Pathologic Study on Amyloidosis

Amyloidosis of the Islets of Langerhans in Diabetes Mellitus

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In Europe and in America as well, biochemical, immunological and morphological studies on amyloidosis have been widely made using various techniques in recent years, and morphological structure, chemical component of amyloid substance and pathogenesis of amyloidosis have gradually been elucidated. In Japan, on the other hand, studies on amyloidosis were insufficiently made probably because of the rare occurrence of this disease. In a recent few years the study on this problem, however, have been actively done by Nakagawa,[1] Uchino[2)3)] and other investigators,[4)5)6)] and publications on amyloidosis are increasing even in our country.

In 1965, the author noticed that hyaline like material observed in the islets of the pancreas of an autopsied diabetic patient revealed the similar staining reaction and the polarizing microscopic appearance to amyloid substance commonly encountered in cases of the primary and experimental amyloidosis previously confirmed in our institute. That case was already reported as amyloidosis of the islets of Langerhans.[7)] The author had doubt everafter that some cases which had been regarded as hyalinization of the islets might contain amyloidosis, and then attempted to examine on diabetic pancreas as a part of pathological studies on amyloidosis in our department.

Fortunately 205 cases of diabetic pancreas were available for this study by favor of pathological department of many medical schools and hospitals in Japan. Moreover 17 cases experienced in our university were added to this study, and the total of 222 cases of diabetic pancreas were examined in detail. The main purpose of this paper is to describe the histological appearance of diabetic pancreas and to point out high incidence of amyloidosis of the islets of Langerhans.

MATERIALS AND METHODS

The materials examined in this study were 222 cases in all, consisting of 220

diabetic pancreas of autopsied cases and 2 diabetic one of surgically biopsied cases. Sections of the head and tail of autopsied pancreas, and the tail of biopsied cases were examined. All these diabetic patients had clinically been diagnosed as diabetes pancreaticus, and bronze diabetes and steroid diabetes were excluded. Two biopsy cases were consisted of a case of obstructive jaundice and a case of gastric ulcer, both of which being complicated with diabetes mellitus. As the control materials for staining reaction of amyloid, the liver taken from human primary amyloidosis and from experimental amyloidosis of the mouse were similarly examined. Hyalinized glomerulus of the arteriosclerotic contracted kidney, hyalinized artery of the spleen and of the pancreas were also used as the control of hyalinization. The pancreas tissues of generalized amyloidosis were also examined as to confirm the involvement of the islets of Langerhans.

Most materials used in this study had been fixated with 10 % formalin and then embedded in paraffin. Sections were stained with various dyes and examined as follows : haematoxylin-eosin, van Gieson, P.A.S, alcian blue, silver impregnation, toluidine blue with and without peptic digestion, crystal violet with and without peptic digestion, alkaline congo red method of Puchtler, Sweat, and Levine.[8] Formalin-fixed frozen sections were used for Sudan III stain.

All sections stained with congo red were examined by the Nippon Kogaku polarized microscope of S–Po type.

In the cases in which a diagnosis of amyloidosis was established, the ratio of amyloid positive islets to the total islets in the section were estimated.

As to prove amyloid fibrils, a few cases of the islet-amyloidosis were moreover examined by the electron microscope. Small pieces of tissue sections previously fixed with formalin were rinsed in the running water and then dehydrated in a graded series of ethanol and embedded in Epon 812 by Luft's method finally. Thin sections were cut with the Porter-Blum microtome and stained with uranyl acetate and lead citrate doubly. The stained sections were examined with the Japan Electron Optical LAB. JEM 5–HS or JEM–7 electron microscope.

The institutes which provided the materials for this study were as follow : Hokkaido Univ., Sapporo Med. College, Kanazawa Univ., Nagoya City Univ., Gifu Univ., Mie Pref. Univ., Nara Pref. Med. College, Wakayama Pref. Med. College, Kyoto Univ., Kansai Med. College, Osaka Univ., Kobe Univ., Okayama Univ., Tottori Univ., Hiroshima Univ., Tokushima Univ., Kyushu Univ., Kurume Univ., Nagasaki Univ., Kumamoto Univ., Kagoshima Univ., Yamaguchi Univ., Hiroshima Atomic Bomb Casualty Commission, Yamaguchi Pref. Central hospital.

RESULTS

On the bases of staining reaction with the various dyes, and also from the

findings obtained with the polarized microscope, the fluorescent microscope and the electron microscope, hyaline like materials of the islets in the diabetic pancreas have been decided to amyloid substance. The results of morphological observation of amyloid substance in the islets have been described as follows.

Staining Reaction of Amyloid Substance in the Diabetic Islets ;

Staining reactions of amyloid substance in the islets of Langerhans are shown in Table 1. Amyloid observed carefully at high magnification is neither homogeneous nor amorphous substance, but frequently contains small fibers. As shown in Table 1, staining reaction of amyloid material in the islets in completely similar to that observed in human liver of the primary amyloidosis and mouse liver of experimental amyloidosis, and is easily distinguished from hyalinized renal glomerulus and hyalinized artery of the spleen and the pancreas.

Table 1. Staining Reaction of Islet-Amyloid

		Islet-Amyloid	Amyloid as control	Hyaline
H. E		light pink	light pink	light red
PAS		light red	light red	crimson
van Gieson		yellow	yellow	reddish brown
silver impregnation		reddish brown	〃	〃
alcian blue		blue	〃	light blue
sudan III		negative	〃	〃
metachromasia	toluidine blue	〃	〃	〃
	methyl violet	〃	〃	〃
Peptic digest	toluidine blue	positive	〃	negative
	methyl violet	〃	〃	〃
congo red		orange	〃	〃
※dichroic birefringency (congo red)		positive	〃	〃
※fluorescence (thioflavine T)		〃	〃	〃

Morphology of Amyloid Substance in the Islets ;

Morphology of amyloid substance in the islets may possibly be divided into following four types ; nodular, linear, fascicular and massive type (Fig. 1). Though the coexistence of these four types of amyloid substance is revealed in many cases, nodular type is most frequently observed (Figs. 6, 12, 18), followed by linear type (Figs. 7, 10, 11, 19). Fascicular (Fig. 8) and massive type (Fig. 14) are infrequent and are only observed in several cases of amyloid involvement. Slight fibrosis in the islets are frequently noticed in linear type, but rarely encountered in nodular type. Amyloid substance is mostly situated at the capillary wall of the islets (Fig. 11) and is adjacent to the capillary endothelial cells

(Fig. 7). Such relation of amyloid substance with the capillary endothelial cells is most apparent in nodular type. Amyloid substance deposited between the endocrine cells and the endothelial cells (Fig. 5), compresses the endocrine cells (Fig. 4), occasionally. The distribution of amyloid substance in the islets of diabetic pancreas is not different between the head and tail of the pancreas.

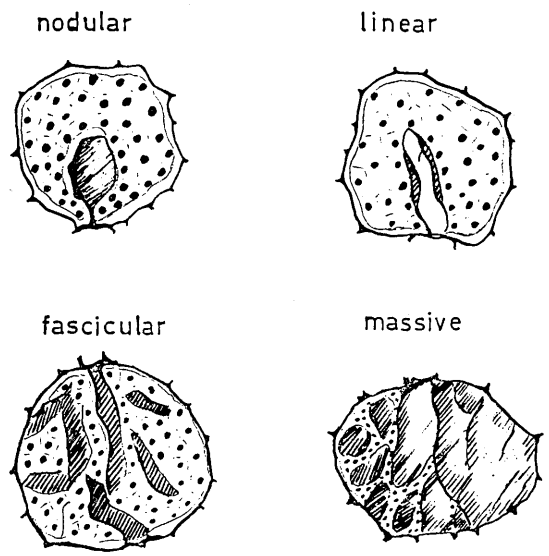

Fig. 1. Schematic figure of four types of amyloid deposit in the islet.

The Polarized Microscopic Appearance of Amyloid Substance in the Islets ;

The polarized microscopic appearance of amyloid stained with congo red exhibits dichroic birefringence of yellow and green color exactly under the crossed polar of analyzer and polarizer. Dichroism is changed by the rotation of specimen, namely, dichroism is reversed from green to yellow color or from yellow to green by the rotation of specimen through an angle of 90°, and the colors return as before at each angle of 180°. The colors are vanished at an angle of 45° (Fig. 2). The polarized microscopic appearance of small nodular amyloid exhibits fan-shaped arrangement of green and yellow color (Figs. 9, 11, 14). Amyloid substance of linear, fascicular and massive type shows interlaced appearance of green and yellow color (Figs. 9, 11, 14, 15). Birefringence of collagen fiber reveals white color. Hyalinization of control demonstrates no polarized light.

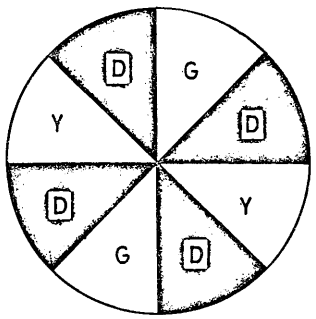

Fig. 2. Schematic figure of polarized microscopic finding of amyloid by rotation of the specimen.

The Fluorescent Microscopic Appearance of Amyloid Substance in the Islets ;

On the fluorescent microscopic examination of amyloid material in the islets stained with thioflavine-T, second fluorescence of brilliant yellowish green color has been observed. The exocrine and endocrince do not reveal any specific fluorescence. Hyalinization of control cases does not present fluorescence.

Fluorescence of massive and fascicular (Fig. 16) amyloid in the islets is found readily, but it is difficult to find fluorescence of small linear amyloid (Fig. 19).

The Electron Microscopic Appearance of Amyloid Substance in the Islets ;

Ultrastructural examination of amyloid substance in the islets of Langerhans demonstrates fibrillar structure, that is a characteristic to amyloid. The fibril has an approximate width of 100 Å (Figs. 22, 23). Bundle composed of parallel arrangement of these fibrils (Figs. 20, 21, 22) and felt-like structure (Figs. 20, 21, 23) formed with irregular interlacing of them, are clearly observed. These filamentous substances are situated between the endocrine cells and the capillary endothelial cells in the islet (Fig. 21).

The Distribution of Age of Diabetic Patients ;

The distribution of age of 222 diabetic patients (Table 2) are ranged from 6 years old to the eldest of 83 years old. There are the cases that are only a few under the age of thirty, and the case increases in number after the age of 40 years with the advancing age. The greatest number have developed diabetes in the 6 th decade, but show the tendency to decrease over the age of 70 years. The cases are few over the age of 80 years. The total of 222 cases examined are consisted of 131 males and 86 females and in five cases sex is unknown.

The Relation of Age to Amyloidosis of the Islets ;

Amyloidosis of the islets of Langerhans is ranged from the youngest female of 28 years old to the eldest of 81 years old (1 male, 1 female). Amyloidosis of the islets is noticed only in one case under the age of twenty-nine, and it is

Table 2. Incidence of the Amyloid Islets in Diabetic Patients with Respect to Age and Sex.

age	Diabetic Patients			Cases of amyloid islets			percentage with evidence of amyloidosis
	male	female	total	male	female	total	
0～29	5	4	9	0	1	1	11.1 %
30～39	4	11	15	0	5	5	33.3
40～49	12	12	24	3	5	8	33.3
50～59	39	18	57	21	9	30	52.6
60～69	51	23	74	36	12	48	64.8
70～79	18	15	33	16	8	24	72.7
80～	2	3	5	1	3	4	80.0
unknown			5			4	
total	131	86	222	77 (55.8 %)	43 (50.0 %)	124 (55.8 %)	

remarkably rare occurrence under the age of 40 years. In the 5 th decade, increasing number of cases appears and in the 6 th decade the greatest number develops amyloidosis of the islets, followed by the 7 th decade. Amyloidosis of the islets of the diabetic pancreas shows the tendency to increase with the advancing age. As to the sex difference, the incidence is 55.8 % in male and 50.0 % in female (Table 2). The ratio of amyloid-islets to total islets of Langerhans reveals the tendency of increasing with the advancing age and the highest incidence is found in the 7 th decade (Fig. 3).

The Histopathological Appearance of Diabetic Pancreas ;

Of 222 diabetic pancreas examined, amyloidosis of the islets of Langerhans has been observed in 124 cases (55.8 %). Other histological changes are as follows : degeneration and atrophy in 27 cases (12.1 %), fibrosis in 19 cases (8.5 %), fatty infiltration in 13 cases (5.8 %), no pathological findings in 26 cases (11.7 %), and autolysis in 9 cases (Table 3). Most cases in which amyloid substance has been observed in the islets shows proliferation of the islet cells, whereas fibrosis in the islets has rarely been found. The diabetic pancreas, in which amyloid substance is not demonstrated, frequently reveals atrophy and degeneration of the islet cells. Although histological changes of the exocrine part are almost similar in both amyloid group and amyloid abscence one, amyloid abscence group shows fatty infiltration in many cases. In amyloid abscence group, round cell infiltration, fibrosis and fatty infiltration are obviously found in the interstitium. In amyloid positive cases abnormal findings of pancreas vessels, such as intimal hypertrophy, hyalinization and calcification, are frequently observed. Intimal hypertrophy of the vessel is particularly prominent (Table 4).

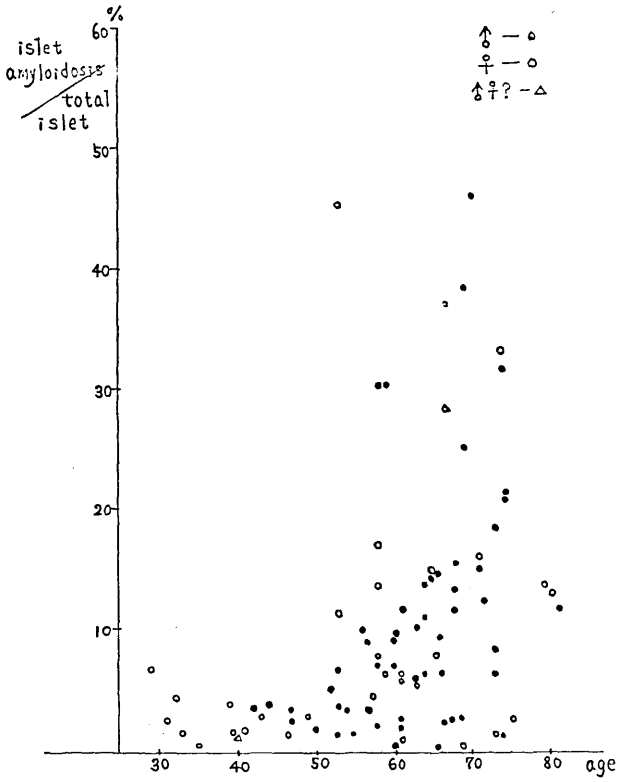

Fig. 3. Relation of age to amyloidosis of the islets (Diabetics).

Table 3. Microscopic Diagnosis of Diabetic Pancreas

Examined cases of diabetic pancreas	222 cases	
Amyloidosis of the islet	124 cases	55.8 %
Atrophy · Degeneration	27 〃	12.1
Fibrosis	9 〃	8.5
Fatty infiltration	13 〃	5.8
Inflammation	4 〃	1.8
No pathological changes	26 〃	11.7
Autolysis	9 〃	

Table 4. Morphological Changes of Diabetic Pancreas with or without Amyloid
(cases selected at random)

Case	Age · Sex	Endocrine							Exocrine			In terstitium					
		Amyloid	Cell infilt.	Deg. Atrophy	Fatty Infilt.	Hyalinization	Fibrosis	Hypertrophy	Cell infilt.	Fatty infilt.	Fibrosis	Cell infilt.	Fatty infilt.	Fibrosis	Vessel		Int. hyperrtro.
															Hyalini -zation	Calci fication	
1	43 ♂	−	−	+	−	−	−	−	±	−	##	+	−	##		−	−
2	57 ♂	−	−	##	−	−	−	−	−	−	±	−	−	±	−	−	−
3	76 ♀	−	−	##	+	−	−	+	−	###	+	+	##	##	+	−	+
4	62 ♀	−	−	##	±	−	−	−	−	+	−	−	+	−	+	−	−
5	67 ♂	−	−	##	−	−	−	##	±	+	##	+	+	##	−	−	−
6	62 ♀	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−
7	35 ♀	−	−	##	−	−	−	−	−	−	±	−	−	±	−	−	−
8	67 ?	−	−	##	−	−	−	−	−	−	##	−	−	###	−	−	−
9	24 ♀	−	+	+	−	−	−	−	##	−	±	##	−	##	−	−	−
10	32 ♀	−	−	+	−	−	−	−	−	±	##	−	−	###	−	−	−
11	62 ♀	−	−	##	−	−	−	−	−	−	+	−	−	−	+	−	−
12	59 ♂	−	−	##	−	−	−	−	−	##	+	−	##	−	##	−	−
13	65 ?	−	−	##	−	−	##	−	−	−	##	−	−	###	−	−	−
14	79 ?	−	−	+	−	−	−	−	−	###	+	−	##	+	−	−	−
15	61 ♂	−	−	+	−	−	−	−	−	##	−	−	−	−	−	−	−
1	38 ♀	+	−	+	−	−	+	±	−	−	+	−	−	−	+	−	+
2	64 ♂	###	−	##	−	−	+	##	−	−	−	−	−	−	−	−	−
3	81 ♂	###	−	+	−	−	+	##	−	±	±	−	−	−	−	−	+
4	59 ♀	##	−	+	−	−	±	−	−	−	±	−	−	−	+	+	+
5	68 ♂	###	−	+	−	−	−	+	+	±	+	+	−	−	−	−	+
6	59 ♂	###	−	##	−	−	##	−	−	+	##	−	+	−	−	+	+
7	47 ♂	+	−	+	−	−	−	−	−	−	±	−	+	−	+	+	+
8	58 ♀	##	−	±	−	−	−	−	−	−	±	−	−	−	+	−	+
9	70 ♂	###	−	+	−	−	−	+	−	±	+	−	+	−	−	−	−
10	57 ♂	+	−	−	−	−	−	−	−	±	±	−	+	−	−	+	+
11	57 ♂	##	−	+	−	−	±	+	+	−	+	−	−	−	−	+	+
12	69 ♂	+	−	+	−	−	−	+	−	−	+	−	−	−	+	+	+
13	69 ♀	+	−	+	−	−	±	−	−	−	+	−	−	−	+	−	+
14	68 ♀	###	−	##	−	−	−	##	−	−	−	−	−	−	−	+	+
15	68 ♂	###	−	+	−	−	±	±	−	±	+	−	−	−	−	−	+

DISCUSSION

In 1901, Opie[9] recognized homogeneous and amorphous material of the islets of Langerhans in diabetic pancreas. He regarded the material as hyalinization and emphasized that hyalinization was one of main morphological changes of diabetic pancreas. He also suspected that hyalinization of the islets might have closely been related with amyloid degeneration. But, then it was impossible to decide whether it was amyloid or hyalin. The homogeneous and amorphous materials frequently observed thereafter in the islets of Langerhans have been described as hyalinization by many authors[10-14] until the present time. In 1943, Ahronheim[15] and Arey[16] reported that the morphological change which appeared like hyalinization of the islets of Langerhans was amyloid, as the staining reaction with methyl violet and gentian violet exhibited metachromatic change.

Ehrlich and Ratner,[17] in 1961, emphasized that the changes seemed like hyalinization of the islets in the diabetic pancreas were amyloid on account of denoting dichroic birefringence with the polarizing microscope. Recently, Lacy[18][19] examined hyaline-like changes of the islets with the electron microscope and demonstrated the similar fibrillar structure to that of amyloid.

The Staining Reaction of Amyloid Substance in the Islets ;

In this study, staining reaction of amyloid substances in the islets of Langerhans was similar to amyloid examined as control, and was definitely differentiated from hyalin (Table 1). By Braunstein and Buerger,[20] Cohen[21] et al., Thompson[22] et al. and other investigators,[23-25] histochemical characteristics of amyloid have been established as follow ; it is stained yellow in color with van Gieson as a reaction for protein, and it exhibits positive reaction with alcian blue and P.A.S. staining as a reaction for polysaccharide. In this study, amyloid substance in the islets of Langerhans showed faint pink color with haematoxylin eosin, faint red color with P.A.S., brilliant blue color with alcian blue, and presented strongly yellow color with van Gieson stain. Metachromatic reactions by the routine method of crystal violet and toluidine blue staining were negative or weekly positive. It had generally been believed by many investigators[20-22][26] that metachromatic reaction is one of the characteristic findings of amyloid. Arey[16] and Ahronheim[15] diagnosed hyaline-like degeneration of the islets, which exhibited brilliant red color with gentian violet or methyl violet, to be amyloid. Bell[12] objecting against them, reported that the positive reaction with gentian violet or methyl violet was not chemical difference of islet haylin from amyloid. Kramer and Windrum[27] pointed out that metachromatic staining reactions with using impure dyes, which could been differentiated by the paper electrophoresis into the several components, were insufficient to determine amyloidosis.

They reported in addition, that P.A.S., positive protein of amyloid had an ability to mask a metachromatic component and, therefore, metachromatic staining with previous peptic digestion of the specimen presented true reaction.[28] Using the method of Kramer and Windrum[28], and Larsen,[29] Braunstein and Buerger[20] indicated that metachromatic staining reaction of amyloid was due to the presence of non-sulfated acid mucopolysaccharide, probably of carboxylated nature. The author recognize positive metachromatic findings of amyloid in the islets stained after the peptic digestion using the method of Kramer and Windrum.[28] Thompson[30] et al. described that though crystal violet and methyl violet were useful dyes for the demonstration of amyloid, they were not always capable of demonstrating a true metachromasia. The author regard that even if positive metachromatic reaction of hyaline-like material in the islets of diabetic pancreas is not definite evidence to prove amyloid, but these reaction should be estimated to be valuable for one of the proofs of amyloid.

The Polarized Microscopic Appearance of Amyloid Substance in the Islets ;

The polarized microscope has been utilized for the study of amyloidosis by many investigators, since Missmahl and Hartwig[31] established the method of polarized microscopic examination in 1953. They reported that the double refraction of amyloid was consisted of form and strain birefringence, and was related with thickness of section or arrangement of fibrils.[31] Wolman and Bubis[32] experimentally examined the cause of the green polarization color of amyloid stained with congo red and reported that the green polarization color occurred only with congo red dye, and that the green polarization color was produced with a near perfect parallel arrangement of the molecules of congo red and with an adequate thickness of the object to produce a $\frac{\lambda}{2}$ retardation. They obtained the conclusion that dichroic birefringence of amyloid was caused by congo red molecules arranged parallel to the length of the amyloid fibril.[32] According to Uchino's experimental investigation of amyloidosis,[2] the early stage of amyloid produced in the reticuloendothelial cell consists of bundles of amyloid fibrils arranged in parallel fashion. Amyloid extracellularly excreted still forms bundles for a while, but they are gradually loosened and are finally interlaced forming felt-like structure. Amyloid star revealed by the light microscopy is composed of bundles of fibrils. The polarized microscopic examination of amyloid star represents dichroic birefringence, which is apparently distincted as green and yellow color by the direction of orderly arranged amyloid fibril. As for the polarizing microscopic appearance of felt-like structure of amyloid visible by the electron microscopy, it is rather difficult to clearly demarcate each interlaced color of green and yellow. Even in such a state, however, the intensity of dichroic birefringence does not change and normal dichroism of green and yellow color is observed by the rotation of specimen. Nakagawa[1] recognized

atypical orange colored or yellow colored double refractivity and divided amyloid into three types. The author could follow amyloid substance in various stage of development from small nodular type in the early stage to large massive type in the final one, and the brilliant dichroic birefringence of green and yellow color were usually observed (Figs. 9, 11, 12, 13, 14, 15). Dichroic birefringence of amyloid is recognized logically in accordance with the theory of Wolman and Bubis.[32] They described that congo red stained amyloid appeared yellow, orange and finally red with increasing thickness of section under the crossed polar, and appeared faint reddish in the thin section of 1 to 2 microns.[32] The author, therefore, supposes that no orange color is shown, if the specimen of amyloid obtained by routine section is examined exactly under the crossed polar. Pearse[33] described that dichroic birefringence was characteristic for amyloid, but not specific and had deen observed in cellulose fiber stained with congo red. Cohen et al.[21] reported that unstained section of amyloid revealed weak birefringence and its intensity was markedly increased after congo red staining, and they suggested that amyloid substance was apparently distinguished from collagen fiber with the polarizing microscope. They, furthermore, mentioned that amyloid was not completely amorphous substance, as it had orderly intrinsic molecular arrangement with congo red binding, and suggested that elucidation of these subject should be carried out by the electron microscopic study. As birefringence of amyloid is readily recognized with the relation of congo red dye molecules to amyloid fibrils, the polarizing microscopic examination for amyloid has logically been evaluated. It this study, the author could clearly differentiate amyloid from collagen fiber and hyalinization and even small amount of amyloid in the islets of Langerhans was easily detected with the use of polarizing microscope (Fig. 11).

The Fluorescent Microscopic Appearance of Amyloid Substance in the Islets ;

Amyloid of the islets of Langerhans stained with thioflavine–T revealed brilliant yellowish green color with the fluorescent microscope. Saeed and Fine[34] described that long staining and the differentiation of specimen did not noticeably affect thioflavine–T fluorescence of amyloid. Various fluorescent color due to amyloid were, however, observed by some different techniques in staining procedure and also fluorescent color of amyloid were undistinguishable from one of false positive materials.[35] The technique of this staining should usually be attempted under certain condition. In this study, nothing but amyloid showed brilliant yellowish green fluorescence and the endocrine and exocrine part exhibited no fluorescence (Figs. 16, 19). The majority of amyloid found by polarizing microscope exhibited fluorescence. By fluorescent microscope, however, it was difficult to found small linear amyloid observed by the polarized microscope (Fig. 19). The fluorescent microscopic examination was therefore slightly inferior to polarized microscopic obsevation in the detection of small amount to amyloid.

The Electron Microscopic Appearance of Amyloid Substance in the Islets ;

Recently, Lacy[18)19)] described that hyalin of the islets of Langerhans on diabetic pancreas was composed of bundles and masses of fine fibrils radiating in various directions, and the ultrastructural appearance of hyalin within the islets was similar to amyloid. Kawanishi et al.[36)] reported that amyloid fibrils first appeared in the capillary endothelial cells, and then were observed in perisinusoidal areas with resultant destruction of the basement membranes, followed by intracytoplasmic invasion of the islet cells. Ultrastructure of the endocrine cells in the islets of the pancreas, which was previously fixed with formalin and refixed for this study, was moderately damaged and was difficult to be examined in detail. But ultrastructure of amyloid composed of characteristic filamentous fibrils with a width of 100 Å was recognized between the capillary endothelial cells and islet cells (Figs. 20, 21, 22). These fibrils were arranged in parallel fashion forming amyloid bundles (Figs. 21, 22) and felt-like structure consisted of interlaced fibrils was also demonstrated (Figs. 21, 23). From findings, amyloid fibrils were distinctly differentiated from collagen and elastic fiber. As for the electron microscopic appearance of hyaline degeneration, Spiro[37)] examined arteriosclerotic blood vessels and he recognized amorphous mat-like structure at 6,000 magnifications, but these findings were completely dissimilar to amyloid. With quotation of Aagenaes and Moe's paper,[38)] Lacy[18)] described that electron microscopic appearance of amyloid in the islets was distinctly differentiated from homogeneous material observed around peripheral capillaries of diabetic patient. In the present study, the large amyloid mass showed only compression of the islet cells and were clearly demarcated from these cells (Fig. 21). No intracytoplasmic invasion of amyloid into the islet cells as Kawanishi et al.[36)] reported was encountered. As shown by Cohen,[39)] however, the possibility of amyloid to invade into the parenchymal cell is undeniable. Uchino[40)] also recognized amyloid fibrils in the cytoplasm of liver cell. The author could not observe amyloid fibrils in the cytoplasm of capillary endothelial cells, but amyloid was frequently found adjacent to the endothelial cells (Figs. 20, 21).

The Nature of Hyaline-like Substance in the Islets ;

The author examined 222 cases of diabetic pancreas in the expectation that a few cases commonly accepted as hyalinization of the pancreatic islets might be amyloidosis. Most hyaline-like materials of the islets in 124 cases were decided to be amyloid substance from a result of examination with various staining methods, the polarizing microscope, the fluorescent microscope, and the electron microscope. Opie, in 1901,[9)] supposed that hyaline-like material observed in the islets of diabetic pancreas might be amyloid, but he could not exactly prove his conception. Reitman,[41)] in 1905, reported that amyloid was found in the islets of Langerhans. Arey[16)] and Ahronheim[15)] described that

the hyaline-like material found frequently in the islets of the diabetic pancreas was amyloid. Ehrlich and Ratner,[17] in 1961, reported amyloidosis of the islets from the results of examination by polarized microscope and various staining methods. Electron microscopically, Lacy[18][19] found amyloid fibrils in the hyaline-like material. Warren et al.[42] observed the same staining reaction of hyaline-like material in the islets as that of amyloid. In this study, all specimens which seemed like hyaline material with haematoxylin-eosin staining revealed to be amyloid and no hyalinization of the islets was found in this investigation. Warren et al.[42] however, still use the term "hyaline" and they regard that all homogeneous substance in the islets are not amyloid. The author also can not completely deny the presence of hyalinization of the islets, because of the possible occurrence of hyalinization sencondary to fibrosis of the islets. Even if it may be impossible, however, to exclude the existence of hyalinized islets, hyalinization can be definitely differentiated from amyloidosis by the examination of the light microscope, the polarizing microscope, the fluorescent microscope and the electron microscope. All hyaline-like materials in the islets examined in this study have been disclosed to be amyloid substance, and amyloidosis has been observed at high rate in the diabetic pancreas. Therefore the author regards that so-called hyalinization of the islets in the diabetic pancreas, which has been described in many publications, must be a amyloidosis. Klintworth[43] reported that morphologic alteration of the corneal stroma in lattice corneal distrophy was disclosed to be amyloid. Ravid et al.[44] also found high incidence of non-systemic microdeposit of amyloid in many autopsied cases, and reported that amyloidosis of the islets was found in 27 cases of 281 pancreas tissues. Schwarts et al,[45] in addition examined 111 autopsy cases between the age of 50 to 90 and reported that pancreatic-islet amyloidosis was observed in 75 %. The author in conclusion presumes that amyloid may be more frequently contained in such cases as are considered to be hyalinization and collagen degeneration by the examination of specimens stained only with haematoxylin and eosin.

The Morphology of Amyloid Substance in the Islets of Langerhans ;

The author classified various shapes of amyloid substance in the islets into four types, namely, nodular, linear, fascicular and massive type (Fig. 1). Nodular type of amyloid (Fig. 6) were most frequently observed, generally within such islets, which the secretory cells were relatively well preserved, and showed proliferation of the cells with resultant enlargement of the islets. Secondarily, linear type of amyloid substance were frequent, followed by fascicular and massive type. According to Smetana[46] and Uchino,[2] who followed the process of amyloid formation experimentally, small nodular amyloid first appeared in the capillary endothelium became linear in shape with spreading along the pericapillary space, and then fascicular and massive type of amyloid were formed with

increase and fuse of fibrils. In the diabetic pancreas, amyloid were situated adjacent to the walls of the capillaries of the islets and also between the endocrine cells and capillary endothelial cells (Figs. 5, 6, 7, 20, 21). No invasion of amyloid into the exocrine and interstitial parts were observed even in the advanced case, and amyloid was only localized within the islets of Langerhans (Figs. 4, 8). These findings were also confirmed by Ehrlich and Ratner.[17] Ratio of amyloid present islets to a total one were almost similar at the tail and head parts of pancreas, but the difference of ratio were occasionally recognized in each lobule in which amyloid positive islets were numerous or a few. Distribution of the small vessels in the lobule might be related to the difference of amyloid deposit in a lobule. Moschcowitz[14] mentioned that hyalinization of the islets of Langerhans was related with disturbance of the pancreatic vessels. According to Ravid[44] et al., in 11 of 27 cases of islets-amyloidosis, local deposit of amyloid was observed in other organs. In this study, however, amyloid substance was not observed in other organs such as spleen, liver, and kidney, which are reported the slight involvement of the capillary of the islets in a few cases of systemic and secondary amyloidosis. The author examined 10 cases of systemic amyloidosis as a control, and recognized amyloid substance on the capillary of few islets in 2 cases, in which the blood vessels in the interstitium were severely affected. In other 8 cases, amyloid substance were sufficiently observed in the various organs, but not in the islets of Langerhans. From these results, it is presumed that amyloidosis of the islets of Langerhans is localized type of amyloidosis, and that secretory function of islet cells may be associated with the deposit of amyloid.

The Relation of Diabetes Mellitus to Amyloidosis of the Islets of Langerhans ;

In this study, amyloidosis of the islets of Langerhans were observed in 124 (55.8 %) of 222 cases of diabetic pancreas (Table 2). The incidence of amyloidosis of the islets obtained in this investigation was predominant comparing to the reports of Ehrlich and Ratner,[17] in which islet-amyloidosis were observed in 45 (49.4 %) of 91 cases of diabetes mellitus over the age of fifty years. If these materials were limited over the age of fifty years, amyloidosis was more frequent occurrence and was observed in 106 (62.7 %) of 169 cases. It was therefore considered that amyloidosis of the islets had an intimate relation to diabetes mellitus. In the previous study, however, amyloidosis of the islets was observed in 18 (23.3 %) of 77 cases of clinically non deabetics.[47] After Opie[9] had pointed out hyalinization of the islets in diabetic pancreas, Cecil,[10] Wright[11] and other investigators reported that hyalinization of the islets was observed even in non diabetics. On the contrary, Bell[13] objected against them emphasizing that hyalinization of the islets might be a morphological expression of clinically unrecognized of potential diabetes. Non diabetic cases associated with amyloidosis of the islets revealed slightly higher blood sugar level than in amyloid negative

cases.[47] Amyloid positive ratio in non diabetic pancreas was markedly decreased comparing to that in diabetic pancreas. These results might therefore also show a close relation of amyloidosis of the islet with diabetes mellitus.

The Histopathological Difference between Amyloid Positive Cases and Negative Cases ;

In amyloid absent cases, degeneration and atrophy of the endocrine of the islets were predominantly observed, and the majority of islets were diffusely involved without showing any difference in the lobules (Table 4). In amyloid positive cases, on the other hand, particularly in the early stage of amyloidosis, the endocrine cell had comparatively large cytoplasm and revealed nearly normal structure (Table 4). Moderate difference of presence of amyloid positive islets in each lobule was noticed. Amyloid substances were also observed in the islets where proliferation of the endocrine cells, which might be one of histologic manifestation of compensatory hypertrophy, were observed (Table 4). The islet cells compressed by marked deposit of amyloid were apparently atrophic, and in such a case, it was presumed that secretory function of the islets were completely destroyed and that deposit of amyloid localized only in the islets might be influenced by the function of the endocrine cells. In conclusion, the most predominant difference between amyloid present and absent cases might be supposed that the endocrine function were comparatively maintained in amyloid positive cases compared with amyloid absent ones.

The Pathogenesis of Amyloidosis of the Islets in Diabetic Pancreas ;

Symmers[25] has introduced various opinions by many investigators on pathogenesis of amyloidosis but this problem are not yet solved. A few years ago, the view[25][48] that deposition of serum protein in the slit of connective tissue might play a significant role in pathogenesis of amyloidosis had been prevailing. Recently, however, it has been emphasized by Teilum,[49] Cohen,[39] Uchino[2] and other investigators that amyloid substance is produced by the reticuloendothelial cells. Kennedy[50] also reported that endothelial cells might play an important role in secretion of amyloid. In this study, the author presumed that three factors, namely, ageing, diabetes mellitus and capillary endothelial cells of the islets, had some relation with production of amyloid substance in the islet. According to Bell,[12] hyalinization of the islets was related only to the age and appeared to be an associated phenomenon of diabetes rather than a cause or an effect of the disease. Examining senile cardiac amyloidosis, Thung[51] and Pomerance[52][53] reported that amyloidosis had a close relation to ageing. Although the author indicated an apparent relation between amyloidosis of the islets and ageing, it was not concluded if the increasing of age was the direct cause of occurrence of amyloid. As mentioned before, it was apparent that diabetes mellitus played an important role for occurrence of amyloidosis of the

islets. So the structure of the islets in the amyloid positive pancreas were comparatively preserved, diabetes mellitus associated with amyloidosis of the islets might be occurred not as a result of absolute lack of insulin due to atrophy and destruction of the islet cells, but probably because of relative decrease of insulin due to increased insulin antagonist of insulin. Porta et al.[54] recognized amyloidosis in 5 of 6 cases with functioning islet cell adenoma, which were consisted of similar cells to the normal beta cell. Their observation might be regarded as an evidence which suggested that diabetes mellitus with amyloidosis were not always occurred as a result of disturbance of the islet cells. Early small nodular amyloid substances were situated at the capillaries of the islets and revealed a close relation to the adjacent endothelial cells rather than collagen fiber and islet cells. From these findings, the author thought that the capillary endothelial cells of the islet had associated with the occurrence of amyloid. The capillary endothelial cell might have ability to produce amyloid substance as the nature of reticuloendothelial cells pointed out by Uchino[2] and Kennedy.[50] It might be an significant evidence which support the theory of endothelial production of amyloid that Kawanishi[36] demonstrated amyloid fibrils in the cytoplasm of endothelial cell. Recently, the investigation of insulin antagonist, particularly insulin antibody and humoral insulin antagonist have made the great progress. With the use of fluorescent-antibody method, Blumental[55] indicated that disturbance of blood vessels in diabetes mellitus were intimately related to hyalinization of the islets of Langerhans. Lacy[18] suggested moreover that hyalin of the islets might have a relation to secretion of insulin and might be a combined substance of protein and insulin or might be resulted from antigen-antibody reaction. If the occurrence of amyloidosis of the islets is supposed to be a result of antigen-antibody reaction as Lacy's theory,[18] the relation of secreted insulin to the occurrence of amyloidosis of the islets of Langerhans should be elucidated in future.

SUMMARY

(1) Amyloidosis of the islets in 222 diabetic pancreas was examined by using various stainings, fluorescent microscope, polarized microscope and electron microscope. In 124 cases (55.8 %), amyloidosis of the islets of Langerhans was found, but hyalinization of the islets was not encountered.

(2) Amyloid substance was closely situated at the wall of capillaries in the islets and was adjacent to the capillary endothelial cells.

(3) Deposit of amyloid in the islet was divided into four types ; nodular, linear, fascicular, massive type. Nodular type of amyloidosis was most frequently observed.

(4) Amyloidosis of the islets of Langerhans showed the tendency to increase with advancing age.
(5) It was presumed that secretory mechanism of the islet cells was related to the occurrence of amyloid substance.

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EXPLANATION OF FIGURES

Fig. 4. Amyloid in the islets. The endocrine cells are compressed by massive deposit of amyloid in the islet. H.E. stain ×100

Fig. 5. Amyloid substance is situated between the endothelial cells and the endocrine cells, and is clearly distinguished from collagen fiber and endothelial cells. A few erythrocytes are seen in the capillary blood vessels (arrow). Van Gieson stain ×400

Fig. 6. Nodular type of amyloid, which contained small fibers, is observed in center of the islet. Congo red stain ×400

Fig. 7. Linear type of amyloid is situated closely adjacent to the endothelial cells. Congo red stain ×400

Fig. 8. Amyloidosis of the many islets in a lobule. Fascicular type of amyloid is observed (arrow). Congo red stain ×100

Fig. 9. Polarized microscopic finding of amyloid presented in Fig. 8 shows dichroic birefringence. Congo red stain ×100

Fig. 10. Linear type of amyloid is situated adjacent to the capillary in the islet. Congo red stain ×100

Fig. 11. Polarized microscopic finding of linear type of amyloid presented in Fig. 10 Dichroic birefringence is clearly observed in the same portion as shown in Fig. 10. Congo red stain ×100

Fig. 12. Polarized microscopic finding of nodular type of amyloid at early stage shows fan-shaped deposit. Congo red stain ×400

Fig. 13. Polarized microscopic finding of nodular type of amyloid shows radiating shape. Congo red stain ×400

Fig. 14. Polarized microscopic finding of massive type of amyloid in a hypertrophic islets. At the right side, fan-shaped amyloid is observed. Congo red stain ×100

Fig. 15. Polarized microscopic finding of the pancreas taken from the biopsy case. Amyloid is observed at the peripheral region of the islet. Congo red stain ×400

Fig. 16. Fluorescent microscopic finding of amyloid in the islets reveals brilliant yellowish green color. No amyloid substance is observed in the exocrine part. Thioflavine-T stain ×100

Fig. 17. Fluorescence of amyloid is observed closely adjacent to the capillary. Thioflavine-T ×100

Fig. 18. Fluorescent microscopic finding of nodular type of amyloid in the islet. Thioflavine-T ×400

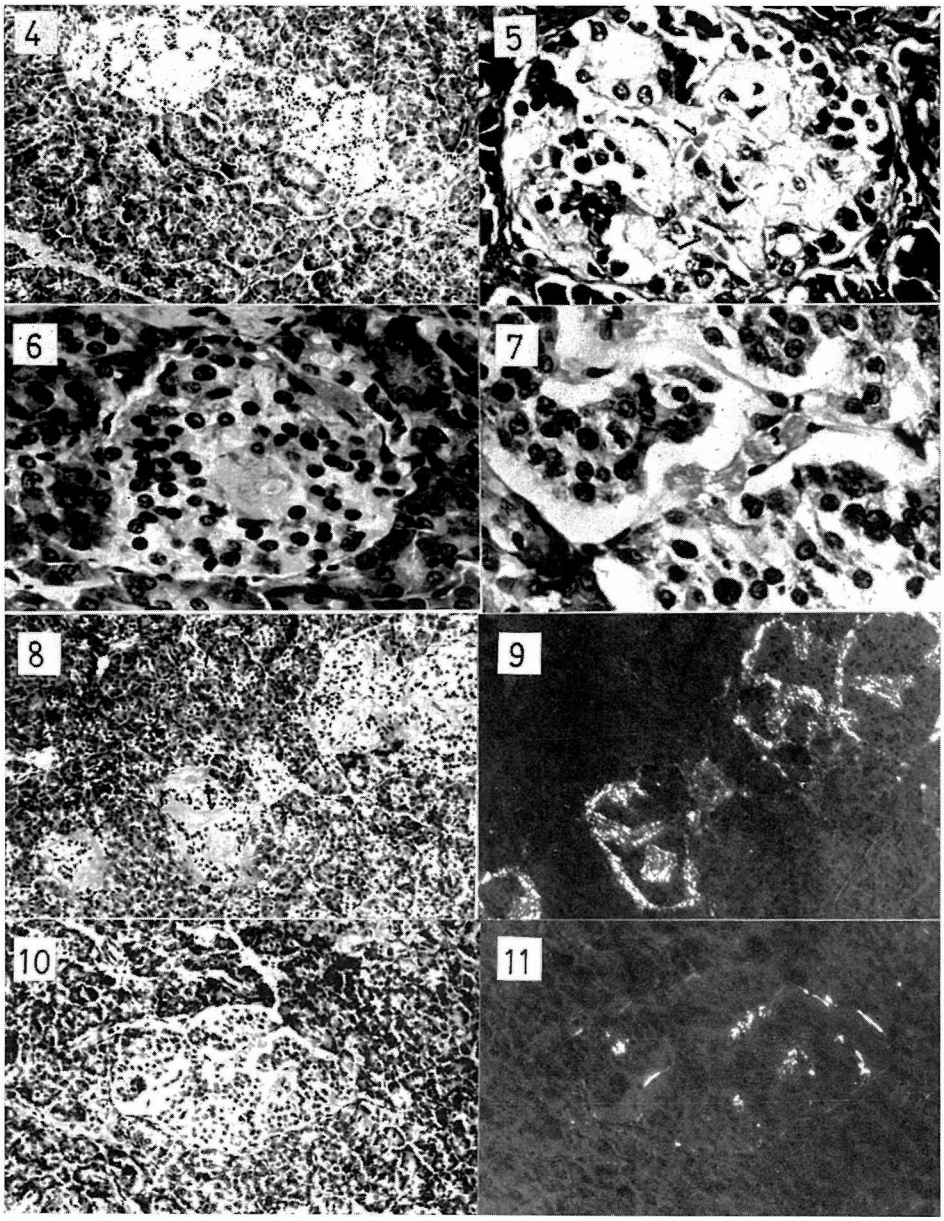
Fig. 19. Fluorescent microscopic finding of linear type of amyloid. Amyloid is localized at the periphery of the islet. Thioflavine-T ×400

Fig. 20. Electron microscopic finding of amyloid substance near the endothelial cell. ×7500

Fig. 21. Electron microscopic finding of amyloid substance between endothelial cell and islet cell. At the right upper side, amyloid fibrils form bundles, and at the left side, felt like structure is visible. Amyloid substance is clearly demarcated from the islet cell. ×10500

Fig. 22. Electron microscopic finding of amyloid which is consisted of fibrils with 100Å in width. Bundles of amyloid are easily visible. ×25000

Fig. 23. Electron microscopic finding of amyloid which shows felt like structure of amyloid fibrils. ×30000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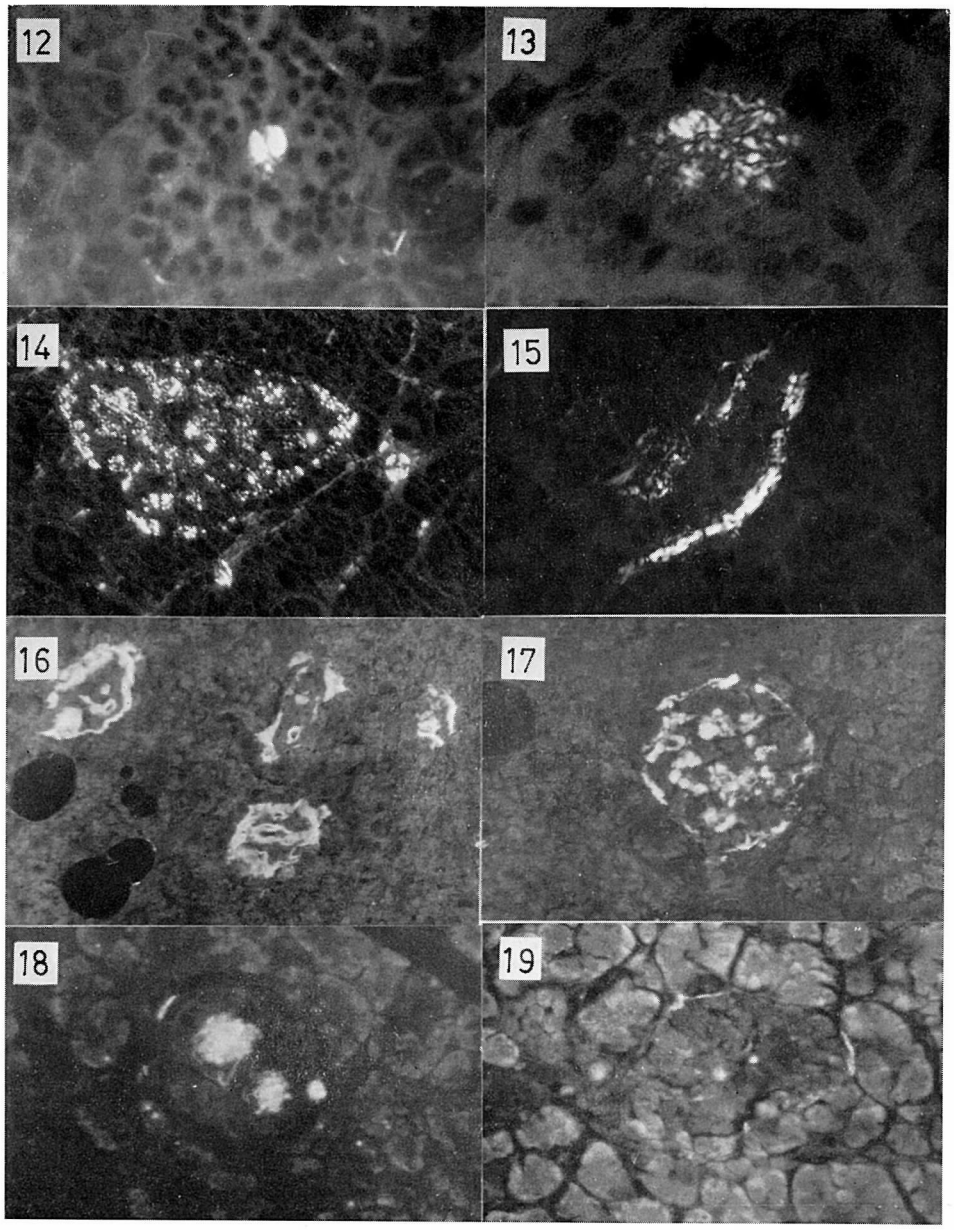
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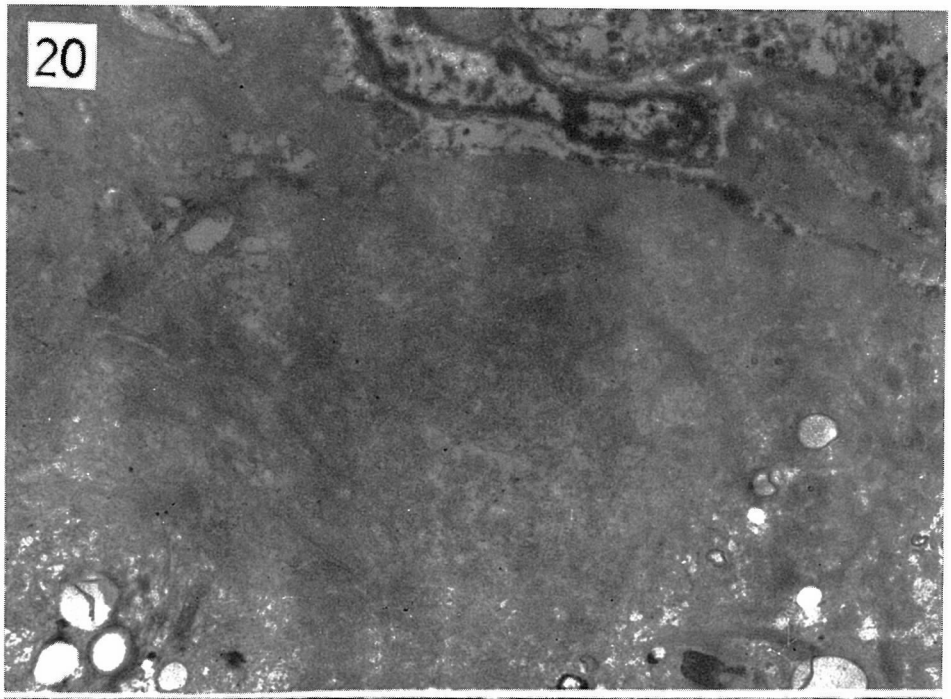
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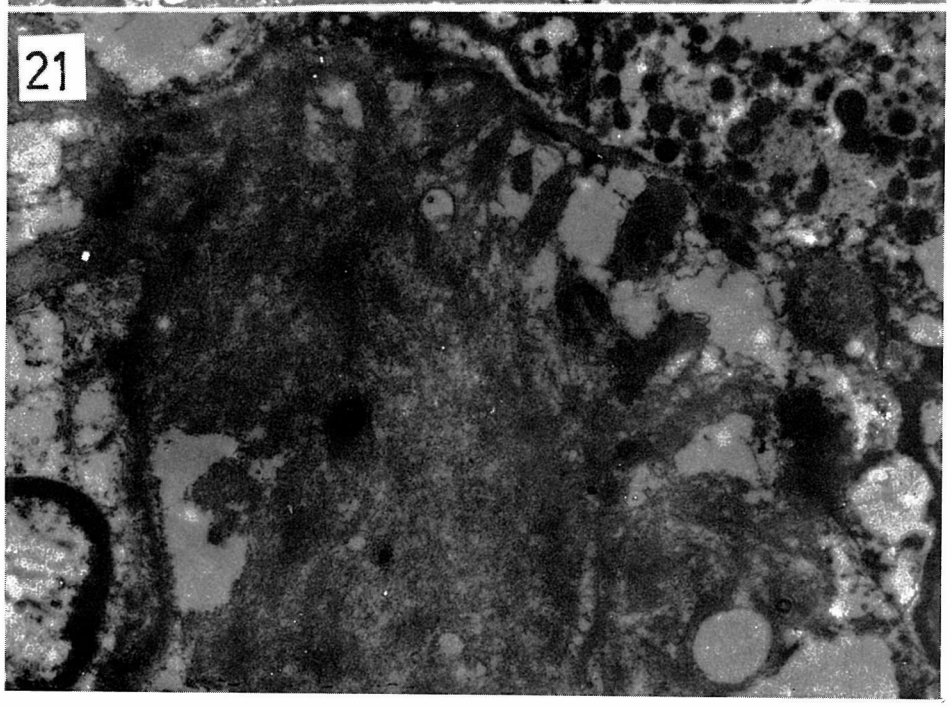
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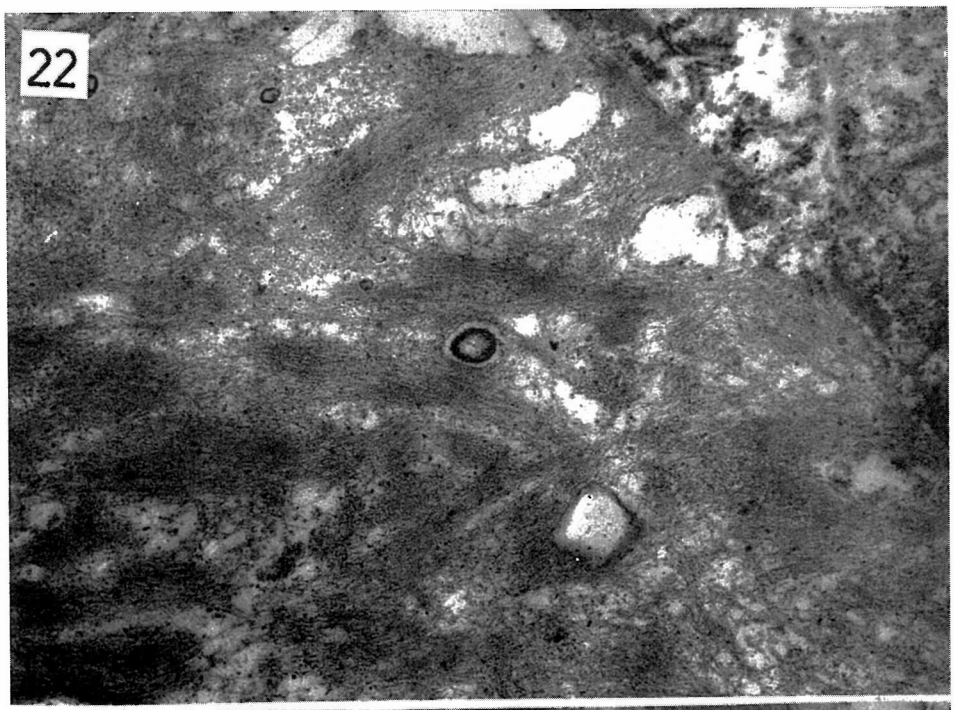
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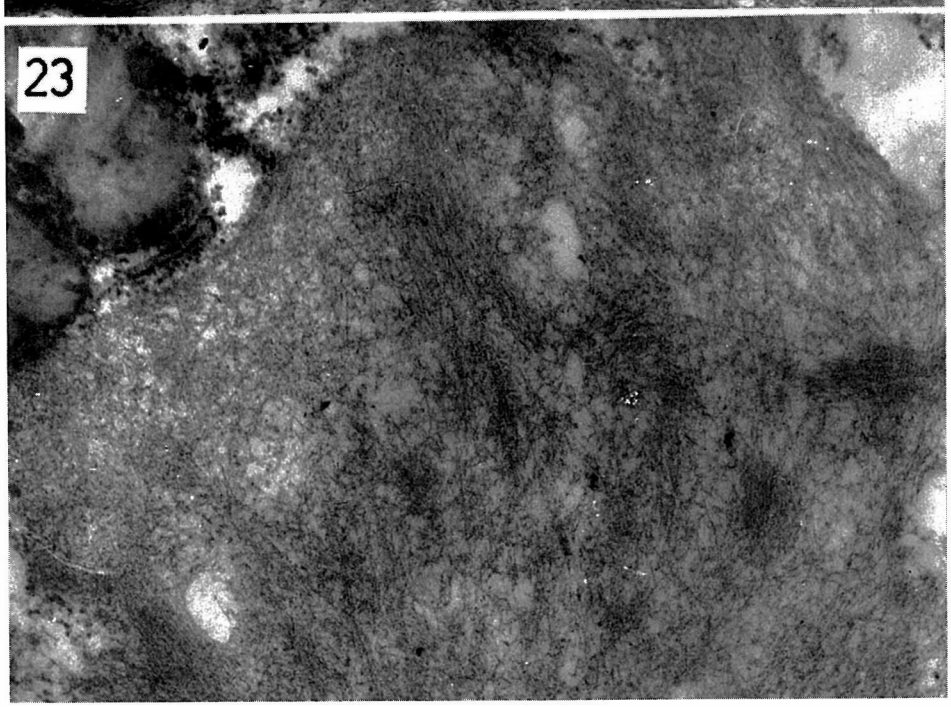
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