PHYSIO-HISTOLOGICAL STUDIES ON THE PHYSIOLOGICAL OBESITY OF THE MEAT PIGS (REPORT I), ESPECIALLY ON THE OCCURRENCE OF THE GLYCOGEN WITHIN THE NUCLEI AND CYTOPLASMS OF THE ZONA RETICULARIS IN THE ADRENALS

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PHYSIO-HISTOLOGICAL STUDIES ON THE PHYSIOLOGICAL OBESITY OF THE MEAT PIGS (REPORT I), ESPECIALLY ON THE OCCURRENCE OF THE GLYCOGEN WITHIN THE NUCLEI AND CYTOPLASMS OF THE ZONA RETICULARIS IN THE ADRENALS

By

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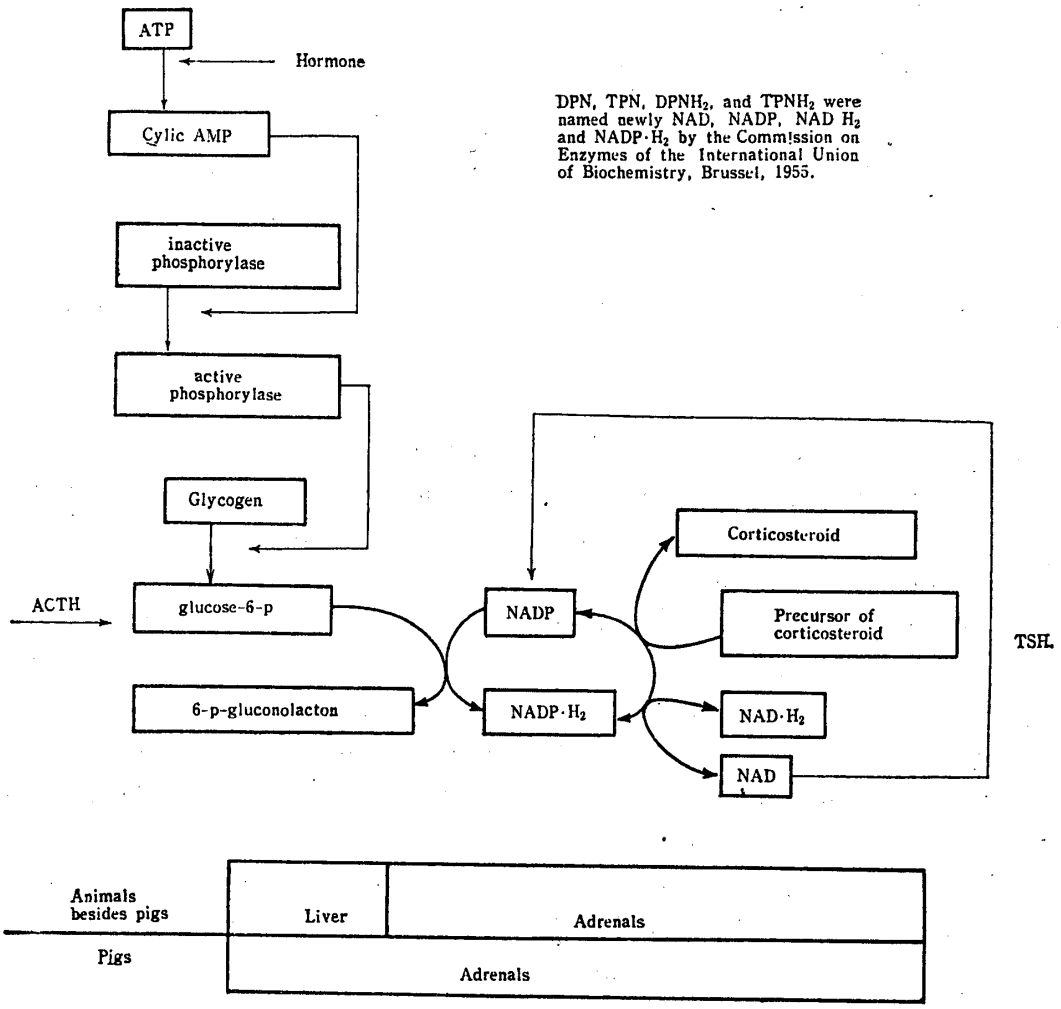
Introduction

Physio-histological studies on the mechanism of adipositas and the effect of fattening has been done histochemically on the various organs of Yorkshire pigs used for experiment of the feeding standard on the meat pigs. Through these results, meat pigs were considered to have different structure in comparison with the other domestic animals. Accordingly, the pigs might be interesting animals from the point of physiology. Hereafter the authors will report on some problems in connection with the physiological adipositas (1).

By the present study there was found the occurrence of glycogen in the nuclei and cytoplasms of the zona reticularis in the adrenals, and it was planned to examine the mode of glycogen-bearing nuclei, the relationships between glycogen and fat deposition related to the production of ketosteroid, the shape of intranuclear glycogen and the intracytoplasmic one, and the vacuolization in the nuclei.

No one has found glycogen in the nuclei of the adrenals. In the present investigation special attention was also paid to the presence of glycogen in the nuclei and also in the cytoplasms of the adrenal cells of the pigs, because the deposition of glycogen in the cells was demonstrated. With regard to this physiological meaning, it is expected that it will become clarified in the future. However, it has been shown that the working hypothesis as the regulation of the intracellular reaction by the hormone (SUTHERLAND (2-3) 1960 and KONDO (4) 1963) might be established. According to SUTHERLAND's opinion (2-3), there were found the following metabolic systems: glycolysis from the 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 relations are closely connected with glycolysis, coenzymes and steroid hormone (Table 1).

Table 1. Our opinion based on KONDO's hypothesis (1963) with regard to regulation of cellular reactions by the hormone. (ITIKAWA et al 1964)



The chemical reactions shown in Table 1 are related to each other. In the animals besides pigs the glycolysis was done in the liver and the production of corticosteroids in the adrenals. The presence of glycogen in the nuclei and cytoplasms of the swine adrenals found recently by us, might be effective to produce the corticosteroids.

According to WILLIAMS (5) (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 syntheses and secretion of corticosteroids formed in the adrenal. 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 TPNH was produced. This co-factor 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 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. These fat-containing cells corresponded to the ketosteroid-producing cells, but the certification of ketosteroid in detail will be described at another occasion.

Materials and Methods for Studies

Eleven sows and nine hogs of Yorkshire were used. Boars were castrated at the age of 23 to 24 days after birth. These hogs and sows were used for 123 days. Animals were studied on the feeding standard by the administration with various feeds such as medium protein-high energic group (E ane F), medium protein-medium energic group (AC and M) and low protein-medium energic group (B) in the Miyagi Prefectural Agricultural Experiment Station in Sendai in 1963. The rations of the feed are shown in Table 2:

Pigs in each lot were fed with the ration above given in Table 2. Rations were administered under the dry lot-feeding conditions and the pigs were set for about two hours each day. All the pigs showed on increase of body weight and rapid growth and good appetite. Autopsy was done at the age of 196 days after berth, and the body-weight and feed intake according to sex and experimental periods are shown in Table 3:

Table 2. Ration used for the experiment

Administered Feeds		Feeding \ Exp. groups \ Period	Former period E	Former period F	Former period AC	Former period B	Former period M	Later period E	Later period F	Later period AC	Later period B	Later period M
		Concentrated combined : Barly feed	6 : 4	6 : 4	7 : 3	7 : 3	7.2:2.8	6 : 4	6 : 4	7 : 3	7 : 3	7.2:2.8
Administered Feeds	Basal ration	yellow corn	36	37	16	18	25.2	40	39	20	20	28.0
		Bran	6	2	20	11	4.2	8	2	22	11	5.6
		Defattend rice-bran	2	1	7	8	1.4	2	1	7	8	1.4
		Soybean oil meal	7	3	6	3	4.9	3	1	2	1	2.1
		Fish meal	3	3	4	3	2.1	1	1	2	1	0.7
		Starch meal	—	8	11	21	—	—	10	11	23	—
		Others	6	6	6	6	4.2	6	6	6	6	4.2
		Barley	40	40	30	30	28	40	40	30	30	28
		Alphalpha meal					30					30

Table 3. Body weight and feed intake of the pigs according to sex and experimental periods.

Lots	Sex (Cases)	Age (days) Initial	Age (days) Middle period	Age (days) Final period	Body weight (kg) Initial	Body weight (kg) Former period	Body weight (kg) Middle period	Body weight (kg) Final period	Feed intake(kg) during all periods Former period	Feed intake(kg) during all periods Final period	Feed intake(kg) during all periods Whole period	Remarks
E	♀(2)	70	140	196	22.5	50.7	73.2	114.7	318	307	625	Medium protein-High energic ration
	♂(2)	70	140	196	20.6	49.7	70.2	109.4	307	360	667	
F	♀(2)	70	140	196	23.0	44.3	67.3	97.4	306	283	589	Medium protein-High energic ration
	♂(2)	70	140	196	21.5	53.5	75.0	114.8	331	338	669	
AC	♀(2)	70	140	196	21.0	44.1	65.1	100.6	297	343	640	Medium protein-Medium energic ration
	♂(2)	70	140	196	22.7	52.8	75.5	120.6	357	443	800	
B	♀(2)	70	140	196	21.8	39.8	61.6	98.1	286	360	646	Medium protein-Medium energic ration
	♂(2)	70	140	196	22.3	48.6	70.9	112.5	322	385	707	
M	♀(2)	70	140	196	21.3	44.9	66.2	108.1	531	478	1009	Low protein-Medium energic ration with alphalpha-meal
	♂(3)	70	140	196	20.2	43.0	63.2	99.7	514	452	966	

The total adrenals from these sows and hogs were fixed either in buffered formol or in CARNOY's fluid, enbedded in paraffine and cut into 6μ sections, and cut with frozen microtome for fat-staining. The stains employed were as follows: Hematoxylin eosin stain and CROSSMAN's stain for general histology: PAS-hemetoxylin stain with or without saliva digestion for the glycogen; and FEULGEN's nuclear reaction counterstained with light green for th DNA.

Results

1. Glycogen occurred in the cytoplasms and nuclei, and vacuolized giant nuclei.

According to OKAMOTO (6) (1958), there are no reports on the presence of glycogen in the cortex of adrenals. However, WILLIAMS (5) (1962) described that the recent investigations have helped in the mechanisms involved in the synthesis and the release of corticosteroids as follows: relatively high concentration of glycogen in the zona fasciculata and zona reticularis, plentiful presence of glucose-6-P dehydrogenase in the zona fasciculata and zona reticularis and etc.

It was noticed that a large amount of the nuclei and cytoplasms of the swine adrenals showed a strong positive reaction to PAS stain. This reaction

Table 4. The ocurrence of glycogen-laden nuclei and cytoplasms, and that of the vacuolized giant nuclei in the zona reticularis of swine adrenals

Sex	Cortex	Cytoplasmic Glycogen	Nuclear Glycogen	Feeding / Group / Name	Medium protein-High energic group				Medium protein-High energic group					Low protein, Medium energic	
				Group	E		F		AC		M			B	
				Name	22	24	11	27	21	7	2	14	25	1	8
Sows	Z. reticulares	+	+	−	—	○1	—	○1	—	—	○1	○6	○7	◎29	—
		−	+	−	○5	○9	○14	○3	◎35	○12	○3	⊚592	◎26	◎52	○
		+	−	+	—	—	—	—	—	—	○	○	○	○	○
		+	−	−	⊚467	⊚792	○65	○76	○84	○92	◎134	○63	○72	○69	◎225
		−	−	+	—	—	○6	—	○2	○	○14	◎97	○16	○16	○4
	Zona fasciculata*				—	—	—	—	—	—	—	◎	—	◎	—
	Zona glomerularis*				—	—	—	—	—	—	—	—	—	—	—
					28	29	4	5	12	23	13	15	3	26	6
Hogs	Z. reticularis	+	+	−	—	—	—	○4	missing	○8	○8	missing	—	⊚140	—
		−	+	−	○14	◎22	⊚478	◎29		⊚99	⊚63		○1	⊚118	◎39
		+	−	+	—	—	○	○		○	○		○	○16	○
		+	−	−	⊚468	○28	○21	⊚1872		◎196	⊚639		◎53	◎80	⊚397
		−	−	+	○12	○1	◎62	○1		○7	○12		○3	⊚115	○7
	Zona fasciculata*				—	—	—	—		○	◎		—	—	—
	Zona glomerularis*				—	—	—	—		—	—		—	—	—

Remarks: * indicated the presence of intranuclear glycogen, ⊚, ◎, ○ in the table showed the degree, and the numbers added to right corner showed the cell numbers, and—no glycogen.

became negative in the sections previously treated with saliva for less than one hour at 37°C. This indicated that the PAS-positive substance in the nuclei was glycogen. Eleven sows and nine hogs contained the glycogen-laden nuclei of the cells in the zona reticularis or zona fasciculata in 100 percentage. There were found various types as follows: glycogen in both cytoplasms and nuclei; no glycogen in the cytoplasms and glycogen in the nuclei; glycogen in the cytoplasms and no glycogen in the nuclei; no glycogen in both cytoplasms and nuclei, and vacuolized giant nuclei with no glycogen. Usually the glycogen deposition in the nuclei was shown in zona reticularis at 100 percent, but in zona fasciculata at 18 percent of 11 sows and at 22 percent of nine hogs. No intranuclear glycogen was found in zona gomerularis. These results were indicated as shown in Table 4, and the cell counts of the glycogen-laden nuclei in one section are shown in the table. The presence of glycogen in the cytoplasms and nuclei seemed to have no relation to the difference with the feeding ration, but it might be related to the sex from the point of more intensive appearance in the hogs than that in the sows.

2. The shape of intranuclear and intracytoplasmic glycogen deposition.

Up to this time it was known that there were no glycogen besides DNA, RNA and histon in the ordinary nuclei. With the exception of the hepatic cells or pancreatic β-cells in diabetes melitus the presence of glycogen was found in these cells. However, recently TORYU *et al* (7) (1960) of Institute reported that a large amount of glycogen was demonstrated histochemically in the cells of the anterior lingual salivary glands of adult laying hens, but not in adult cocks. The occurrence of the glycogen-laden nuclei in the glands was also investigated in growing female chicks and adult female chicks during laying and moulting (TORYU *et al*) (8-9) (1961). They also investigated the presence of glycogen-laden nuclei of the male chicks of from 2 to 180 days of age (TORYU *et al*) (8-9) (1961).

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. Almost all of the intranuclear glycogen present in the juxta medullary zone of zona reticularis adjoins the medullary, and also a large amount of glycogen in the nuclei existed in the boundary zone (temporary cortex or central body). In these regions lipids deposited remarkably.

In severe cases there occurred a large amount of glycogen in the nuclei, this extended to the deep zone of zona fasciculata, and also to the spongy zone. Generally the cells in the zona reticularis were arranged in an irregular network and they possesed fat droplets and glycogen in the cytoplasms. The cells indicated a network of round chain-like appearance in the juxta medullary zone, and that of strand-like appearance in the boundary zone. A large part of the glycogen-laden nuclei were giant in size, but some nuclei were normal.

The size of the normal and abnormal nuclei with or without glycogen in the zona reticularis was various types as shown in Table 5.

Table 5. The size of glycogen-laden nuclei and no glycogen-laden nuclei in some cells of zona reticularis in the swine adrenals

yto- lasms		Nuclei			Size (length and width, square microns)						
at	Glycogen	Glycogen	Vacuo- lization	Normal	(3.97–5.0) × (5.0–7.15) μ	(6.35–7.15) × (5.0–7.15) μ	(7.95–8.7) × (4.76–8.7) μ	(9.5–10.3) × (4.76–10.3) μ	(11.0–12) × (8.7–12) μ	(13–13.5) × (9.5–13.5) μ	(14–19.2) × (16–19.2) μ
-	+	+	—	—	•	•••	••	•		•R	
-	—	+	—	—		•••			•		
-	+	—	+	—					•••	•	
-	—	—	+	—				•	•		
-	+	—	—	+	•••••••	••••••	••••				
-	—	—	—	+	••	•••••	•••				
-	+	+	—	—			•	•••	•		•R
-	—	+	—	—	••I••••	••	•••G••G••R•	••	•R•R	•R	•
-	+	—	+	—					• •	••	
-	—	—	+	—					•••	•	
-	+	—	—	+	•	•					
-	—	—	—	+	•	•	•				

Remarks : I. indicated internal bodies ; R. ring form ; and G. granale form.

Giant nuclei with glycogen were four-five times as large as the normal nuclei. There were various types of intranuclear glycogen as follows: large entire bodies (glycogen occupied entirely in the nuclei), ring form (glycogen deposition near nuclear membrane and empty cavity), multiple granule form (spreading diffusely as the granular bodies in mixture to chromatin), and internal bodies (round bodies, bottle form, and funnel form in the nuclei). The development of these types of intranuclear glycogen deposition will be described in another paper. There existed hyperchromic or hypochromic nuclei which reacted to PAS with glycogen.

The nuclei had hydrops recognized as a simple vacuole in the hematoxylin-eosin stain and these glycogenic vacuolization was similar to that in the diabetes. The vacuolized nuclei without glycogen in the PAS-stain were called" vacuolization in the giant nuclei. Glycogen might be lost from the ring form of nuclei which contained a small amount of glycogen around the nuclear membrane. According to TORYU *et al* (7) (1960) the process of the nuclear vacuolation and the migration of the chromatin granules towards the nuclear membrane, were also ascertained in the sections stained by FEULGEN's technique or by basic dyes.

Various forms of the nuclei were arranged in an order according to their shape and the amount of the intranuclear glycogen. The nucleus, which might be taken as "normal", was approximately 6.0 μ in diameter, containing the chromatin granules and nucleolus. They contained no glycogen. When the vacuoles became larger, they nearly occupied the entire space of the nucleus, pushing aside all chromatin granules towards the nuclear membrane. Consequently, the chromatin granules were found attached to the membrane. Glycogen depostion occurred in such nuclei. They consisted of the one called the entire bodies with glycogen and the other called the ring form which glycogen demonstrated by PAS stain was limited in the largest vacuoles occupying most of the nuclear space. They consisted of the two forms called the large entire bodies and the small entire ones. A few glycogen granules, small in size, appeared in the periphery of the vacuoles close to the chromatin granules attached to the nuclear membrane or near the dispersed chromatin granules in the nuclei. They were called the multiple granular form of glycogen-laden nuclei. The size of the glycogen granules remarkably increased, united with each other, and they formed internal bodies in the whole vacuolar cavity. And then the whole vacuolar cavity was filled with glycogen. They are called the large and small entire bodies.

The occurrence of the glycogen-laden nuclei was examined in zona reticularis (sometimes in zona fasciculata) of the adrenals. Variation of the nuclei containing glycogen and that of the glycogen-laden cytoplasms are shown in Table 6. Whole cells in the zona reticularis were counted in the sections stained by PAS-hematoxylin stain using the square-meter

The variation of the intranuclear or intracytoplasmic glycogen deposition and the glycogen-free vacuolization in the nuclei, was divided into the undermentioned six types by means of observing the combination of intracytoplasmic and intranuclear glycogen deposition and the vacuolized giant nuclei without glycogen:

a) The cells contained glycogen in both cytoplasms and nuclei.

b) The cells contained the glycogen-laden nuclei and glycogen-free cytoplasms.

c) The cells contained the glycogen-laden cytoplasms and the vacuolized nuclei without glycogen.

d) The cells contained the glycogen-laden cytoplasms and normal nuclei without glycogen and vacuoles.

e) The cells contained the vacuolized giant nuclei and the glycogen-free cytoplasms and nuclei.

f) The cells seemed to be normal and contained no glycogen-laden cytoplasms and nuclei and no vacuolized giant nuclei.

Type a happen at the same time in the severe cases of type b (M-14, B-1 in sows and F-5, AC-21. B-26, M-13 in hogs, see in Table 6). The appearance of nuclear glycogen in the sixth type was found in all of cases. In the inten-

Table 6. Variation of the intranuclear or intracytoplasmic glycogen deposition and the glycgen-free vacuolization in the nuɔlei.

Sex	Cytopl. glycogen	Nucl. glycogen	Vac.	Feeding	Medium Protein High Energic				Medium Protein-Medium Energic					Low Protein-Medium Energic		Total	Average
				Groups	E		F		AC		M			B			
				Name	22	24	11	27	21	7	2	14	25	1	8		
♀	+	+	—	Ring form													
				Large entire								1		5		6	0.5
				Small entire										1		1	0.1
				Inner bodies							1	2	3	6		12	1.1
				Multiple gr.		1		1				3	4	7		16	1.4
	—	+	—	Ring form					2	1	1	5				9	0.8
				Large entire								18	3			21	1.9
				Small entire	1		2		2			12	1			18	1.6
				Inner bodies	1	1	5	1	11	3	1	274	2	23	3	325	29.5
				Multiple gr.	3	8	7	2	20	8	1	233	20	29	3	334	30.4
	+	—	+	Whole glyc. bodies													
	+	—	—	Whole glye.	55	785	24	66	36	60	93	25	39	64	45	1292	117.4
				Dodies	412	7	41	10	48	32	41	38	33	5	180	847	77.0
	—	—	+	Large vac.			1		1		4	42	5	6	3	62	5.6
				Small vac.			5		1		10	5	11	10	1	43	4.0
	—	—	—	Free glyc.	6709	4935	8325	10179	8930	19267	6762	32770	17200	10800	8680	134547	12231
					28	29	4	5	12	23	13	15	3	26	6		
♂	+	+	—	Ring form				1	3	missing		missing		3		7	0.8
				Large entire							1			70		71	8.0
				Small entire							1			39		40	4.4
				Inner bodies				2	2		5		1	16		26	3.0
				Multipl gr.				1	3		1			12		17	4.2
	—	+	—	Ring form			26	3	5					1		35	4.0
				Large entire			4	4	5					54	2	69	7.6
				Small entire	1	4	54	1	6		1			38	2	107	12.0
				Inner bodies	7	2	254	11	49		23			15	15	376	41.8
				Multipl. gr.	6	16	140	10	34		39			10	15	270	30.0
	+	—	+	Whole glyc.										6		6	0.7
				Dodies										1		1	0.1
	+	—	—	Whole glyc.	408	18	17	1864	145		651		45	79	387	3614	402.0
				bodies	60	10	4	8	51		38		8	1	10	190	21.0
	—	—	+	Large vac.	4		52	1	3		2		3	104	3	172	19.1
				Small vac.	8	1	10		4		10		0	11	4	48	5.3
	—	—	—	Free glyc.	13220	12027	25422	22457	28737		13289		12497	17408	16800	161857	17984

sive cases there occurred 548/1 section of the adrenal (M 14♀) or 478/1 section (F4 ☿) at maximum and in the slight cases 4-5/1 section or only 1/1 section (M1 ♂) at the mininum. On the average there occurred 67/1 section in sows and 113/1 section in hogs. These types had a tendency to have no glycogen in the cytoplasms. Almost all of type c did not appear, but only one (B 26 ☿) of 20 cases existed. In a word the cells contained the vacuolized giant nuclei had no glycogen in the cytoplasms. On the contrary in type a occurred the glycogen-laden cytoplasms and nuclei, was found in the six cases of 11 sows (54%) and in the five cases of nine hogs (55%). It is of interest to find that M-3 (☿) seemed to be a resting type which occurred in only type a.

Type a consisted of a small amount of large and small entire bodies and internal bodies, but type b contained a large amount of internal bodies and multiple granular bodies and a small amount of large and small entire bodies. It mignt be related to various phases in the carbohydrate metabolism.

Intracytoplasmic glycogen deposition was divided into the following two forms: the cells contained a large amount of glycogen in the whole cytoplasm, called the entire glycogen; the cells contained a small amount of glycogen in the locus near the nuclear membrane as the form of granular aggregates or large globule (it mignt be related to GOLGI apparatus), called the bodies. Diffuse form stained lightly with PAS or occurred as a very minute granules, was omited from the cell-count. The adrenals contained entire glycogen(E 24♀, F 5 ☿, E 28 ☿, M 2 ♀ and B 6 ☿) had a few glycogen-bodies, but the adrenals contained a large amount of glycogen-bodies (E 22 ♀ and B 28 ☿) and had a few entire glycogen. However the intermediate forms contained both entire glycogen and glycogen-bodies, and occupied a large part of the adrenals.

In the cells occurred the vaccuolized giant cells which were indicated as the large vacuole. Some cases (M 14 ♀, F 4☿ and B 26☿) contained a large amount of these cells, but the other cases only a small amount of them. In the cases in which there occurred a large amount of the vacuolized giant nuclei, there was found the severe occurrence of the intranuclear glycogen deposition. Generally, the vacuolized giant nuclei were indicated in the 63 per cent of 11 sows and in the 100 per cent of nine hogs, but the intensity of the degree was parallel to the occurrence of the intranuclear glycogen.

The degree of occurrence of Type a, b, c, d and e to type f (normal cells contained on glycogen) is shown in Table 6. In short, there were found at the average in one section as follows: nuclear glycogen 67, cytoplasmic glycogen 200, vacuolized giant nuclei 10 and glycogen-free nuclei and cytoplasms 12230 in the adrenal of the sow; nuclear glycogen 113, cytoplasmic glycogen 424, vacuolized giant nuclei 24 and glycogen-free nuclei and cytoplasms 17540 in the adrenal of the hog. From this results there might have occurred more intensive glycogen in the cells of the sows than of the hogs.

3. The relationships between the fat-stored cells and glycogen-laden cells in zona reticularis.

With regards to the histochemistry on the corticosteroids 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 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 1953) (10), pseudo-plasmal substance (CHU 1950) (11), peroxide (CAIN 1949) (12), unsaturated fatty acids (BAYLEY 1945) (21), enzyme (GOMORI 1950) (13), unknown unsaponified substance (UI 1957) (14) and cholesterol (SCHULTZ 1924) (15).

MAYEDA (1962) (20) described on some problems in the corticosteroids staining of adrenars as follows: it is indirectly possible to presume the presence of steroids in the sudanophilic granules by means of experiments. According to YOSHIMURA (1962) (21) 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. NAKAO (1962) (19) found a new method which be called $SbCl_3$-reaction to demonstrate corticosteroid, and he stated the antimony-granules to be reactive substance in the sections. However, there remained some doubt in this histochemical regions. However, owing to the correspondence to the sudanophilic part and $SbCl_3$-reactive part, there were considered sudanophilic cells which contained the ketosteroid, and indicated the significance and localization of the fat-stored cells shown in this report. In future the relationships between the sudanophilic granules, $SbCl_3$-granules, and glycogen-laden nuclei and glycogen bearing cytoplasms, may be discussed in other papers.

In the present investigation the fat-stored cells are shown in the tables 7, 8 and 9). The following opinions are summarized from these tables:

a) When the cells of zona reticularis contained a large amount of glycogen laden nuclei and cytoplasms, this zone tended to enlarge by means of the increase of cells.

b) There were divided into two types of rich fat-stored cells and poor fat-stored cells in the zona reticularis with no enlargement.

c) The ratio of nuclear glycogen to cytoplasmic glycogen was various, but they were divided into three types such as 1:1 (0.03–1), 1:50 (2–49) and 1:100 (53–93). In the zona reticularis of the ratio 1:1, the cell number of glycogen-laden nuclei was more abundant than that of glycogen-stored cytoplasms. In the ratio of 1:100, the cell number of glycogen-laden nuclei was

Table 7. The fat-stored cells, glycogen-laden cells and enlargement of zona reticularis of the hogs hrom the points of cell-counts.

Types	Fat +	glycogen +		glycogen −			Medium Protein-High Energic Feeding				Medium Protein Medium Energic Feeding					Low Protein-Medium Energic Feeding		Total (average)
							E		F		AC		M			B		
	Cytopl.	Cytopl.	nuclpl.	Cytopl.	Normal nucl.	vacuol nucl.	28	29	4	5	12	23	13	15	3	26	6	
a	+	+	+	—	—	—										57		
b	+	—	+	+	—	—		2										
c	+	+	—	—	—	+												31092 (3452)
d	+	—	—	+	—	+										34		
e	+	+	—	—	+	—	408	894	21	1864	22	Missing	157	Missing	45	1640	445	
f	+	—	—	+	+	—	612	4100	7136	4161	152		2719		2827	3600	196	
g	—	+	+	—	—	—				4	8		8			140		
h	—	—	+	+	—	—	14	22	478	29	99		63		1	118	34	
i	—	+	—	—	—	+										7		142543 (15838)
j	—	—	—	+	—	+	12	1	62	1	7		12		3	115	7	
k	—	+	—	—	+	—	468	28	21	1872	196		689		53	80	397	
l	—	—	—	+	+	—	13220	12027	25422	22457	28737		13289		12497	17408	16800	

Table 8. The fat-stored cells, glycogen-laden cells and enlargement of zona reticularis of the sows from the points of cell-counts.

Types	Fat +	glycogen +		glycogen −			Medium protein-High energic feeding				Medium protein-Medium energic feeding					Low protein Med. energic feeding		Total (average)
							E		F		AC		M			B		
	Cytol.	Cytol.	Nucl.	Cytol.	Nonmal nucl.	Vacuol. nucl.	22	24	11	27	21	7	2	14	25	1	8	
a	+	+	+	—	—	—							1	15	3			
b	+	—	+	+	—	—										6		
c	+	+	—	—	—	+												30427 (2766)
d	+	—	—	+	—	+									159			
e	+	+	—	—	+	—	931	785	5	60	9	51	5	91	11	969	225	
f	+	—	—	+	+	—	7920	3801	4365	2349	7910	6605	2265	95	147	857	796	
g	—	+	+	—	—	—		1		1			1	6	7	19		
h	—	—	+	+	—	—	5	9	14	3	35	12	3	542	26	52	6	
i	—	+	—	—	—	+												128581 (11689)
j	—	—	—	+	—	+			6		2		14	47	16	16	4	
k	—	+	—	—	+	—	467	792	65	76	84	97	134	63	72	69	225	
l	—	—	—	+	+	—	6709	4935	8325	10179	8930	19267	6762	32770	17200	10800	8680	

Table 9. Relationships between glycogen- and fat- metabolism in the cells of zona reticularis of swine adrenals

Sex	Feeding / Groups / Name / Changes	Medium Protein-High Energic Feeding				Medium Protein-Medium Energic Feeding					Low Protein Med. Energic Feeding	
	Groups	E		F		AC		M			B	
	Name	22	24	11	27	21	7	2	14	25	1	8
♀	Nuclear glycogen	5	10	14	4	35	12	4	548	33	71	6
	Cytoplasmic glyc ogen.	467	792	65	76	84	92	134	63	72	69	225
	Vacuol. giant nuc lens.			6		2		14	47	16	16	4
	Free glyc. in nuc. and cyttpl.	6709	4935	8325	10179	8930	19267	6762	32770	17200	10800	8680
	Fat-stored cells	8851	4586	4370	2409	7919	6656	2271	201	321	1832	1021
	Total cells in z. retc.	16032	10323	12780	12668	16960	26028	9185	33629	17642	12788	9936
	Enlargement of z. retc.	◎	○	○	○	◎	⊚	○	⊚	◎	○	○
	Nucl.-Glyc. : Cyt-Glyc	1 : 93	1 : 79	1 : 5	1 : 19	1 : 2	1 : 8	1 : 34	1 : 0.1	1 : 2	1 : 1	1 : 38
	Nucl.-Glyc. : Free Glyc. Vac.	1 : 0	1 : 0	1 : 0.4	1 : 0	1 : 0.1	1 : 0	1 : 4	1 : 0.1	1 : 0.5	1 : 0.2	1 : 0.7
	Nucl. Cyt. Glc. : Free Glyc.	1 : 14	1 : 6	1 : 105	1 : 127	1 : 75	1 : 183	1 : 50	1 : 53	1 : 164	1 : 78	1 : 37
	Fat stored cells	8851	4586	4730	2409	7919	6656	2271	201	321	1832	1021
	Free Fat Stor. Cells	7181	5737	8410	10259	9051	19371	6914	33428	17321	10956	8915
	Fat Cells : Free Fat Cells	1 : 0.8	1 : 1.2	1 : 2	1 : 4	1 : 1.1	1 : 3	1 : 3	1 : 166	1 : 54	1 : 6	1 : 8
	Appearance of fat strage	⊚	⊚	⊚	⊚	⊚	⊚	⊚	○	○	⊚	⊚
Sex	Name / Changer	28	29	4	5	12	23	13	15	3	26	6
♂	Nuclear glycogen	14	22	478	33	107	Missing	71	Missing	1	258	34
	Cytoplasm. glycogen	468	28	21	1872	196		689		53	87	397
	Vacuol. giant cells	12	1	62	1	7		12		3	115	7
	Eree glycogen in nuc. cytpl.	13220	12027	25922	22457	28737		13289		12497	17408	16800
	Fat. stored cells	1020	4996	7157	6025	174		2876		2872	5331	641
	Total cells in z. retic.	14734	17074	33140	38388	29221		16937		15426	23199	17879
	Enlargement of z. ret.	◎	◎	⊚	⊚	⊚		◎		◎	⊚	◎
	Nucl.-glyc. : cyto. glyc.	1 : 33	1 : 1	1 : 0.04	1 : 57	1 : 2		1 : 10		1 : 53	1 : 0.3	1 : 12
	Nucl.-glyc. : free glyc.	1 : 1	1 : 0.05	1 : 0.1	1 : 0.03	1 : 0.07		1 : 0.2		1 : 3	1 : 0.4	1 : 0.2
	Nucl.-cyt. glc. : Free glyc.	1 : 27	1 : 40	1 : 51	1 : 12	1 : 94		1 : 17		1 : 230	1 : 52	1 : 39
	Fat stored cells	1020	4996	7157	6025	174		2876		2872	5331	641
	Free fat stored cells	13714	12078	25983	24363	29047		14061		12554	17868	17238
	Fat cells : free fat cells	1 : 13	1 : 2	1 : 3	1 : 4	1 : 170		1 : 5		1 : 4	1 : 3	1 : 27
	Appearance of fat storage	◎	⊚	⊚	⊚	○		⊚		⊚	⊚	◎

Remarks : Nucl. indicated Nucleus or nuclear ; cytpl. or cyt, cytoplasma or cytoplasmic ; vac. or vacuol., vacuolized ; z. retc., zona reticularis ; glyc, glycogen ; ⊚ high or strong degree ; ◎ medium degree ; ○, low or weak degreee.

more scanty than that of glycogen-contained cytoplasms.

d) The ratio of the nuclei with glycogen to the vacuolized nuclei without glycogen was various, but there were divided into three types such as 1:0 (0~0.03~0.7), 1:1 and 1:4 (3-4). In the ratio of 1:0 were indicated no appearance of vacuolized giant nuclei without glycogen, and the ratio of 1:4 showed a small amount of nuclear glycogen in comparison with a large amount of cytoplasmic glycogen.

e) The ratio of glycogen in the cells (nuclei and cytoplasms) to no glycogen in the cells was various, but they were divided into three types such as 1:20 (6-17); 1:50 (21-94) and 1:100-200 (105-240). In the ratio of 1:20 were indicated the appearance of a large amount of nuclear and cytoplasmic glycogen in comparison with the whole cells.

f) The adrenals contained a large amount of the fat-stored cells which tended to have a few glycogen-bearing cells, and on the contrary the ones contained a few fat-stored cells tended to have a large amount of glycogen-bearing cells.

From these result it is important to find that the relation between nuclear and cytoplasmic glycogen was close and also the relation between cytoplasmic glycogen and fat-storage existed.

In coparison with the cell counts of the fat-stored cells and the free fat-stored cells, the difference was as shown in the following Table 7, 8 and 9. These ratio was various as 1:0.8~1:166 (average 1:23) in the sows, but it was 1:2~1:170 (average 1:14) in the hogs. Accordingly the hogs might contain more plentiflul fat-stored cells than the hogs. There existed at the average value of 2766 of the fat-stored cells and that of 11689 of the free fat-stored cells in the zona reticularis of the sows; and 3452 of the fat-stored cells and 15838 of the free fat-stored cells in the hogs.

The combination of the fat-stored cells, glycogen in the cytoplasms and nuclei, and vacuolized giant nuclei are shown in Tables 8 and 9 and the cells in the zona reticularis were divided into 12 types as Table 10:

Table 10. Various types of the cells in the zona reticularis of the sows and hogs

Type	Cytoplasmic Fat	Cytoplasmic Glycogen	Nuclear glycogen	Free glycogen in cytoplasms	Normal nuclei	Vaccuolized giant nuclei
a	+	+	+	−	−	−
b	+	−	+	+	−	−
c	+	+	−	−	−	+
d	+	−	−	+	−	+
e	+	+	−	−	+	−
f	+	−	−	+	+	−
g	−	+	+	−	−	−
h	−	−	+	+	−	−
i	−	+	−	−	−	+
j	−	−	−	+	−	+
k	−	+	−	−	+	−
l	−	−	−	+	+	−

In the sows there were found a large amount of type l and f, and secondly abundant in types e and k; but in the hogs a large amount of types e, f and k, and next abundant in types h and g. Accordingly the function in the hogs might be accelerated in comparison with that in sows.

Summary and Discussion

Physio-histological studies on the mechanism of adipositas and the effect of fattening has been investigated histochemically on the various organs of Yorkshire pigs used for experiment of the feeding standard on the meat pigs. 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.

The present study described the frequency of occurrence of glycogen-bearing cytoplasms and nuclei, their shape and form, and the relationship between the glycogen-laden cells and fat stored cells. The results investigated are summerized as follows:

1) It was noticed that a large amount of the nuclei and cytoplasms of the swine adrenals showed a strong positive reaction to PAS-stain. This reaction became negative in the sections previously treated with saliva digestion. Accordingly this indicated that the PAS-positive substance in the nuclei and cytoplasms was glycogen. Eleven sows and nine hogs contained the glycogen-laden nuclei of the cells in the zona reticularis in the 100 per cent. In the animals which contained remarkable large amounts of the glycogen-laden nuclei there were found also in the zona fasciculata 18 percent of the sows and 22 percent of the hogs and none in the zona glomerulosa.

2) The appearance of intranuclear glycogen seemed to be not related to the feeding, but it was clear that it is related to the sex. The intranuclear glycogen tended to appear more intensive in the hogs than in the sows.

3) The nuclei contained glycogen genarally were giant in size, but some glycogen-laden nuclei were small. Usually the size of the normal nuclei of the cells in the zona retirularis was $5.3 \times 5.3\mu$ in diameter, but that of glycogen-laden nuclei arranged in the range of $8.0 \times 8.0\mu$ to $19.0 \times 19.0\mu$ in diameter as the giant form. At first there occurred glycogen like form of granules or bedies in the normal-sized nuclei, and then gradually they developed to large nuclear bodies increasing the storage of glycogen. Afterwards there appeared the internal bodies, funnel like bodies and ring form of glycogen in the vacuolized giant nuclei. At last the vacuolized giant nuclei without glycogen appeared by the decrease of glycogen.

4) The modes of the intranuclear glycogen deposition were various as follows: glycogen occupied in the whole large or small nuclei (large entire bodies or small entire bodies), the glycogen attached to the nuclear membrane

and the internal space was empty (ring form), round internal bodies in the vacuolized nuclei (internal bodies), the flask-like bodies, club-like bodies, dumb-bell-like bodies or funnel-like bodies attached to the one part of nuclear menbrane within the vacuolized giant nuclei (one kind of internal bodies), several granules or rods contained glycogen near the chromatin dispersed within the nuclei (multiple granular form), hyperchromic or hypochromic glycogen-laden nuclei by the stainability of PAS-stain.

The vacuolized nuclei with free glycogen divided to giant nuclei and small nuclei. It was possible to presume that the vacuolized nuclei lost glycogen from the ring form.

5) The modus of the intracytoplasmic glycogen were divided into three types as follows: a large amount of glycogen in the entire cytoplasms (entire glycogenic form), glycogen deposition formed the round solitary islet near the nuclear membrane (glycogenic bodies), and a small quantity of glycogen in the cytoplasms (diffuse form).

6) On an average in the section of one zena reticularis of the sows, 67 of intranuclear glycogen deposition, 200 of intracytoplasmic glycogen deposition, 10 of vacuolized glycogen-free giant nuclei, 12230 of no glycogen deposition in both nuclei and cytoplasms were found. On the contrary, in the hogs, 113 of intranuclear glycogen, 424 of intracytoplasmic glycogen, 24 of vacuolized nuclei, and 17540 of free glycogen in both nuclei and cytoplasms were existed. From these results the zona reticularis of the hogs seemed to contain more plentiful glycogen than that of the sows.

7) Sudanophilic granules existed in the cytoplasms of zona reticularis of adrenals. The authors desired to 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. Accordingly in the present investigation the numbers of the fat-stored cells were calculated, but this problem will be diversed in another paper. On the average, in one section of adrenal of the sow there were existed 2766 of the fat-stored cells and 1538 of the free fat-stored cells. From this point the zona reticularis of the hogs seemed to contain fat-storage more plentiful than that of the sows.

8) From the points of the numbers of cells in the whole zona reticularis, the enlarged zone contained a large amount of cell-numbers with a large quantity of glycogen-laden nuclei and cytoplasms. The non-enlarged zone was divided into two types such as a rich type and a poor type of the fat-stored cells. The rich type of the fat-stored cells tended to have a few glycogen-laden cells, and in opposition the poor typs of fat-stored cells tended to have many glycogen-laden cells.

In short, it is important to observe that the intranuclear glycogen related to the cytoplasmic glycogen, and the cellular glycogen (in both nuclei and

Table 11. Relationships between cellular glycogen and fats in the zona reticularis of the pigs

Cytoplasms			Nuclei	Vacuol-ized giant nuclei	Medium Protein High Energic Feeding				Medium Protein Med. Energic Feeding					Low Protein Medium Energic	
sex	Fats	Glyco-gen	Glyco-gen		E		F		AC		M			B	
					22	24	11	27	21	7	2	14	25	1	8
Sows		+	+	−	−	○	−	○	−	−	○	◎	○	◎	−
		−	+	−	○	○	○	○	◎	○	○	⊚	◎	◎	○
		+	−	+	−	−	−	−	−	−	−	−	−	−	−
		+	−	−	⊚	⊚	◎	◎	◎	◎	⊚	◎	◎	◎	⊚
		−	−	+	−	−	○	−	○	−	○	◎	○	○	○
		−	−	−	◎	○	○	○	◎	⊚	○	⊚	◎	○	○
	+				⊚	⊚	⊚	⊚	⊚	⊚	⊚	○	○	◎	◎
					28	29	4	5	12	23	13	15	3	26	6
Hogs		+	+	−	−	−	−	◎	◎	Missing	◎	Missing	○	◎	−
		−	+	−	○	○	⊚	◎	◎		◎		−	◎	○
		+	−	+	−	−	−	−	−		−		−	○	−
		+	−	−	⊚	○	○	⊚	⊚		⊚		◎	◎	⊚
		−	−	+	○	○	◎	○	○		○		○	⊚	○
		−	−	−	○	○	⊚	⊚	⊚		○		○	◎	◎
	+				◎	⊚	⊚	⊚	○		⊚		⊚	⊚	◎

Remarks, ○ 1—20, ◎ 21—100, ⊚, 101—200—400 of cells.

cytoplasms) were related to the fat-stored cells in inverse proportion.

As the recent biochemical theories of the regulation of cellular reaction by the hormone owing to SUTHERLAND (1960), WILLAMS (1962) and KONDO (1963) have published, they state that the production of ketosteroid and glycogenolysis in the adrenals have been becoming the interesting problems to study the mechanism of fattening or physiological obesity.

WILLIAMS (1962) described that the recent investigations have helped the mechanisms involved in the synthesis and release of corticosteroid as follows: relatively high concentration of glycogen in the zona fasciculata and zona reticularis, plentiful presence of glucose-6-P dehydrogenase in the zona fasciculata and zona reticularis, and the others. The following sequence of events seemed significant in synthesis and secretion of corticosteroids from the adrenal. ACTH stimulated the formation of cyclic adenylic acid, which, with various co-factors, led to the activation of phosphorylase. Phosphorylase accelerated glycogenelysis, 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 was increased, with the result that TPNH (same as $NADH_2$) was produced. This co-factor accelerated splitting of the cholesterol side-chain and the 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 an excess deposit of fat, particularly with the excess food ingestion. Insulin produced an increase in lipids in each and an enlargement of each. Insulin was known to increase the output of glucosteroids, which in turn played an important role in stimulating the increased production of insulin antagonists. Obesity tended to produce hyperinsulinism and hyperadrenocorticism, which can in turn significantly further the obesity.

Our present report described the facts that played a role of understanding morphologically for biochemical studies on the mechanisms of regulation in the cellular reaction by the hormone as the above mentioned.

The relationships between glycogen in the cytoplasms and nuclei and fat in the cytoplasms, in the cells of zona reticularis of the pigs are shown in Table 11.

In the cases of 11 sows and 9 hogs there were observed the presence of glycogen in the cytoplasms and nuclei, glycogenolysis to glucose-6-phosphate, and the increase of fat related to the ketosteroids, so it seemed to be closely related to the physiological obesity. The production of insulin was accelerated by the production of ketosteroid. If the obesity would develope by the hyperinsulinism and hyperadrenocorticism, the glycogen deposition in the cytoplasms and nuclei might play an important role to hyperadrenocorticism. In this meaning, it seemed to be important to have the glycogen-deposition in the cytoplasms and nuclei of the cells in the zona reticularis of the swine adrenals. Moreover it is very important to solve histochemically the problems of co-factor and corticosteroid, and this way become the subject for future investigation.

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Plate 1

Explanation of Figures

Fig. 1. Small entire bodies of glycogen-laden nuclei, and vacuolized giant nuclei with the intracytoplasmic glycogen Hematoxylin stain, ×400.
Small entire bodies (mark : s), vacuolized giant nuclei (v), vacuolized giant nuclei with the intracytoplamic glycogen (v-w), and fat-stored cells (f).

Fig. 2. Fat-stored cells in the wide regions of zona reticularis. Hematoxylin-eosin stain, ×400.
Fat stored cells(f) with a large amount of fat and no glycogen.

Fig. 3. Glycogen-laden body in the cptoplasms of the cells of the zona reticularis. Hematoxylin-eosin stain, ×400.
Glycogenic bodies were similar to GOLGI's apparatus(B).

Fig. 4. Intracytoplasmic glycogen-laden cells and fat-stored cells. Hematoxylin-eosin, ×400.
Intracytoplasmic glycogen-laden cells (G) with no glycogen in the nuclei.

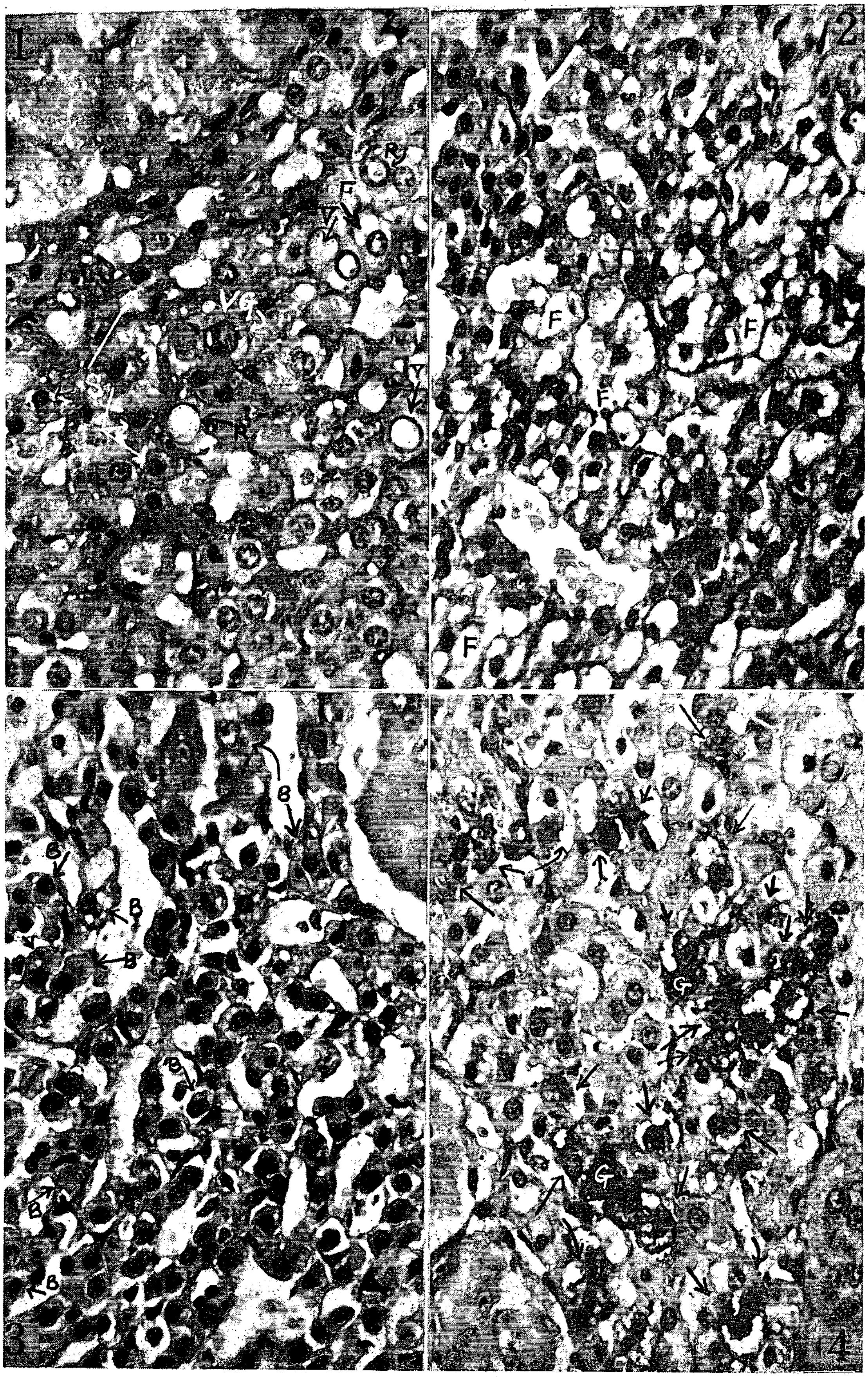

1
2
3
4

Plate 2

Explanation of Figures

Fig. 5. Inner bodies of the glycogen-laden nuclei. Hematoxylin-eosin, stain, ×400.
The inner bodies (I), multiple granules (MG), small entire bodies (S) in the glycogen-laden nuclei. The cells with the inner bodies of nuclei, contained the glycogen-laden cytoplasms.

Fig. 6. Multiple granules of the glycogen-laden nuclei. Hematoxylin-eosin stain, ×400.
Multiple granules of the glycogen-laden nuclei (MG) appeared in large amount.

Fig. 7. Cytoplasmic glycogen depositlon. Hematoxyline-eosen strin, ×40.
The glycogen deposition in the cytoplasms. These glycogen-bealing cells(G) are indicated by the arrow.

Fig. 8. Cytoplasmic glycogdn deposition in the zona reticularis. Hematoxylin eosin, ×40.
The glycogen deposition (G) in the cytoplasm and inner bodies of glycogen-laden nuclei (I), fat-stored cells with the cytoplasmic glycogen (FG), glycogen deposition in both nuclei and cytoplasm (NCG), fat-strored cells (F).

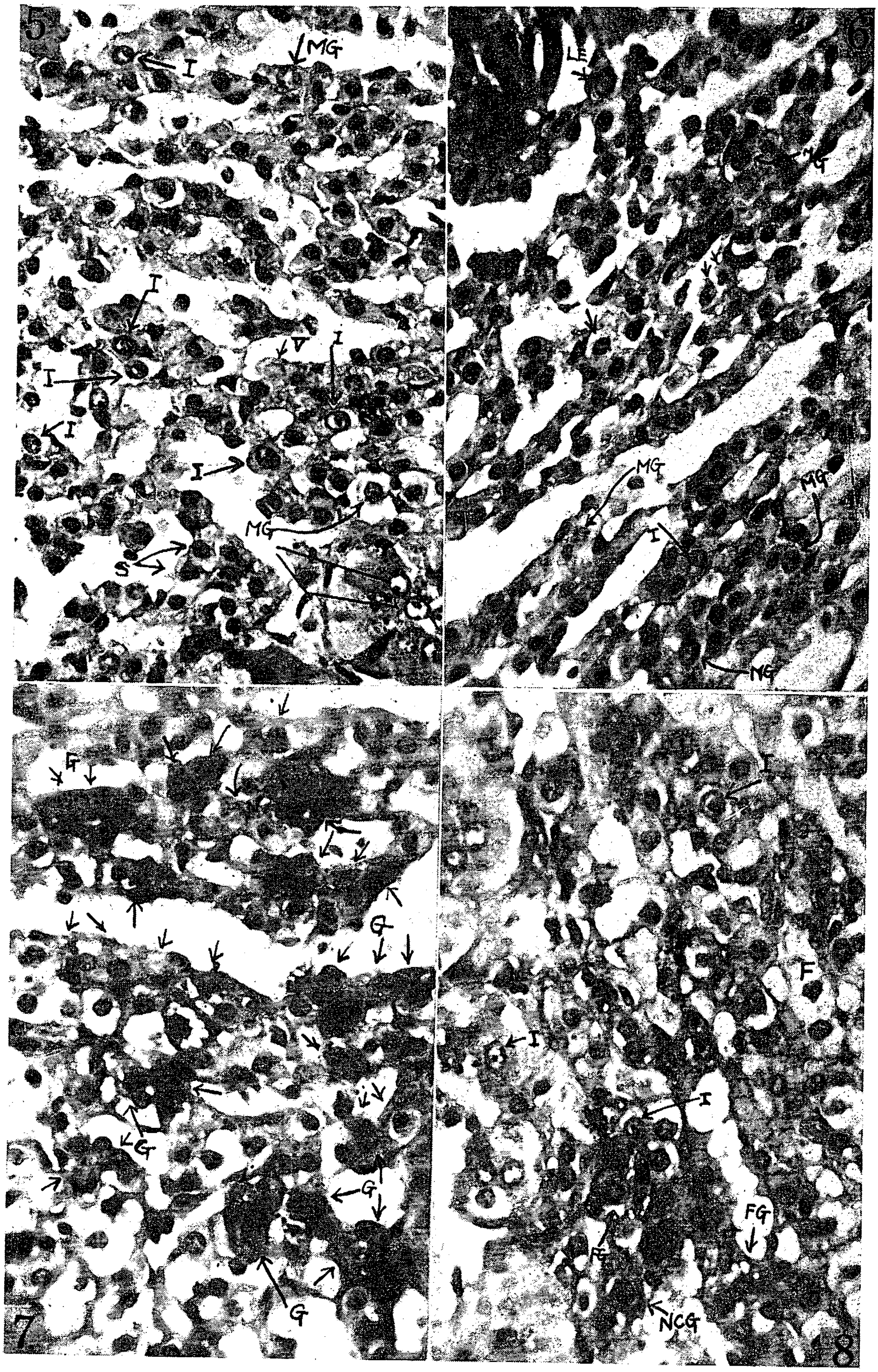

5
MG
I
S
6
LE
MG
I
7
G
8
I
F
FG
NCG

Plate 3

Explanation of Figures

Fig. 9. Small entire bodies or small vacuolized nuclei. PAS-Hemat oxylin-stain ×900.
Small entire bodies with glycogen(S), small vacuolized nuclei and intracytoplasmic glycogen(SV-G), and multiple granular forms with nuclear glycogen(NG).

Fig. 10. Multiple granular forms with nuclear glycogen. PAS-Hematoxylin-stain, ×900.
This type of intranuclear glycogen is rodlike(R). Some cells contained glycogen in both nuclei and cytoplasm(NC).

Fig. 11. Multiple granular forms with nuclear glycogen. PAS-hematoxylin stain, ×900.
Multiple granular forms(MG) with nuclear glycogen were various. Some nuclei consisted of granular glycogen(MGCH) near chromatin, and others formed irregular confluent masses with glycogen(MGC). The cells with these nuclei contained no glycogen in the cytoplasms.

Fig. 12. Multiple granular forms and small entire bodies with nuclear glycogen. PAS-hematixylin stain, ×900.
In the cells of multiple granular forms (MG) and small entire bodies(S) with nuclear glycogen.

Fig. 13. Ring forms with nuclear glycogen near the nuclear membrane. PAS-hematoxylin stain, ×900.
Glycogen attached around the nuclear membrane(R) like ring form, and the intranuclear spaces enlarged in the form of vacuole. Those cells contained fat. Some nuclei contained minute glycogen granules near the chromatin and formed vacuoles in the center. One of the ring form showed a flask-like process within the vauole from the membrane(F).

Fig. 14. Large entire bodies with band and vacuole (LE), and fat deposition in the cytoplasm(F).

Fig. 15. Inner bodies of nuclear glycogen with cytoplasmic glycogen. PAS-hematoxylin stain, ×900.
Three cells contained glycogen in both nuclei and cytoplasms, and formed inner bodies in the nuclei. Two cells also contained fats.

Fig. 16. Small entire bodies of nuclear glycogen. PAS-hematoxylin stain, ×900.
The small entire bodies (S), ring form (R), and the small entire bodies of nuclear glycogen with fat-storage(SF).

Fig. 17. Large entire bodies of nuclear glycogen and muluiple granular forms of nuclear glycogen. PAS-hematoxylin stain, ×900.
Large entire bodies (L) and multiple granules (MG) with nuclear glycogen which contained fat droplets.

Fsg. 18. Large entire bodies, ring form and flask-like process with ring form. PAS-Hematoxylin stain. ×900.
Large entire bodies (L), ring form (R) and flask-like process with ring form (FR).

Fig. 19. Large entire bodies, multiple granular forms and cytoplasmic bodies. PAS-Hematoxyin stain, ×900.
Large entire bodies(L), multiple granular form(MG) and intranuclear glycogen-cytoplasmic bodies(B).

Fig. 20. Large bodies in the cytoplasm and ring form of nuclei. PAS-Hematoxylin stain, ×900.
Large bodies in the cytoplasm (LB) and ring form of nuclear glycogen (R).

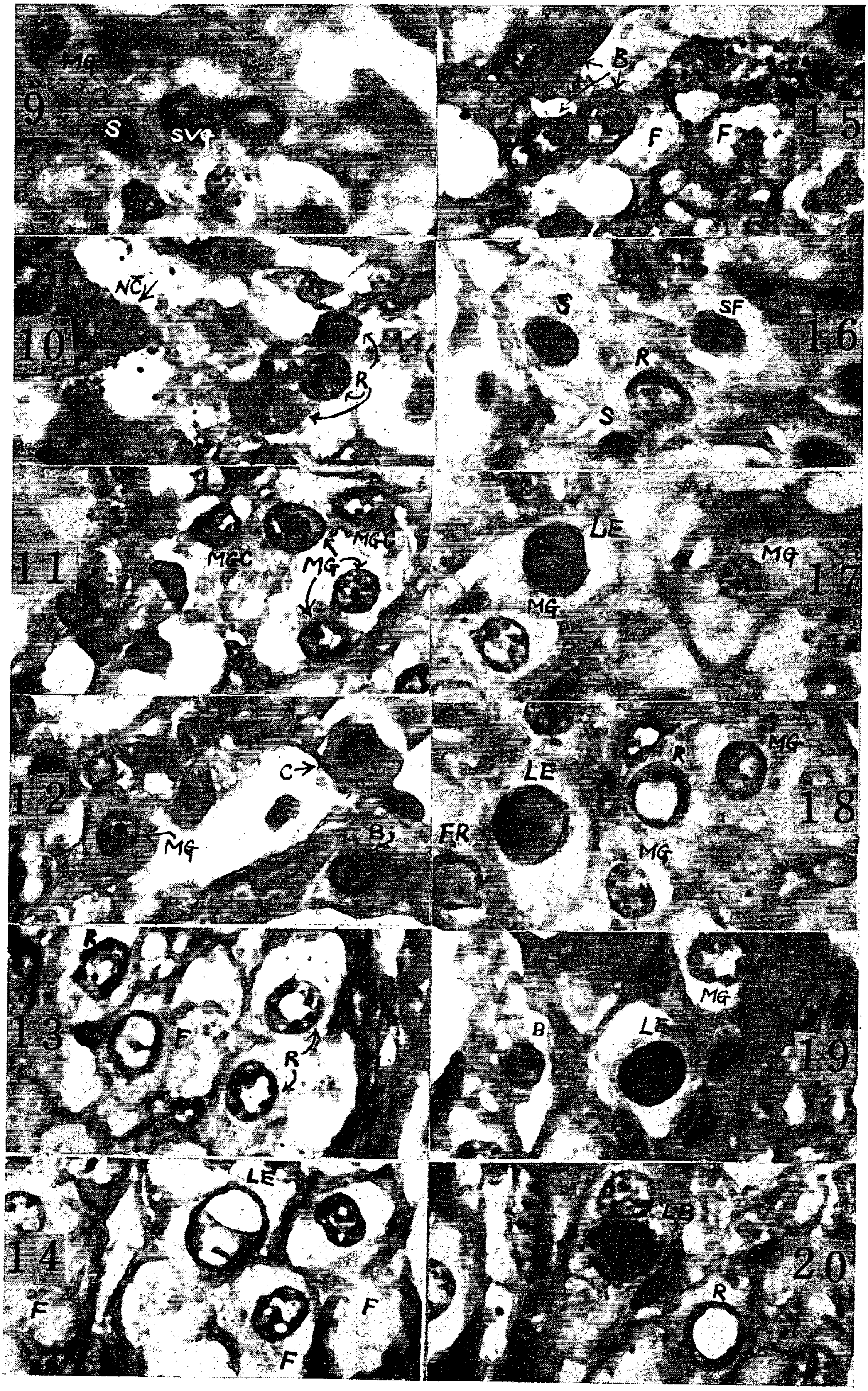

9
MG
S
SVG
15
B
F
F
10
NC
R
16
S
SF
R
S
11
MGC
MGC
MG
17
LE
MG
MG
12
C
MG
B
18
FR
LE
R
MG
MG
13
R
F
R
19
MG
B
LE
14
LE
F
F
F
20
LB
R

Plate 4

Explanation of Figures

Fig. 21. Intranuclear glycogen deposition and fat or glycogen-storage in the cells of zona reticularis. PAS-Hematoxylin stain, × 200.
Intranuclear glycogen deposition (shown by arrow), fat-storage (F) or glycogen-storage(G).

Fig. 22. Intranuclear glycogen deposition and fat-storage in the cells of zona reticularis. PAS-Hematoxylin-stain × 900.
Intranuclear glycogen deposition (NG), fat-storage (F) and fat-glycogen-storage with nuclear glycogen(FGN), and cellar bodies in the cytoplasm(B).

Fig. 23. Ring form of nuclear glycogen and fat-storage. PAS-Hematoxylin, × 900.
Ring form of nuclear glycogen (R) and fat-storage (F).

Fig. 24. Polysacharide-bodies in the cytoplasm of the fat-stored cells. PAS-Hematoxylin stain, × 900.
Polysaccharide-bodies (PB) in the cytoplasm of the fat-stored cells (F).

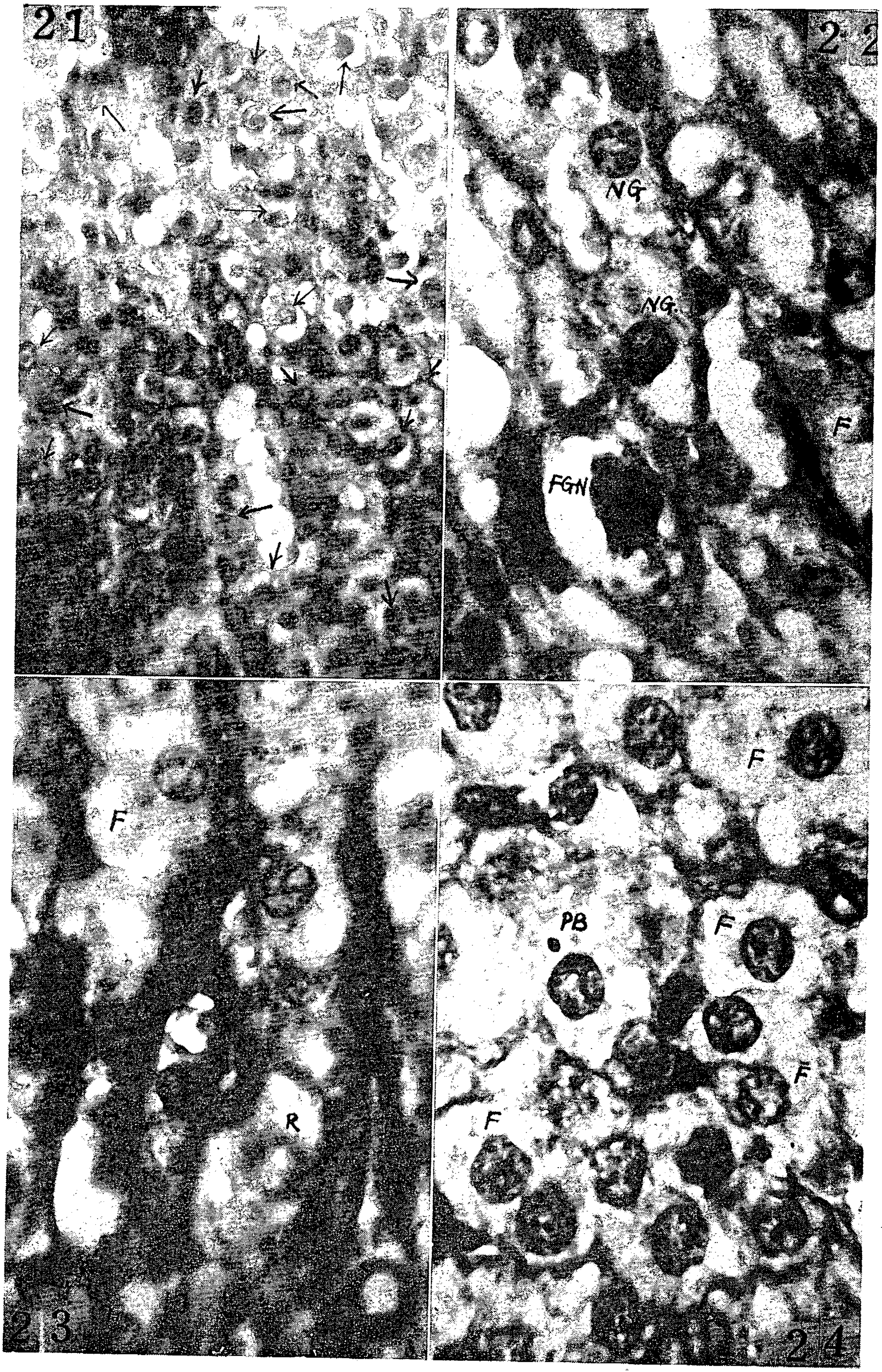

21
22
NG
NG.
F
FGN
F
R
23
F
PB
F
F
F
24

Plate 5

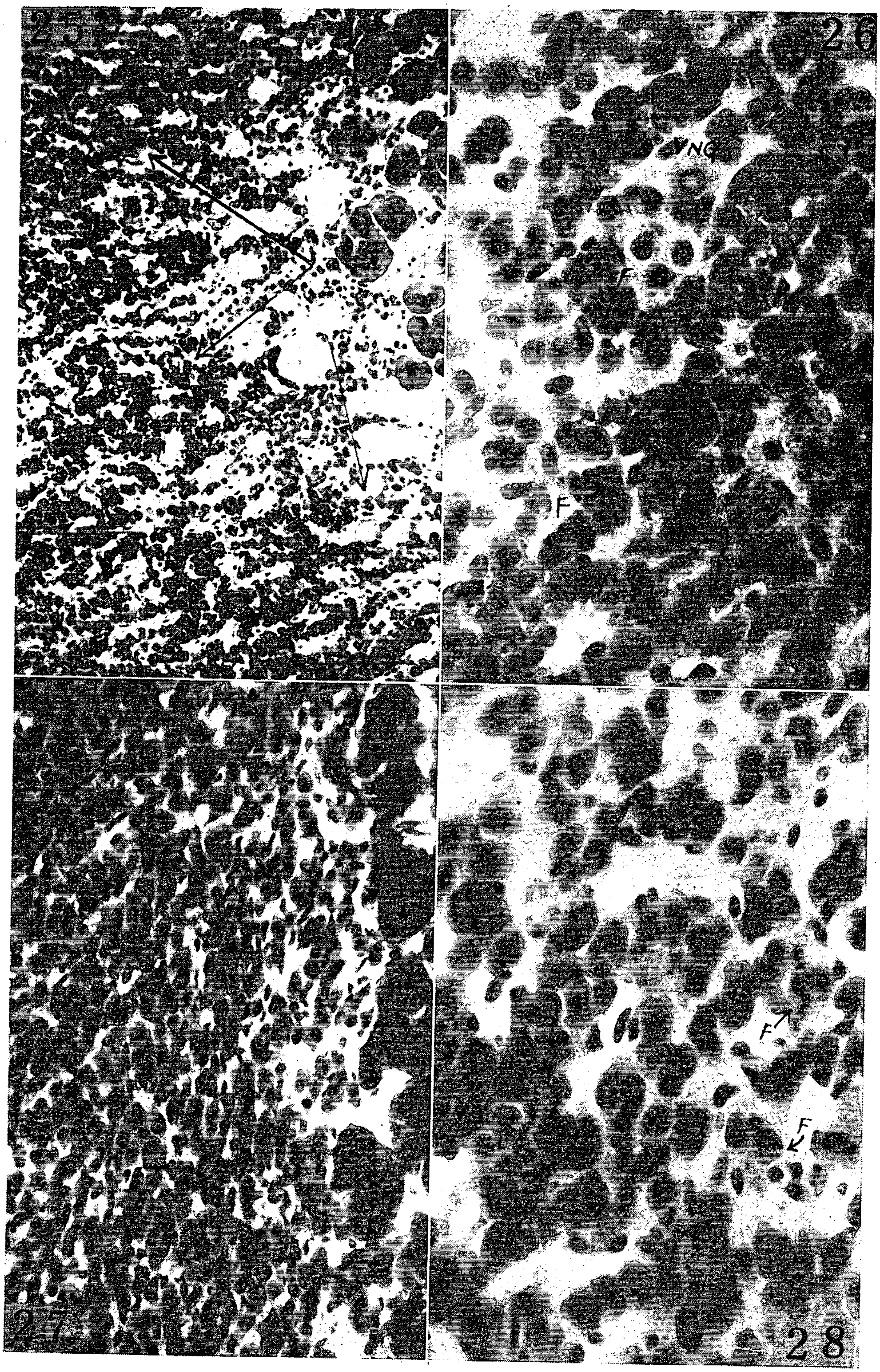
Explanation of Figures.

Fig. 25. A large amount of fat-stored cells in the zona reticularis and zona fasciculata. Sudan III-Hematoxylin stain, × 200.
Blackish cellular strangs with fat-stored cells.

Fig. 26. Fat-stored cells and vacuolized giant nuclei. Sudan III-Hematoxylin stain, × 300.
Fat-stored cells shown by arrow-mark and the vacuolized giant nuclei (V).

Fig. 27. A little quantity of fat-stored cells in the zona reticularis. Sudan III-Hematoxylin stain, × 200.
This microphoto showed no fat-stored cells.

Fig. 28. Free fat-stored cells in the zona reticularis. Sudan III-Hematoxylin stain, × 400.
A few cells contained a little quantity of fat granules in the cells.


26
NG
F
F
27
F
F
28