PHYSIO-HISTOLOGICAL STUDIES ON THE PHYSIOLOGICAL OBESITY OF THE MEAT PIGS (REPORT VI), ESPECIALLY ON THE MECHANISMS OF THE PANCREATIC FAT NECROSIS, FATTY ACID CRYSTALIZATION AND FOCAL PANCREAS NECROSIS

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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 the Pig-Feeding-Standard revising test in the case of pork production. In the previous reports (1, 2, 3, 4, 5, 6, 7 and 8), there were described the following studies: the occurrence of glycogen-bearing nuclei and cytoplasms in the swine adrenals; the relationships between glycogen deposition and fat storage in the swine adrenals; the comparison between the diabetic lesions and obesitic alteration; the diminution of the intranuclear and intracytoplasmic glycogen deposition in the adrenals of the pigs immunized with hog-cholera virus; the deposition of ketosteroid stained with $Sbcl_3$-reaction in the swine adrenals; and the distribution of succinic dehydrogenase, alkaline phosphatase and alkaline phosphatase on the course of corticosteroid production in the swine adrenals.

As the food intake increases, the β cells of the pancreas are stimulated, producing more insulin. This insulin is highly important to lipogenesis, and when it is present in excess it promotes an excess deposit of fat, particularly with excess food ingestion. Insulin, particularly in large quantities, is known to increase the output of glycosteroids, which in turn play an important role on stimulating the increased production of insulin antagonists. Obesity tend to produce hyperinsulinism and hyperadrenocorticism, which can produce in turn significantly further

obesity.

By the present study there was found physiologically the occurrence of the fat necrosis, fatty acid crystalization and focal necrosis in the pancreas of the meat-pigs. HAGAN (9), HOFLUND (10) and SMITH (11) have recognized the necrosis of fat in the fatty tissue of the gland, in the omentum, in the pancreas and in the mesenterium of the cattle. According to their opinions when the adipose tissue undergoes necrosis fat is not infrequently decomposed into its two constituent radicals, fatty acid and glycerine, and fatty acid then combines in various proportions with metalic ions such as sodium, potassium and calcium. The result is the formation of a soap within what is a fat cell, and the soap is not dissolved out, as fat is, by the fat solvents used in the sectioning technique. These facts are called necrosis of fat. The lesions of fat necrosis in the pancreas of the meat pigs the focal pancreatic necrosis and fatty acid crystalization.

According to HOFLUND (10) fat necrosis in cattle is characterised histologically by lipolytic processes with the precipitation of clacium salts of fatty acids, and the surrounding fibrocytic reaction; and with stasis in, or rupture of, the pancreatic duct; and with tranmatic injuries or acute inflammation of the pancreas; and with the release of pancreatic lipase and spreading via the lymphatics to the surrounding tissues. Several authors, such as BALSER (1882), OLT (1898), JOEST (1921), RICH-DUFF (1936) HAAS (1914), LIUM-HADDOCK (1938), NEAL (1946), HOWARD (1948), DRAGSTEDT-HAYMOND-ELLIS (1934) and POPPER-NECHELES (1949, 1951), have been able to produce experimentally fat necrosis through various surgical interferences with the pancreas and its duct. HOFLUND (10) described that fat necrosis in cattle is often found at ordinary slaughter as a secondary finding or at autopsy after other diseases. And he found no changes in the pancreas or its duct in cases of fat necrosis, and only in one case the changes were localized to the peripancreatic fat.

No one has found the pancreatic fat necrosis in the swine. The present authors have found fat necrosis in the meat pigs at ordinary slaughter, and have come to the conclusion that the pathogenesis in these animals might be brought about by the pancreatic necrosis by the release of pancreatic enzymes due to the destruction of the inspissated materials in the pancreatic ducts and fat embolus in the blood vessels of the interlobular interstitium.

In the future it might be that the increase of β cells of the pancreas will produce more insulin for lipogenesis.

Materials and Methods for Studies

The materials used for the studies were 11 sows and 11 hogs. Yorkshire pigs were used for the Pig-Feeding-Standard revising test in the case of pork production. They were administered with various feeds such as medium protein-high energic group (E and F) ,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 the previous report (2). All pigs showed by the increase of the body weight, rapid growth and good apetite. Autopsy was done at the age of 196 days after birth, and the body weight and feed intake according to sex and experimental periods are given in the previous report (2).

All work was done on the pancreas of 196 day old sows and hogs. Animals were killed by the complete (venesection) of the pancreas dissected out and immediately fixed in buffered formol or in CARNOY's fluid, enbedded in paraffine. The paraffinized tissues were sectioned at 6 micra. After fixation the tissues were cut at 15 micra with the freezing microtome for fat-staining. Deparaffinized sections were stained as follows: Haematoxylin-eosin stain and MARROLY's azan stain for general histology; PAS-hematoxylin stain with or without saliva digestion for glycogen and polysaccharide; DUIJN's acrolein-SCHIFF reaction for protein; FEULGEN's HCL-SCHIFF reaction for DNA; BRACHET's pyronine-methyl green stains with or without ribonuclease digestion for RNA, depolymerized DNA and high-polymer of DNA. The sections cut with the freezing microtome were stained as follows: OKAMOTO's procedures for fatty acids and ones with K, Na and Ca salt; Nile blue staining for neutral fat; SCHULTZ method for cholesterol; SMITH-DIETRICH's method for lipid; BAKER's method or Sudan Black B-stain for phospholipid; and Sudan III stains for general fats.

Results

1. Relationship between pancreatic necrosis and obstruction of blood vessels.

According to ANDERSON (12) acute pancreatitis is characterized by edema, necrosis, hemorrhage, and suppuration in varying degrees of predominance. The effects are due to the escape of active lytic pancreatic enzymes which act on the pancreatic parenchyma, blood vessels, and fatty tissue. This condition appears to be brought about by the increased pancreatic secretion, with partial or complete obstruction of outflow and raised intraductal pressure. As the histo-pathologic appearance, the tissue is edematous and infiltrated by erythrocytes but few leucocytes, and the blood vessels showed necrosis of their walls and thrombi in their lumina, and the pancreatic ducts were found with inspissated secretion in them. Chronic pancreatitis is manifested by perilobular and interacinar fibrosis. As the histo-pathologic appearance, the tissue indicated the following changes: the presence of a variable degree of acinar and islet atrophy; dilatation of acini with the appearance of a central space or channel which suggested some duct obstruction, as the presence of inspissated material in the lumina of the ducts; dilatation of the ducts, hyperplasia of the ducts, and the presence of lymphocytic infiltration; formation of calcium salts precipitated in the pancreatic ducts; and fibrosis of the pancreas with hemochromatosis.

Table 1. Pancreatic necrosis and obstruction of the inspissated

Lesions		Feeding		Medium proteinhigh energic group							
		Group		E				F			
		Animals		Sows		Hogs		Sows		Hogs	
		Human Pancreatitis									
		acute	chron.	22	24	28	29	11	27	4	5
Focal necrosis in Acinus	Karyorrhexis			+	+	+	+	+	+	++	+
	Karyopyknosis			+	+	+	+	+	+	++	+
	Kayolysis			+	+	+	+	++	+	++	+
Isolation of acinar cells		+		++	++	++	++	+	$+_s$	++	+
Homorrhage & hyperemia		+	−	−	−	−	−	−	−	−	−
Acinar and islet atrophy		−	+	−	−	−	−	−	−	−	−
Fat embolus		+	−	+	++	+	+	++	++	++	++
Necrosis of blood vessels		+	−	−	−	−	−	−	−	−	−
Organized thrombi, Arteritis obliterans		+	−	−	−	−	+	−	−	+	++
Pancreatic duct	Obstruction	−	+	++	$+_s$	+	+	+++	s +	++	+
	Inspissated material in	+	−	++	++	++	++	++	+	+++	+
	Hyperplasia	−	+	+	−	+	−	−	−	−	−
	Squamous metaplasia	+	−	−	−	−	−	−	−	−	−
Precipitation of calcium salt in duct		−	+	−	−	−	−	−	−	−	−
Perilobular fibrosis inter-acinar fibrosis		− −	+ +	+ +	+ +	− +	− +	− +	− +	− +	− +
Acinar dilation		−	+	−	−	−	−	−	−	−	−
Lymphocytic infiltration		−	+	−	−	−	+	+	−	−	−
Fat Necrosis				+++	+++	+++	+++	+++	+++	+++	+++

Remarks: +++, ++, +, + indicated the degree of intensity, and -negatively

Alsmot all of the acute and chronic pancreatitis, such as hemorrhage, hyperemia, acinar atrophy, precipitation of calcium salts in the ducts, acinar dilation, and metastasia of the duct epithelium with associated acinar dilation, were not found in the swine adipositas; however there commonly existed the alteration such as the presence of focal necrosis in the acinar necrotic area with karryopyknosis, karyorrhexis and karyolysis, isolation of the acinar cells, fat embolus, obstruction of the pancreatic ducts, presence of inspissated material in the duct lumina, necrosis

materials in the pancreatic ducts in the meat pigs.

Medium protein- medium energic group										Low protein medium energic gr.			
AC				M						B			
Sows		Hogs		Sows			Hogs			Sows		Hogs	
21	7	12	23	2	14	25	13	15	3	1	8	26	6
++	+++	++	+++	+	+	+	+++	+++	+	+	++	+	+
++	+++	++	+++	+	+	+	+++	+++	+	+	++	+	+
++	+++	++	+++	+	+	+	+++	+++	+	+	++	+	+
++	+++	++	+++	+	++	+	++	+++	+	+	++	+	+++
−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	++	+	++	−	+	−	−	−	−	−	+	++	+++
−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	+	−	−	−	−	−	−	−	−	−	−	−
+	++	+++	+++	+	+++	+	+++	+	++	++	+++	++	−
+++	+++	+++	+++	++	++	+++	+++	++	+++	+++	++	+++	+
−	+	+	−	+	−	−	−	−	+	−	−	−	−
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− +	+ +	− +	− +	+ +	+ +	+ +	− +	− +	− +	+ +	− +	− +	+ +
−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	++	−	−	−	−
+++	+++	+++	+++	+++	++	+++	++	+++		+++	+	++	+++

of the blood vessels, organized thrombi in the blood vessels, canaliculation in the organized blood vessels, perilobular and interacinar fibrosis and lymphocytic infiltration.

The results of the study on the relationships between pancreatic necrosis and obstruction of the blood vessels and pancreatic ducts in the meat pigs are shown in Table I.

Focal necrosis in the pancreatic acinar area is characterized histologically and

Table 2. Fat necrosis, fatty acid crystalization

	Feeding			Medium protein high energic group							
	Group			E				F			
	Sex			Sows		Hogs		Sows		Hogs	
	Lesions / Human Lesion		Animal number	22	24	28	29	11	27	4	5
Fat Necrosis & Fat Acini	Necrosis of acinus	Ac. +	chr. +	+	+	+	+	+	+	+	+
	Decomposition of zymogen granules			++	++	+	++	+	+	++	++
	Isolation of granular zymogen			++	++	+	+++	+	+	++	++
	Isolation of rod-like zymogen			++	++	+	+++	+	+	++	++
	Fatty cystformation			+++	++	++	++	+++	+	+++	+
	Fat deposition: acinar cells			+ ++	+ ++	− −	− +	− +	− +	− +	− +
	Fat deposition: Langerhans islet			−	+	−	−	−	−	−	−
Fatty Acid Crystalization	Needle-like crystalization	+	+	+++	+++	+++	+++	++	++	++	+++
	Fatty acid	+	−	+++	+++	+++	+++	++	++	++	+++
	General fat by sudan III			+++	+++	++	+	++	++	+	+++
	General fat by sudan black B			+++	+++	+	+	++	+++	+	+
	Neutral fat by nile blue			+++	+++	++	+	+	+++	+	+
	Polysaccharide (by PAS)			++	++	+	+++	++	+	++	++
	Protein (by acrolein SCHIFF reaction)			++	++	+	+++	+	++	+	+
	DNA (by FEULGEN reaction)			−	−	+	−	++	−	−	−
	Symphathetic ganglion & nerve			+++	+++	+++	+++	+++	+++	+++	+++

Remarks: +++, ++, +, +, indicate the degree of intensity, and - negatively

histochemically by the karyolytic processes with nuclear fragmentation and depolymerization of DNA, aggregation of zymogen granules, and the decrease of protein in the cytoplasms. On the other hand, it is common to find the isolation of

and sympathetic nerves in the swine pancreas

Medium protein medium energic group										Low protein medium energic			
AC				M						B			
Sows		Hogs		Sows			Hogs			Sows		Hogs	
21	7	12	23	2	14	25	13	15	3	1	8	26	6
+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	++++	+	++++	+	+	+	++++	++	+	++	++++	+	+
+	++++	+	++++	+	+	+	++++	++	+	++++	++++	−	+
+	++++	+	++++	+	+	+	++++	++	+	++++	++++	−	+
++++	++	++	+	+	+	++	++	++++	++++	+	++	+	++++
− ++	++ ++++	+ ++	++ ++++	− −	− −	+ +	++ ++++	− ++	− ++	− −	++ ++++	− ++	− +
−	++	++	++	−	−	+	++++	−	−	−	−	−	−
++++	++++	++	+	++++	++	++++	+	++++	++	++++	+	++	++++
++++	++++	++	+	++++	++	++++	+	++	++	++++	+	++	++++
++++	++++	++++	+	++++	+	++++	+	++++	++++	++++	++++	++++	++++
++++	++++	++++	++++	+	++	+	++	++	++	+	++++	+	++++
++++	++++	++++	++++	++++	++	+	+	++	++	++++	++++	+	++++
+	++++	+	++++	+	+	+	++++	++	+	++++	++++	+	+
+	++++	+	++	+	+	+	+	++	+	++++	++	+	+
+	−	−	−	−	+	+	−	−	−	−	−	−	−
++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

the small acinar cells in association with regenerative cell mobilization, chiefly which show a tendency to stain with more RNA. It might be proliferative regeneration of the acinar cells surrounding the necrotic area. Accordingly we have found no

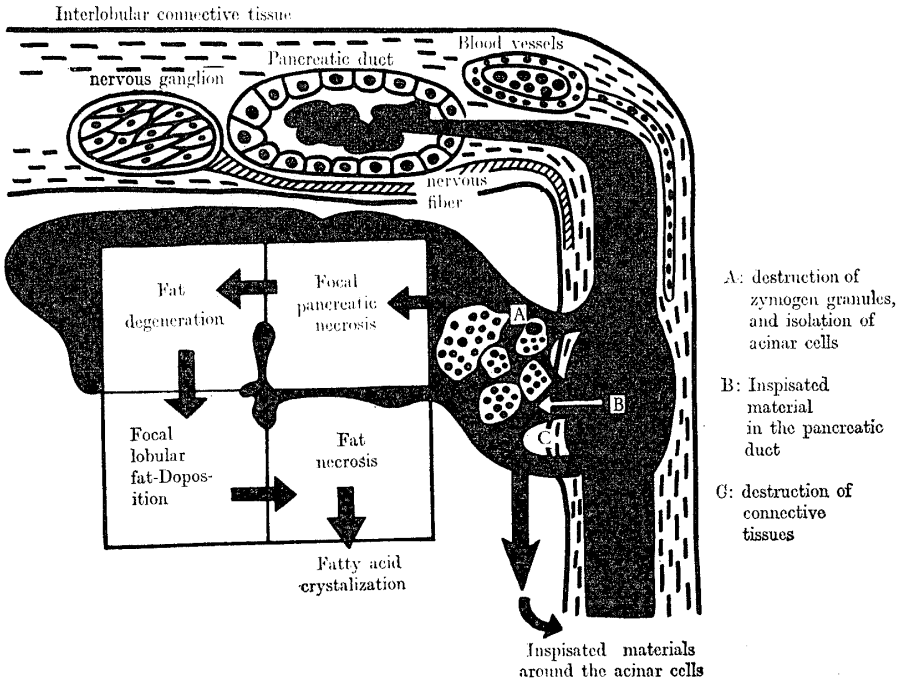
pancreatitis in the swine pancreas, and only pancreatic necrosis localized in the peripancreatic, interlobular and interacinar areas.

It seemed that the conditions appeared to be brought about by increased pancreatic secretion, with partial or complete obstruction of outflow and raised intraductal pressure. The pancreatic ducts were found with inspissated secretion in them, rupture of the interlobular connective tissue around them, outflow of the inspissated materials into the acinar lobuli, the isolation of the acinar cells within the inspissated materials remarkable fat degeneration of the acinar cells or LANGERHANS's islet in the acinar lobuli, destruction of the acinar cells with the zymogen-granules, the aggregates of the isolated zymogen-granules within the infiltrated pancretic fluids, and fat deposition in the center of the acinar lobuli enveloped with the necrotic acinar cells, and the total fat pancreatic acinar lobuli. The results obtained by the present authors are indicated in Table 1 and 2.

The fact that attracted the present author's attention was the remarkable development of many sympathetic ganglion cells and that the mylinated fibers from the vagus nerves in the interlobular connective tissues of the pancreas are from the splauchnic (sympathetic) and the vagus (parasympathetic). The former are nonmedullated and the latter are medullated preganglionic fibers, the cell bodies of their postganglionic fibers lie within the substance of the gland. Nerves are distributed about the secreting acini and on the wall of the blood vessels.

The sympathetic ganglion cells, and myelinated fibers from the parasympathetic

Table 3. Schematic diagram of the pathogenesis of the pancreatic fat necrosis in the meat pigs.



nerves made remarkable growth in the interlobular connective tissues of the swine pancreas in comparison with that of the animals, but these seemed to be mistaken in the organized thrombi, arteritis obliterans and phlebitis obliterans. These ganglion cells and myelinated fibers were certified by the SETO's silver-impregnation method.

Recently there appeared some reports on the relationships between the sympathetic nerve and fat deposition in the damaged liver (BRODIE and MAICKEL, 1963), (MAICKEL and BRODIE, 1963), (BRODY, 1963), (REES and SHOTLANDER, 1963). BRODIE cnd MAICKEL (15), (1963) concluded from the finding that cold-exposure and drugs like alcohol and morphine activate adipose tissue lipase and increase the output of energy-rich free fatty acids only if the sympathetic nervous systems is intact, illustrates a unique confluence of biochemistry with psychology. The body can respond rapidly to an increased demand for energy through the activation of lipase by norepinephrine at the adipose tissue nerve endings. The sympathetic nervous system exercises control over the mobilization of free fatty acids through the activation of lipase. Drugs that upset this control by eliciting a persistent hypermobilization of free fatty acids might set off an important step in a chain of events leading to a fatty liver.

MAICKEL and BRODIE (16), (1963) showed that the pituitary-adrenocortical system, as well as the sympathetic nervous system, is important in the fatty deposition produced by cold-exposure, and by ethanol and morphine. They also described that the cortical hormones appear to have at least two functions, such as maintenance of normal responsiveness of adipose tissue receptors to catecholamines, and the synthesis and deposition of triglycerides in liver.

BRODY (17), (1963) reported on the experiments with the ganglionic blocking compound and stated the antirelease agents would tend to support the hypothesis and that the sympathetic nervous system plays a key role in C Cl_4-induced hepatotoxicity in the rat. REES and SHOTLANDER (18), (1963) reported that non esterified fatty acids are either oxidized or resynthesized to triglyceride and complexed with protein to form lipoprotein. It is in this form that the liver secretes triglyceride into the blood stream via the hepatic sinusoids, a process which probably involves the endoplasmic reticulum.

Development of a fatty liver and necrosis were considered to be related, DIANZANI (19), (1954), and CHRISTIE and JUDAH (20), (1954) observed the mitochondrial damage about ten hours after poisoning with CCl_4, and DIANZANI (19), (1954) believed that as a consequence of this injury, an impairment in the oxidation of the liver lipid led to their accumulation, a theory consistent with that proposed previously by WINTER (23), (1940). These investigations, show that mitochondrial damage proceeded the accumulation of fat.

Recently two theories were proposed, such as the alternative theory proposed by ROBINSON and SEAKINS (21), (1962) and the inhibitory theory by RECKNAGEL (22),

(1960). The former was that of protein synthesis and, hence, lipoprotein formation was inhibited, and this results in the accumulation of triglycerides in the liver. The latter was that the endoplasmic reticulum played a role in the lipid secretory mechanism and CCl_4 dmaged the subcellular structure very soon after its administration. This damage occured at a time when fat had commenced to accumulate to the liver. According to REES and SHOTLANDER, it would appear that the damage to the lipoprotein secretory mechanism and an inhibition of protein synthesis occur concurrently following the administration of CCl_4 to rats. This suggests that CCl_4 exerts its effect by the physical attack on the endoplasmic reticulum. Physical damage to the endoplasmic reticulum and impairment of the secretory mechanism is a much later event in the pathogenesis of intoxication and therefore only plays a secondary role in the development of the fatty liver.

In the present investigation the authors found remarkable growth of the sympathetic nerve system in the interlobular connective tissue of the swine pancreas, and in the previous report they reported, on the morphological findings of the hyperadreno-corticoticism, hypoepinephrism and hyperinsulinism; therefore, the pancreatic fat necrosis following the pancreatic necrosis in the meat pigs might be based on the mechanisms which were described on the above mentioned theories, such as by BRODIE, MAICKEL, REES, ROBINSON, and RECKNAGEL's opinions. But it is very important to solve histochemically the problem of hyperinsulinism and that of the activation of lipase; and this may become the subject for future investigation.

2. Fat necrosis and fatty acid crystalization

CAMERON (13) described the following causes in the fat-necrosis: a) the result of digestion of fatty tissue from liberation of pancreatic juice in acute pancreatitis (WELLS, 1925); b) self digestion by bile leakage alone (REWBRIDGE (1931), (CAMERON, OAKLEY (1932); c) the result of digestion of the fatty tissue from tissue autolysis usually following trauma or impaired blood supply (HADFIELD 1929), d) the proteolytic enzymes of the pancreatic juice digested the cytoplasms of the fat cells and lipase destroyed the fat (NEAL & ELLIS, 1930, 1935); e) pancreatic juice alone will not produce fat necrosis, but needs to be activated by intestinal juice (KESTNER, 1923), (FRUGONI & STRADIOTTI, 1910), f) fresh sterile pancreatic juice can produce fat necrosis, and presumably by tissue kinase (FRUGONI & STRADIOTTI, 1910).

In fat necrosis, there are produced fatty acids and glycerol. The former unite with sodium and potassium salts in the tissue fluids to form soaps which are subsequently converted into calcium soaps and eventually calcium phosphate and carbonate, and the latter is absorbed and metabolized.

The results of the histochemical natures in the fat-necrosis are indicated in Table 2:

Table 4. Histochemical natures in the pancreatic fat necrosis of the meat pigs.

Variety of fatty substance	Staining methods	Needle-like crystal	Granular	Homogeneous
Fatty acid	OKAMOTO's method	###	##~###	–
K Na salt of fatty acid	OKAMOTO's method	–	–	–
Ca salt of fatty acid	OKAMOTO's method	–	–	–
Fatty acid Cholesterol Neutral fat	Nile blue stain	blue ### pink –	##~### –	– –
Cholesterol	SCHULTZ's method	–	+~###	###
Phospholipid	SMITH DIETRICH's method	–	+, –	+
General fat	Sudan III stains	+, –	+~##	###
General fat, particularly phospholipid	Sudan Black B	###	###	###

Fatty substances in the fat necrosis consisted of cholesterol, lipids and fatty acid. The forms of the fat-necrotic area were divided into three types such as needle-like crystal, granular and homogeneous deposition. Fat in the needle-like crystal contained fatty acid, that in the granule indicated a mixture of fatty acid, cholesterol and lipids. But on the other hand there were found no fatty acid or abundant cholesterol. According to DAMERON, in fat necrosis fatty acid unite with sodium and potassium salts in tissue fluids to form soaps which are subsequently converted into calcium soaps. However, the result indicated in Table 4 showed no Ca-or Na-salt in the fat necrosis.

HOFLUND *et al* (10), (1953) investigated on the aetiology, pathogenesis and clinical picture of fat necrosis in cattle. According to their chemical examination of the fat, the necrotic fatty tissue has a higher cholesterol content, and Clostridium was demonstrated in one case of fat necrosis produced lecithinase, an enzyme causing a disturbance of the plasma (and lymph) lipoproteins and flocculation of lipoids occurs. This increase in size of the lipoid particles causes an obstruction of the capillaries, at least locally. If the capillaries of fatty tissue are blocked such a circulatory disturbance occurs and the vital processes are affected with the production of necrosis. The results in Table 2 showed that the fat necrosis contained the granules and rods stained with PAS and acrolein-SCHIFF reaction, and in some places

the nuclear fragments stained with FEULGEN's reaction. Accordingly the fat necrosis might have originated from the decomposed acinar cells because of the presence of mucoprotein and few DNA.

Summay and Conclusion

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 the experiment of the feeding standard on the meat pigs.

No one has found the pancreatic fat necrosis in the swine. The present study described the occurrence of the fat-necrosis, fatty acid crystalization and focal necrosis in the pancreas of the meat pigs. The results are as follows:

1) Focal necrosis in the pancreatic acinar area is characterized histologically and histochemically by karyolytic processes with the nuclear fragmentation and depolymerization of DNA, aggregation of zymogen granules, and decrease of protein in the cytoplasm.

2) There are no pancreatitis in the swine pancreas, and only pancreatic necrosis localized in the peripancreatic interlobular and interacinar area.

3) The pancreatic ducts were found with inspissated secretion in them, rupture of the interlobular connective tissue around them, outflow of the inspissated materials into the acinar lobuli, the isolation of the acinar cells within the inspissated materials, remarkable fat degeneration of the acinar cells or LANGERHANS's islet in the acinar lobuli, destruction of the acinar cells with the zymogen-granules, the aggregates of the isolated zymogen-granules within the inspissated pancreatic fluids, and fat deposition in the center of the acinar lobuli enveloped with the necrotic acinar cells.

4) There is remarkable growth of the sympathetic ganglion cells and parasympathetic myelinated fibers in the interlobular connective tissues of the swine pancreas. These nervous systems in the swine pancreas develops more remarkably than that in the other animals. These seemed to be mistaken in the organized thrombi and angitis obliterans.

5) Fatty substances in the fat necrosis consisted of cholesterol, lipids and fatty acid. From the results investigated by the present authors, the pancreatic fat necrosis consisted of three types, such as Type 1, needle-like crystal contained principally fatty acid and few neutral fat; Type 2, granular form involved fatty acid, neutral fat, cholesterol and few phospholipids; and Type 3, homogeneous form included abundant cholesterol and few phospholipids and no fatty acid.

6) Fatty substances in the fat necrosis contained the granules, chain-like rods stained with PAS reaction for polysaccharides and with acrolein SCHIFF reaction for protein, and in some cases stained faintly with FEULGEN reaction for DNA. These mucoprotein-like substances might have originated from the isolated degenerative zymogen granules in the destructive acinar area within the inspissated

pancreatic fluid.

Recently there appeared some biochemical reports on the relationships between the sympathetic nerve and fat deposition in the damaged liver (BRODIE and MAICKEL), (MAICKEL and BRODIE), (BRODY), (FEES and SHOTLANDER), (ROBINSON and SEAKINS), (RECKNAGEL). Two theories were proposed from these investigations. The one was that protein synthesis and lipoprotein formation was inhibited, and that the triglycerides accumulation resulted in the liver. The other was that the endoplasmic reticulum played a role in the lipid secretory mechanism and become damaged very soon after the intoxication. This damage occured at a time when fat had commenced to accumulate to the liver.

If focal necrosis would occur in the pancreatic acinar area by the inspissated secretion in the pancreatic ducts, rupture of the interlobular connective tissue around them, and outflow of the inspissated materials from them to the acinar lobuli; the protein synthesis and lipoprortein formation might be inhibited and fat might be accumulated to the pancreas by the stimulation of the sympathetic nerve as well as the above mentioned biochemical theories in the fat liver. Focal pancreatic necrosis developed to the fat necrosis and fatty acid crystalization in the destructive pancreatic acinus by the overflow of the inspissated pancreatic fluid.

The present report described the facts that played a role for understanding morphologically the biochemical studies on the mechanism of fat deposition by the sympathetic nerve regulation.

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Explanation of Figures

Plate I

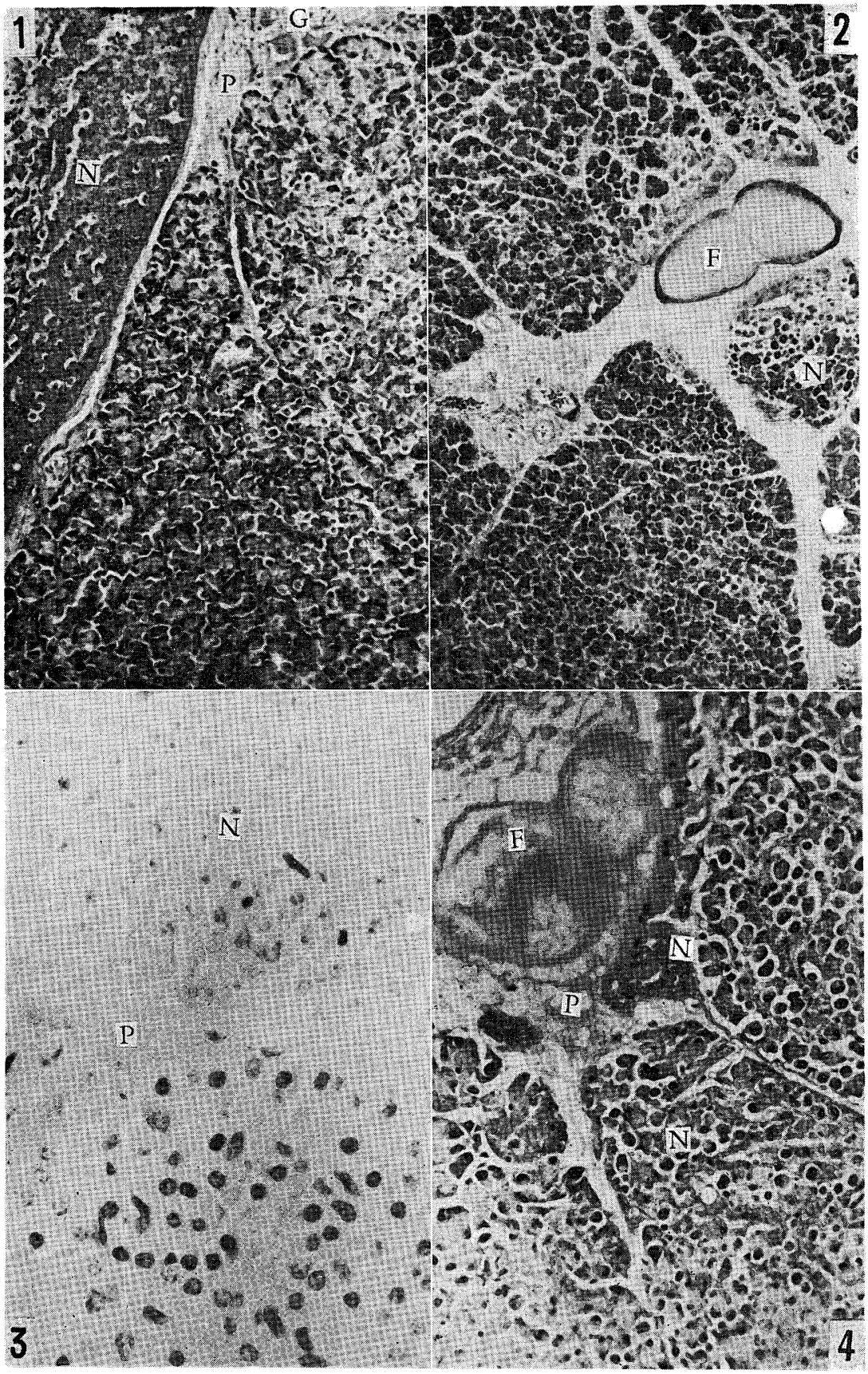
Explanation of Figures

Fig. 1. Acinar pancreatic necrosis. There are shown the karyorrhexis, karyolysis, and destruction of the zymogen-containing cytoplasms (N), the sympathetic ganglion cells (G), and the inspissated materials within the swollen pancreatic duct (P) in the interlobular connective tissue. Pancreas of the meat pig No. 23, stained with MALLORY azan staining, × 200.

Fig. 2. Fat necrosis, pancreatic necrosis and overflow of the pancreatic fluid. There are indicated two fat necroses (F) in which the zymogen granules existed in the periphery (N), and fat deposition in the center, and overflow of the inspissated materials (P) from the pancreatic duct in the swlloen interlobular connective tissue. The necrotic areas in the peripheral parts of the pancreatic acini indicated a loss of stainability. Pancreas of the meat pig, No. 23, stained with Azan staining. × 200.

Fig. 3. Pancreatic necrosis stained with FEULGEN's nuclear reaction for DNA. There are shown the necrotic areas without nuclei or with the fragmented nuclei in the upper part (N), the inspissated part of the pancreatic fluid in the middle (P), and no affected pancreatic acini in the inferior part. Pancreas of the meat pig, No. 13, stained with FEULGEN reaction, × 400.

Fig. 4. Fat necrosis, pancreatic necrosis and overflow of the inspissated pancreatic fluid in the interlobular connective tissue. There are shown almost all of the pancreatic lobuli (N) contained in the necrotic acinar cells and round free ones, complete pancreatic necrosis near the fat necrosis indicated the decomposition of the zymogen granules (N), and the overflow of the pancreatic fluids from duct (P). Pancreas of the meat pig, No. 23, stained with Azan stain, × 200.


1
G
P
N
2
F
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3
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P
F
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P
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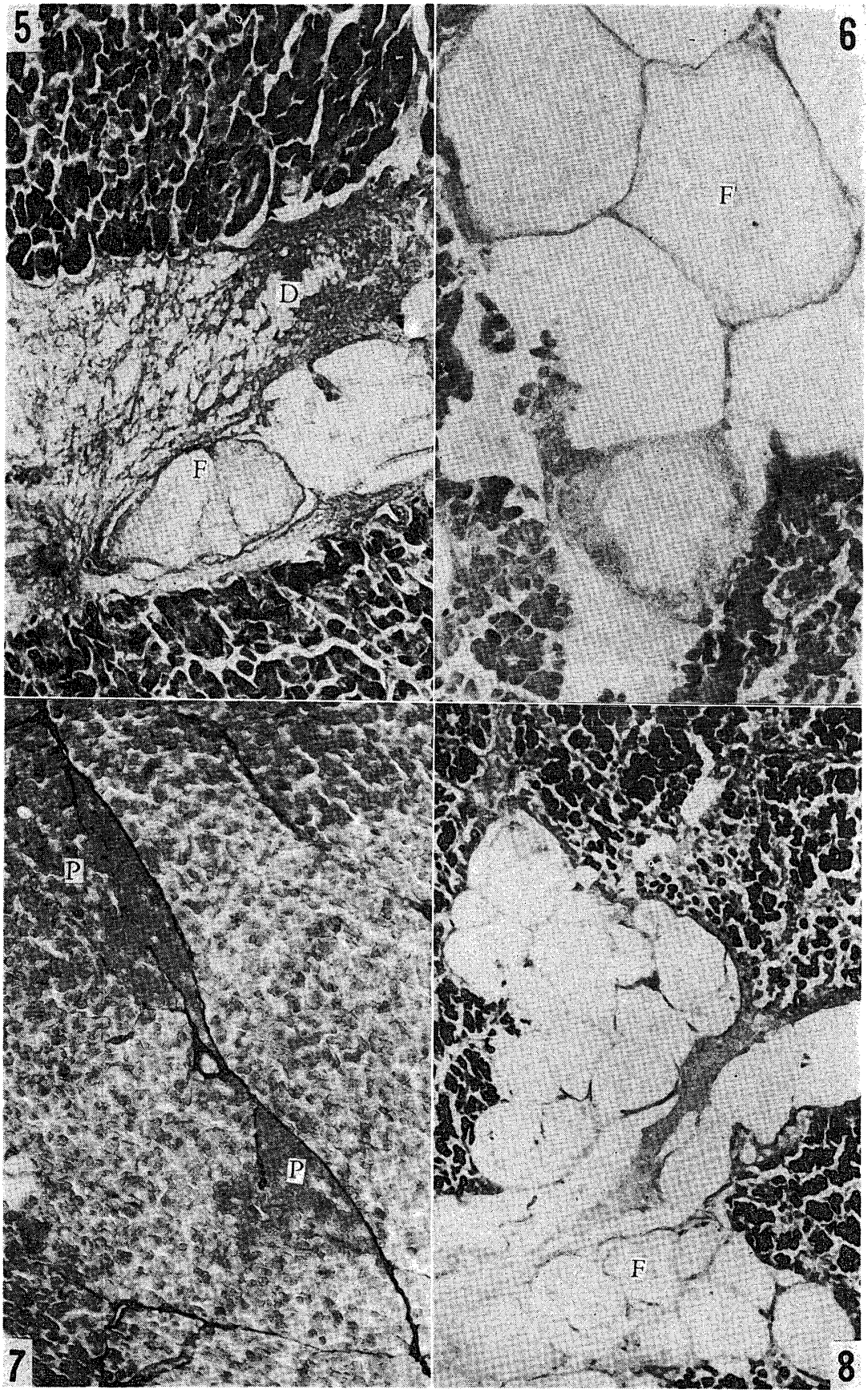

5
D
F
6
F
P
P
7
F
8

Plate II

Explanation of Figures

Fig. 5. Acinar lobuli, overflow of the inspissated materials and fat necrosis, stained with DUJIN's acrolein-SCHIFF reaction for protein. There are indicated strong intensity of stainability in the affected acinar lobuli (upper and inferior parts of Photo) and outflow of the inspissated materials (D) into the acinar lobuli containing granular and long chain-like appearance of destructive zymogen granules, and fat necrosis (F) which enclosed the granular or long chain-like substances. Pancreas of the meat pig, No. 1 stained with acrolein-SCHIFF reaction, × 200.

Fig. 6. Acinar lobuli and fat necrosis, stained with MCMANUS's periodic acid SCHIFF reaction for polysaccharide. There are indicated moderate intensity of stainability in the unaffected acinar lobuli (left and right lower parts of Photo) and fat necrosis (F) which enclosed the granular or long chain-like substances. Pancreas of the meat pig, No. 7, stained with PAS, × 400.

Fig. 7. Pancreatic necrosis caused on the rupture of the interlobular connective tissue and on the outflow of the inspissated materials from the pancreatic duct into the acinar lobuli. There are shown the rupture of the interlobular connective (balckish in Photo), fragmentation of the connective tissue (blackish scattered fragments of fiber in Photo), spindle-like necrotic area (two faint blackish parts, Mark: P in Photo), and the myelinated sympathetic nerve. Pancreas of the meat pig, No. 14, stained with Azan stain, × 200.

Fig. 8. Wide-spreading fat necrosis in the acinar lobuli, overflow of the inspissated materials, and the pancreatic necrotic area near fat necrosis. There are indicated many fat necrotic masses (F) resembling the acinar lobuli, and new-formed pancreatic necrosis near (N) the fat necrotic masses within the inspissated pancreatic fluid, and the overflow of the inspissated materials from the pancreatic duct (P). The pancreas of the meat pig, No. 1, stained with Azan, × 200.

Plate III

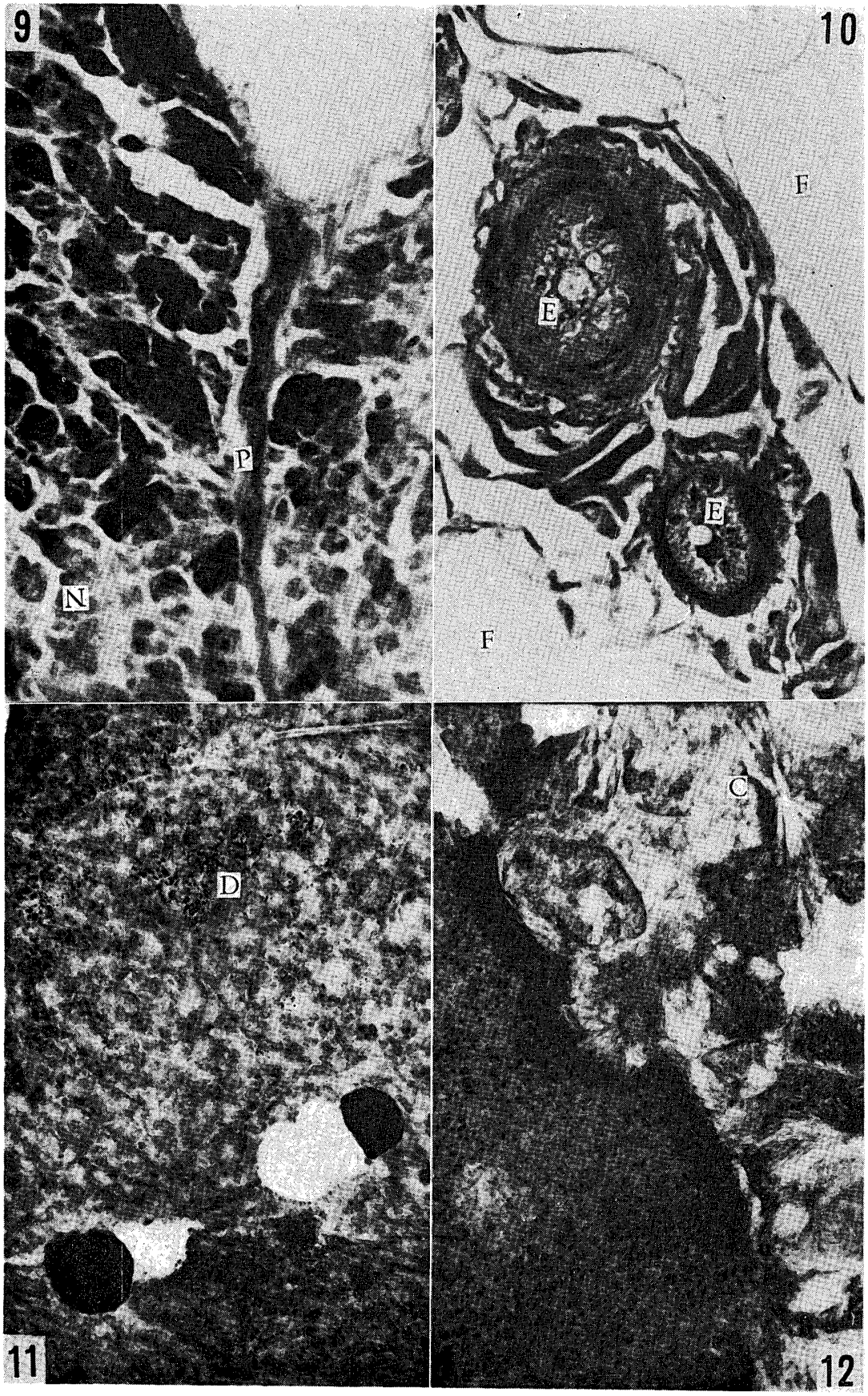
Explanation of Figures

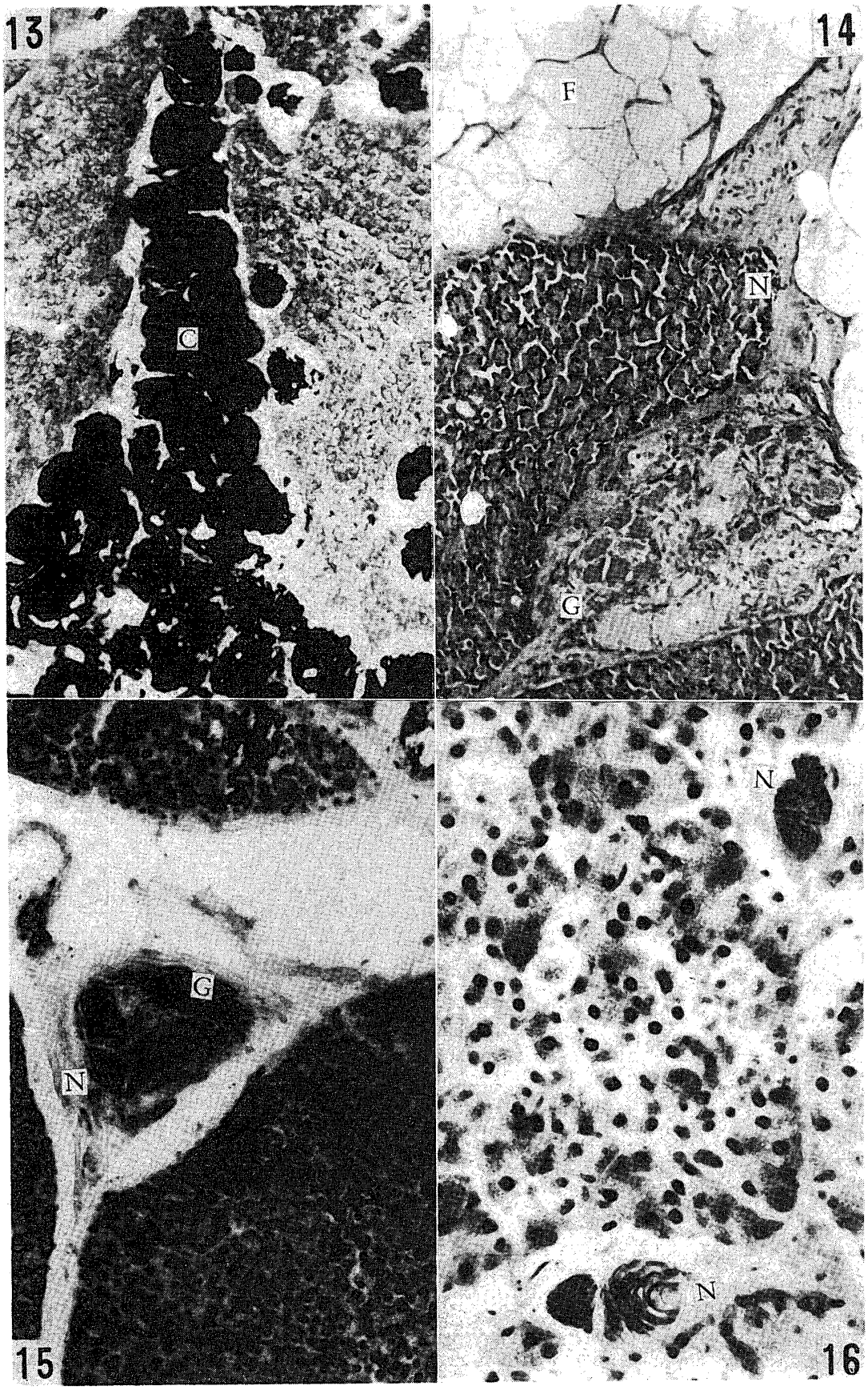
Fig. 9. Glycoproteid inspissated materials in the swollen pancreatic duct of the interlobular connective tissue. These inspissated materials (P) contained the granules that flowed to the neighborhood of the fat necrotic area (F) and pancreatic necrosis (N). The pancreas of the meat pig, No. 14 stained PAS, × 400.

Fig. 10. Fat embolus of the arteries in the interlobular connective tissue around the fat necrosis of the pancreas. There are shown two arteries which narrow lumen contained round fat droplets (E), and fat necrosis (F). The pancreas of the meat pig, No. 5, stained with Azan stain, × 400.

Fig. 11. Fat necrosis and fat deposition in the acinar lobuli. There are shown the fat necrosis (F) and deposition (D) in the acinar lobuli. The pancreas of the meat pig, No. 8 stained with Sudan Black B, × 400.

Fig. 12. Fatty acid crystalization in the fat necrosis of the pancreas. There are found a large amount of needle-like crystalization (C) unstained with Sudan Black B, but some granules within the fat necrosis stained with it. The pancreas of the meat pig, No. 5, stained with Sudan Black B, × 400.


9
P
N
10
F
E
E
F
11
D
12
C


13
C
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F
N
G
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G
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16
N
N

Plate IV

Explanation of Figures

Fig. 13. Fatty acid crystalization within the fat necrosis in the swine pancreas stained with OKAMOTO's procedure for fatty acid. There are shown fatty acid crystalization (C). The pancreas of the meat pig, No. 26, × 200.

Fig. 14. Sympathetic ganglion and myelinated fibers from the parasympathetic nerves in the interlobular connective tissue of the swine pancreas. There are shown remarkable developement of the sympathetic ganglion cells (G) and the myelinated fibers from the vagus nerves (N), and the wide-spreading fat necrosis (F) in the acinar lobuli. The pancreas of the meat pig, No. 26, stained with PAS-hematoxylin stain, × 200.

Fig. 15. Sympathetic ganglion stained with SETO's silver-nitrate staining in the interlobular connective tissue. There are shown the sympathetic ganglion (G) and myelinated nerve (N) in the interlobular connective tissue. The pancreas of the meat pig, No. 26, stained with SETO's silver impregnation, × 2000.

Fig. 16. The parasympathetic myelinated fibers in the interlobular connective tissue. These myelinated fibers (N) were distributed about the secreting acini and on the wall of blood vessels. The pancreas of the meat pig, No. 26, stained with SETO's silver impregnation, × 400.