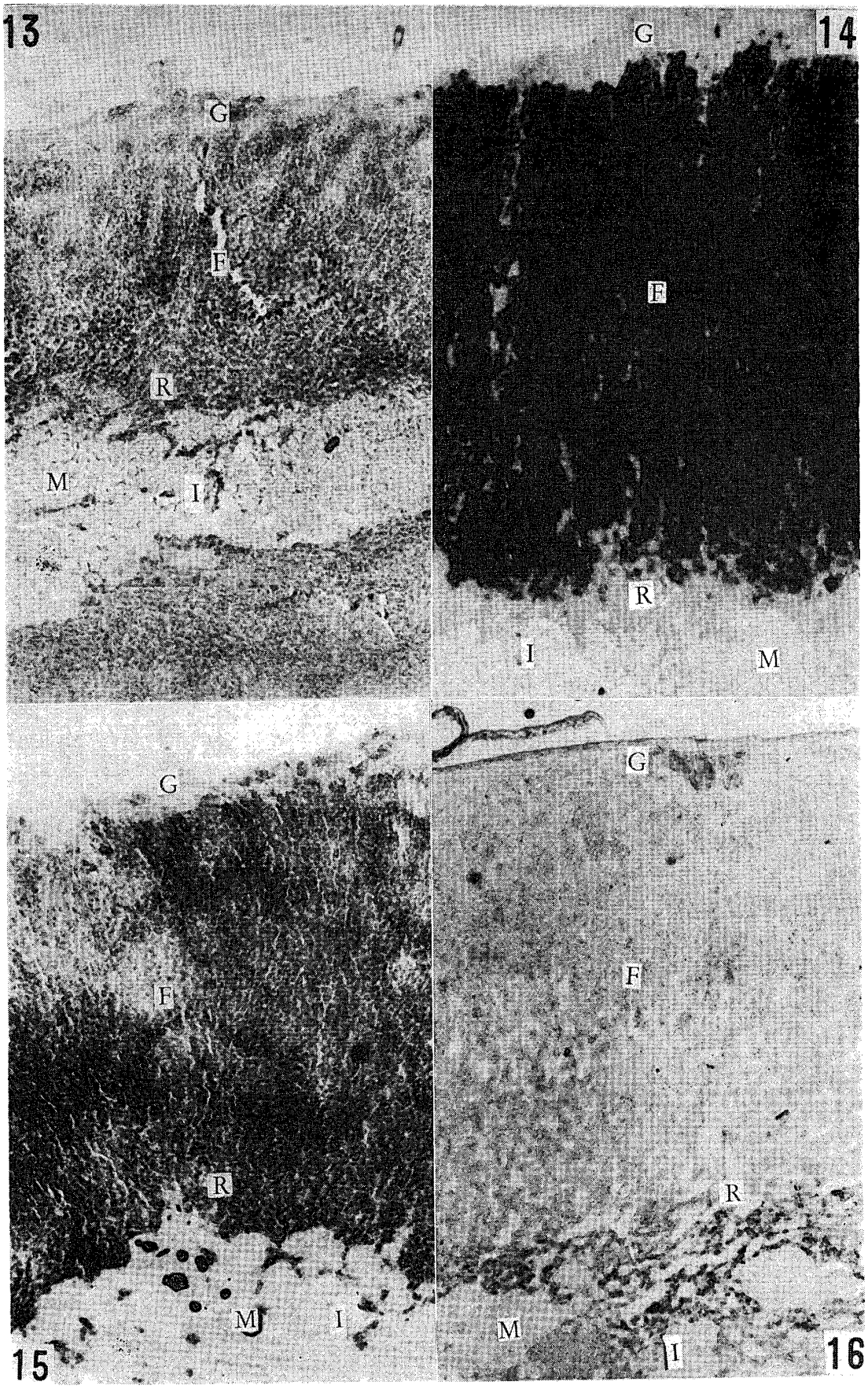
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Plate 4

Explanation of Figures

Fig. 13. Acid phosphatase activity in the zona glomerulosa (G), zona fasciculata (F), zona reticularis (R), adrenocortical cells extended within the medullary (I), and medullary (M) of the adrenal of the Landrace-F_1 meat pig, No. 8. stained with Seligman's azo-coupling method, and enlarged 16 X.

Fig. 14. Alkaline phosphatase activity in the zona glomerulosa (G), zona fasciculata (F), zona reticularis (R) and the adrenocortical islets extended within the medullary (M) and medullary (M), of the adrenal of the Landrace-F_1 meat pig, No. 8, stained with Gomori's metalic method, and enlarged 16 X.

Fig. 15. Alkaline phosphatase activity in the zona glomerulosa (G), zona fasciculata (F), zona reticularis (R) and adrenocortical islets extended within the medullary (I) and medullary (M), of the adrenal of the Landrace-F_1 meat pig, No. 8, stained with Seligman's azo-coupling method, and enlarged 16 X.

Fig. 16. Corticosteroid stained with Nakao's Hcl–$Sbcl_3$ reaction in the zona fasciculata (F), zona reticularis (R) and adrenocortical islets extended within the medullary (I), of the adrenal of the Landrace-F_1 meat pig, No. 14, and enlarged 16 X.