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LIGHT MICROSCOPIC AND ELECTRON MICROSCOPIC STUDIES OF INDIVIDUAL CELLS IN PIGMENTED VILLO-NODULAR SYNOVITIS AND BURSITIS (JAFFÉ)

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Kazushi HIROHATA *Light Microscopic and Electron Microscopic Study of Individual Cells in Pigmented Villonodular Synovitis and Bursitis (Jaffé).* Kobe J. Med. Sci. **14,** 251-279, December 1968——Pigmented villonodular synovitis and bursitis (Jaffé) has been rarely reported and the pathogenesis is still open to study.

In this study, the author could examine the specimens of 18 cases, which were obtained from synovial and bursal tissues at surgery.

First of all, the incidence of individual cells was described by using light microscope and the foam cell was disclosed to play an important role in this disease.

The purpose of the present study is to clarify the cytogenesis of the foam cell by various methods, especially under the electron microscope whose findings have not been reported. Foam cells contain numerous and plemorphic cytoplasmic inclusions of lipid-cholesterol which were also proved by the polarizing microscope. About the cause of this disease, these results do not support the inflammatory theory and the metabolic disturbance of lipid-cholesterol is discussed.

INTRODUCTION

The term pigmented villonodular synovitis and bursitis[19,20] (hereafter referred to as PVS and PVB) which were proposed by Jaffé in 1941, have well been supported by the more recent authors in the world.

At the historical view such diseases were the case of xanthoma which was reported by Simon 100 years ago. On the other hand, Chaissaignac found already in 1852 a case in which similiar symptoms were observed in the tendon sheath and he called it "nodular lesion". The incidence of PVS and PVB has been thought to be lower. In the matter of this fact, only three cases a year on the average are reported in Japan. The opinion about its pathogenesis have been divergent such as inflammatory[6,19,20] (process by Jaffé), neoplastic lesions[11] and metabolic disturbance.[14,17,22]

A number of experimental studies have been performed in order to prove those theories. However, whether these results are connected with the PVS and PVB is much more debatable. Therefore, the cause of PVS and PVB would be still open to speculation.

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In the past 10 years the author has seen 18 cases of PVS and PVB, all of which were subjected to surgical procedures. On the basis of these experiences, the study reported here was undertaken to clarify the extensive pathologic findings in PVS and PVB and to extend this to the comparative studies with our experimental PVS and PVB.

MATERIALS AND METHODS

As shown in the table 1, the age of the patients was distributed from 18 months to 71 years. 10 of the patients were men and 8 women.

No history of trauma was found in our cases, and the period from the onset of symptoms to the date of examination ranges from 2 months to 18 years.

With regard to the site of lesion, 10 cases had occured in synovial membrane of the knee joint, 4 in the bursa of the knee region, one in synovial membrane of hip joint associated with the iliopectineal bursal lesion and the others had multiple synovial lesions of the wrist, the hands, ankle and feet associated with bursal involvement.

In each of these cases, the first clinical sign was the swelling with slight pain. No inflammatory signs were observed in any stage.

The patients complained only of the gradual growth of tumor in case of the bursal lesions. In 12 of 18 cases, chronic joint bleeding was prevalent from the onset. But in the remaining of this disease, no chronic joint hemmorrhage was elicited even in their history. The joint function were quite normal over the years except a few cases.

In 8 cases, X-ray findings indicated the secondary osteoarthritic changes (cyst-formation, bone destruction and bone sclerosis) in which the specific findings were not observed in histological sections.

For the light microscopic observation, the extirpated synovial and bursal tissue were embedded in paraffin, stained with H. E., van Gieson, Gomori, Azan and Berlin-blue solution or on the frozed section, with toluidin blue, sudan III and potassium Jodi-solution (cholesterol staining; Okamoto et al[26] 1944). Some of frozen sections were observed under the polarizing microscope. According to these findings, the cells were preliminarily classified and the incidence of individual cells were investigated by means of a micrometer (Table 2).

Further those sections from 18 cases were able to divided into the villous or nodular tissues, from some of which the specimens were immediately fixed in buffered osmium tetroxide (pH 7.4) for an hour. After the graded dehydration in alcohol and propylene oxide, small pieces of tissues were embedded in Epon 812. Ultrathin sections were cut with glass knives on Porter-Blum ultramicrotome, stained doubly with uranyl acetate and lead citrate.[28]

Before the electron microscopic observation, the thick sections were sometimes stained with toluidin blue[23] and examined under the light microscope. All sections were viewed under the HS-7, HS-11-A electron microscope by magnification of 2,000 to 20,000.

Table 1

Case	Age	Sex	Site	Clinical Diagnosis	Trauma	Duration of Symptomes	Effusion	Value of Blood Cholesterol (mg/dl)		Type of Operation	X-ray Therapy	Recurrence (follow-up)
1	65	M	Knee	Diffuse Synovitis	None	2 months	Bloody	215.0	Osteoathritic change	Partial Synovectomy	——	No rec. (4.2 years)
2	56	F	Knee	Bursitis	None	4 years	Bloody	247.0	Normal	Removed Bursa	——	No rec. (3.8 years)
3	24	F	Knee	Localized Synovitis	None	5 months	Bloody	257.0	Normal	Removed Bursa	——	No rec. (4.8 years)
4	36	F	Knee	Bursitis	None	12 years	Bloody	207.1	Normal	Removed Bursa	——	No rec. (4.9 years)
5	44	M	Knee	Bursitis	None	10 months	Turbid yellowish	——	Normal	Removed Bursa	——	No rec. (2.4 years)
6	42	M	Knee	Localized Synovitis	None	14 months	Turbid yellowish	180.4	Normal	Partial Synovectomy	Total 2000 r.	No rec. (3.6 years)
7	31	M	Knee	Diffuse Synovitis	None	10 years	Bloody	——	Osteoathritic changes	Total Synovectomy	Total 4000 r.	Recurrence 1 year after (Re-operation)
8	25	F	Knee	Diffuse Synovitis	None	3 years	Bloody	160.5	Osteoathritic changes	Partial Synovectomy	Total 2000 r.	Recurrence (1 year after) Re-operation
9	1.6 2.8	M	Knee	Diffuse Synovitis	None	4 months 14 months	Bloody	193.6 155.5	Normal	Partial Synovectomy Total Synovectomy	—— ——	Recurrence (1.6 years after) Re-operation No rec.
10	62	F	Knee	Diffuse Synovitis	None	10 years	Bloody	232.2	Osteoathritic changes	Partial Synovectomy	——	No rec. (4.1 years)
11	64	M	Knee	Diffuse Synovitis	None	2 months	Bloody	198.7	Normal	Total Synovectomy	——	No rec. (1.7 years)
12	70	M	Hand, Wrist Elbow	Multiple lesions	None	18 years	Bloody	185.6	Osteoathritic changes	Removed Bursa	——	No rec. (1.7 years)
13	39	M	Knee	Diffuse Synovitis	None	2 years	Bloody	218.3	Osteoathritic changes	Total Synovectomy	——	No rec. (1.3 years)
14	23	F	Knee	Bursitis	None	5.8 years	Bloody	191.0	Normal	Removed Bursa	——	No rec. (5 months)
15	24	F	Knee Ankle	Teno-synovitis Teno-synovitis	None	3 months	None	191.4	Normal	Removed Tenosynovia	——	No rec. (10 months)
16	71	M	Hand, Wrist Elbow	Multiple lesions	None	10 years	Turbid-yellowish	178.5	Osteoathritic changes	Total Synovectomy	——	No rec. (6 months)
17	25	M	Hip	Diffuse Synovitis Bursitis	None	5 years	Brownish-colored	136.7	Osteoathritic changes	Total Synovectomy	——	No rec. (5 months)
18	30	F	Knee	Localized Synovitis	None	10 years	Turbid-yellowish	172.8	Normal	Partial Synovectomy	——	No rec. (5 months)

Table 2. Incidence of individual cells in P.V.S. and P.V.B.

Finding \ Case	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Villi-formation	###	+	+	−	###	+	###	###	###	###	##	###	###	##	###	+	###	##
Lining cell (100×) proliferation	###	+	+	+	###	±	###	###	###	###	###	##	###	###	###	##	+	###
Giant cell (100×)	0	2	6	1	0	2	5	8	1	0	1	0	2	1	0	3	5	1 *
New (100×) vascularization	13	6	3	6	30	8	6	8	28	18	21	16	8	13	21	9	7	10 *
Foam cell (400×)	0	18	4	20	9	8	4	35	1	0	0	0	16	5	3	22	1	12 *
Cholesterol cleft	0	11	2	0	0	1	0	0	0	0	0	0	3	0	0	1	0	7 *
Hemosiderin-laden cell (100×)	###	###	±	##	##	###	###	###	###	###	###	##	###	###	+	###	###	###

* Cell-Counts in 45mm²

LIGHT MICROSCOPIC AND ELECTRON MICROSCOPIC OBSERVATIONS

a) Findings in a site of villi-formation

It was found to have fibrocellular proliferation consisting mainly of F-type of synovial lining cells (Fig. 1). The electron microscopic observation of individual cells showed no significant difference from rheumatoid arthritis and osteoarthritis. (reported by the present author et al[18,16]). No characteristic findings in F-type of cells except numerous siderosomes and scattered hemosiderin granules in cytoplasm were found (Fig. 2). Innumerable filopodia and pseudopodia of lining cells were protruding into the joint cavity, and the interdigitation of these cellular processes coincided with the finding of synovial clefts under the light microscope. In addition, few histiocytes, lymphocytes, erythrocytes, and fibrinlike substances were observed in the lining cell layer.

New vascularization was conspicuous, but this will be described later because this can be seen frequently in nodules.

b) Findings in a site of nodule-formation

Histological views and their cellular incidence were notably different from those of the villous region. Its light microscopic finding was similar to that of the granulation tissue, being represented by remarkable vascularization, foam cells, giant cells, macrophages, fibroblastic cells and collagenization (Fig. 3-A, 3-B, 3-C). Plasma cells, lymphocytes and leucocytes were scarecely observed in their intercellular space. But these findings were also subject to some differences in location where were included a great variety of cells, such as sudanophilic cells and groups of the cells stained with Berlin-blue. In the weight-bearing synovial joint, contrary to bursa, the complex mesenchymal reactions are caused by the high irritability and hypersensitivity of the tissue. Out of these findings, only those which have been historically reviewed as a pathogenetic point will be described in the following:

(i) New vascularization

This is observed in all cases. The endothelial cells of newly formed blood vessels have fine stellate cytoplasmic processes, which interdigitate to form luminal tubules (Fig. 4). Some of observation include erythrocytes which are immigrating from these tubules and also are scattering into the extravascular region. There is a tendency that the new vascularization is often found in a cluster of foam cells or that it invades such a mass. Electron microscopic observation on the endothelial cells was characterized by the findings of its basement membranes and perivascular sites. The luminal tubules are rather widened and vessel walls are quite thin. The connection between endothelial cells are loose, and sometimes microvilli are observed on luminal surfaces. Cytoplasm are generally bright and the development of organelles is poor. The pinocytotic granules are scant and lipid granules are never found in perivascular cells, as will be described later. But such lipid granules are observed neither in endothelial cells nor in smooth muscle cells of larger arterioles. In newly formed blood vessls, the basement membrane was poorly

developed and unable to be clearly visible. As in rheumatoid arthritis and the other joint inflammation, none of the amorphous electron-dense fibrinlike substance, lymphocytes, plasma cells nor leucocytes were observed around these vessels.

(ii) Multinuclear giant cell

In some cases innumerable giant cells are found so that such cases can sometimes remind the entity of "giant cell synovioma[6]", but in other cases these cells are scarcely observed. The cells are variable in size, with the number of nuclei ranging from several to more than ten. Under the light microscope, no inclusions which could be stained by the sudan III and Berlin-blue method were found in their cytoplasm. The site where the giant cells appear is indefinite. They did not appear in the lining cell layer, but they did appear diffusely in the deeper layer with the coarse collagenization.

Under the electron microscope, the giant cells form innumerable filopodia, and well developed intracellular Golgi apparatus and smooth surfaced endoplasmic reticulum are observed. A lot of free ribosomes and numerous mitochondria are found in the entire cytoplasm. As in light microscopic examination, neither siderosomes nor lipid granules are observed. But sometimes phagosomes of dead cells are observed as shown in the figure.[14] In the intercellular space, giant cells are also observed around cholesterol crystals which seem to have been precipitated and agglomerated, but the giant cells which include indigested cholesterol crystals were not observed elsewhere.

c) Foam cell

A large number of foam cells which were called xanthomatous cells[5] are observed in a cluster, but in some sections these cells are scanty. There was a case where the section from the first operation showed only a small number of these cells, while in the repeated operation carried out three years later, innumberable foam cells were observed, accompained by the growth of nodules. The incidence of these cells seems to be high in the section from large nodules, but it is sometimes difficult to identify such foam cells under the light microscope. Therefore, it is required to select the adequate sections preliminarily by staining with toluidin blue.

The size of foam cells is variable, ranging from the immature cells of $1.2\mu \times 3.4\mu$ to large differentiated or mature cells having a diameter of more than 20μ. Immature cells are spindle-shaped or oval, as shown in the Fig. 6-A. They gradually differentiate to a globular form. It is immposible to find out small foam cells under the light microscope because of its limited resolving power.

Sometimes immature cells are indistinguishable from undifferentiated mesenchymal cells or from fibroblastic cells. In the electron-microscopic view, the large mature cells which show more than 20μ in diameter include a large quantity of lipid granules in their cytoplasm (Fig. 6-B). Therefore, the futher details of each foam cell which was in the process of development should be described. These cells have a tendency to scatter when they are immature but they tend to cluster together as they are maturing. Of course, the cells in a cluster are those

in various stages of development.

Irrespective of their maturity, dense collagen fibers are observed around the foam cells, forming the matrix of these cells. Sometimes these extracellular matrices directly envelope the cell. Such a group of foam cells rarely contains inflammatory cells, but new vascularizatisn is often observed. Immature cells usually have a few tongue-shaped or pseudopodia-like cytoplasmic processes and they gradually change to globular or polyhedral mature cells, which have minute filopodia and microvilli. These cells are interwined with each other by these processes, but they do not have any desmosome. In their cytoplasm there are many electron-dense lipid granules of various sizes and electron-lucent granules surrounded by a single membrane. The latter is vesicular and its content is considered to have been dissolved out by organic solvents during tissue-dehydration process. In some of their lucent granules, there remains less dense homogeneous substance (Fig. 6-B). The authors named the former "P-ch granules" and the latter "Ve granules"[14,17] (Fig. 6-B, 7-A).

A P-ch granules is oval or round or in some instances polygonal formed, having various diameters up to 2μ, and they are found in any of the immature (Fig. 7-B), intermediate (Fig. 7-A) and mature type of cells (Fig. 6-B, 8-A). In a higher magnification of these cells, these granules were seen like the collection of electron-dense homogeneous fine granules. Generally such a granule is enveloped by a single membrane such as lysosomes, but very small granules have an obscure boundary, looking like pinocytotic granules. It should be noted that these are all fundamental structures but many granules seem to show polymorphous changes, corresponding to the cellular functions and its maturity. In these granules there appear denser, speck-like granules or substance showing indeterminate structure. Also concentric circle, onion-like figure and spiral structures or the myelinated structure are observed in some sections, as shown in figure 6-B. In this membraneous figure, there appear less-dense homogeneous small vesicles, or sometimes also electron-lucent small vacuoles. It was the most interesting finding that there are electron-lucent, rarely less dense areas in these granulus showing some crystals of rectangular, diamond-shaped, or needle-like form. Such crystals show a minimum width of 0.05μ but mostly a width of 0.1μ and sometimes of 0.2–0.6μ (Fig. 7-A, 7-B). Generally no unit membrane is found around such figures. The maximum length of crystals is 2μ, but depending upon the direction of section, various features appear. These figure are considered to be cholesterol crystals which have been dissolved out during the dehydration and sectioning. Granules containing a number of crystals sometimes are variable such as a circle, lozenge or polygonal. A P-ch granule commonly contains several crystals. Large foam cells having a diameter of more than 20μ contain fewer P-ch granules than immature cells do. According to this observation, an immature foam cell having a size of $1.2\mu \times 3.4\mu$ already contains such crystals, as shown in the figure 6-A.

The Ve-granule, on the other hand, is electron-lucent and is enveloped by a single membrane. In contrast to P-ch granules, few homogeneous, electron-less-dense substances in these granules can be observed (Fig. 8-A, 8-C). It is also probably that the substance in Ve-granules has been dissolved during the sectioning

process. Ve-granules are scarce in immature cells, while in large cells they occupy the entire cytoplasm. Occasinally not only the nucleus but also other organelles are compressed by these granules. They are round or discoid shape and the same size as P-ch granules, ranging to a maximum of 2μ. But some of the granules fuse together and became larger. When the granules grow larger, they are visible under the light microscope. Therefore, these cytoplasmic granules are considered to be cholesterol esters which is soluble during alcoholic dehydration processes.

In immature cells, the nucleus is large as compared with the cytoplasm in amount and has a renal shape or 2–3 indentations (Fig. 9). With maturation, the nucleus becomes ovoid or round and is eccentric in location. In maturing cells, chromatin granules are clearly visible and are accumulated along the nuclear membrane. In mature cells having a diameter of more than 20μ, the nucleus is small as compared with the cytoplasm in quantity and the nucleoplasm becomes homogenous. The nucleolus is often seen in cells which are in a process of maturation, normally as a thread-like structure. Golgi apparatus and other cytoplasmic organelles are poorly developed in immature cells. Nevertheless, P-ch granules and Ve-granules are sometimes observed in this stage (Fig. 9). As cells gradually mature, however, well-developed Golgi apparatus and other organelles are observed. Especially rough surfaced endoplasmic reticulum is highly developed in foam cells which are maturing and show a variety of P-ch granules, and they sometimes show cystic enlargement. Free ribosomes are abundant in a series of these cells, but agranular vesicles are few. Mitochondria having rod-like or oval shapes are few. They are mostly round. Immature cells have not so many mitochondria and they increase their number as the cells mature. Cristae are arranged somewhat radially. This is characterized by its small number, shortness in length and less dense matrix (Fig. 7-B). They collect together sometimes around P-ch granules. In cells more than 20μ in diameter, cytoplasm is entirely filled with Ve-granules, the number of mitochondria become fewer and mitochondria as well as the Golgi apparatus are compressed and not clearly visible.

d) Fibroblastic cell

Besides the foam cell, fibroblastic cells are another type of cell showing the most vigorous activity in this disease. Both rough surfaced endoplasmic reticulum and Golgi apparatus are well-developed and are the same as those observed in other type of connective tissue diseases (Fig. 10). But sometimes less dense, homogeneous substance is contained in the rough surfaced endoplasmic reticulum, which therefore must be distinguished from the immature type of foam cells containing more dense lipid granules.

e) So-called hemosiderin-laden cell

In light microscopic observation, blue-stained granules or mass can be seen in cytoplasm which has been stained with Berlin-blue. Such granules are often observed in the F-type of lining cell (Fig. 1, Fig. 2) and histiocytic macrophage of the synovia and bursa. But they are not observed in the giant cells or in the aforementioned foam cells. In electron-micrograph, these granules appear as the

siderosomes of the cytoplasmic inclusions which have a clear-cut unit membrane. Often they appear as irregular collections of fine electron-dense granules. These are hemosiderin granules originating from erythrocytes which have been caused by intraarticular hemorrhage. Unlike P-ch granules contained in foam cells, this type of granules has a large size, form the electron-dense and irregular mass enveloped by a unit membrane and do not show any lamellar structure like a myelin figure.

f) Extracellular lipid granules

Frozen section treated by sudan III or cholesterol-staining represents the existence of extracellular lipid and cholesterol crystals, whereas the paraffin section shows only the so-called cholesterol cleft like a needle, lancet or oblong. The extracellular cholesterol cleft observed under the light microscope shows a fairly largs size and various shape. On the electron microscopic observation (Fig. 11), free lipid granules could not be observed in those matrices but electron-lucent areas of more than 2-3μ in width was recognized as corresponding to the above-mentioned cleft, surrounded with giant cells and large phagocytes. An electron micrograph of pure cholesterol crystal was measured in the figure, according to which the crystal is rectangular of 0.05μ in width.[14] The mass of granules appearing in the extracellular matrix are probably of accumlations of such cholesterol crystals.

BIOCHEMICAL ANALYSIS OF LIPID, CHOLESTEROL IN AFFECTED TISSUE

Biochemical analysis of lipid and cholesterol in affected tissues were undertaken. Some of these resected tissues were homogenized, and this was soaked in a mixed solution of absolute alcohol (50%) and ether (50%) for 2-3 days. The extract was evaporated and the dried residue was analysed to measure the total quantity of free cholesterol and esters. As the results are already reported before,[14] this indicates that the tissue-cholesterol content in these diseases is much higher than that in the other diseases.

Further, the X-ray diffraction of the residue of this extract was examined for comparison of crystal shape with free cholesterol crystal, but typical cholesterol diffraction curves were not obtained. This may be attributed to the contamination of lipid and cholesterol ester. Then a qualitative analysis of lipid was conducted by the thin-layer chromatography. The result indicated that principal components as phospholipid were lecithin, sphingomyelin and lysolecithin, while as a neutral lipid, the main component was cholesterol, followed by cholesterol ester and then free fatty acid.

DISCUSSION

This disease has been given a variety of names. This may be due to its unknown etiology. Xanthoma, PVS and recently, resorption granuloma, fibroblastoma giantocellulaŕe pigmentosa and giant cell tumor, are synonyms. The advanced study

of its pathogenesis was described in some of the reports.[4,5,6,14,19,20,35] But the reporters differ in opinion. In spite of this, the disease now has a history of 100 years. Its pathogenesis is roughly divided into inflammatory and neoplastic hypotheses.

Prior to Jaffé's report[19,20] the cause of this disease was already discussed. De Santo[5] made a pathological study of 41 cases and Galloway[9] reported detailed biochemical investigation about 68 cases. Later, Young[40] and Volz[36] attempted to clarify the pathogenesis of this disease by animal experiments. These did not produce definite conclusions, but were important as clinical researches.

Some people gave the name "xanthoma[5]" as it is found in multiple lesions of the tendon sheath of the hand. From the viewpoint of the clinical findings as well as pathological and biochemical findings in these cases, the origin of these diseases may be regarded as the same one. The synovial and bursal tissues are genetically of mesechymal origin and belong to reticuloendothelial system (RES) as we have already reported. In this regard we would agree with Jaffé's opinion[19,20] that this disease occurring in the bursa, synovia and tenosynovia should be put into the same category. However, this disease should be distinguished from the disturbance of systemic lipid metabolism such as xanthoma multiplex tuberosum[7,18,39] (to be discribed later).

The author has been carrying out pathological researches over years on synovia, bursa and tenosynovia in various diseases.[13,14,15,16,21] Now we should continue to discuss the pathogenesis of this disease. Is it due to the metabolic disturbance or inflammation or tumor itself? This is a subject of my present study.

The author made detailed cytological studies of the 18 cases, referring to clinical findings. The histological findings show no great difference between the lesion of synovia and that of bursa, but a little difference is inevitable, depending upon the stage of disease and the site of specimens obtained. Jaffé[20] who reported twenty cases and named PVS stressed that villi- and nodular formation are most prevalent in synovial membrane. But these changes are not the specific finding of this disease. "The synovial cleft was named by Collins[4] and he described the origin of the stromal cell which is from the synovial cell. The villi-formation is a result due to interdigitation of the cytoplasmic processes. The siderosome in cytoplasm is nothing but the inclusion of hemosiderin after hemorrhage. Such siderosome has often been observed in hemophilic arthropathy reported by us.[21] Therefore, the villi-formation is histologically an unspecific finding and does not show the cardinal synovial change.

Thus the cytological examination of this tissues should be carried toward the lesion of nodule-formation. In other words, the pathological study should be directed to the appearance of new vascularization, foam cell, giant cell, many undifferentiated mesenchymal cells and fibroblastic cells.

First of all, the author should turn the attentions to study of foam cells containing innumerable Ve-granules and P-ch granules in their cytoplam; the foam cell being a large, sudanophilic cell having a diameter of 30–40μ. These cells have been found in great numbers in the affected tenosynovial membrane of the hand and have been called a "xanthoma cell."[5] However, it is well known that these

cells can be found also in other than the subject disease. For example, in various metabolic bone disease, leprosy, xanthoma multiplex tuberosum, in the aortic wall in atherosclerosis[2,8,10,27,33] and in degenerated renal tissue.[25] However, these diseases are not a local affection like this but are mostly accompanied by systemic lipid-metabolic disturbances. Furthermore, this type of cell, in spite of extensive investigation in various diseases, still remains as a controversial problem as to its orign. Some reporters were of the opinion that the foam cell is a cell of modified RES and able to derive from the synovial cell[4,5,6] and others assumed its origin from macrophages and fibroblasts.

Bozdéch[1] reported that in fibroblastic cells stained with sudan III, lipid was observed but hemosiderin was not. The author's findings also revealed that foam cells do not belong to macrophages, contrary to Volz's report,[36] because they do not have phagocytic ability. These electron microscopic observations were undertaken on the cells from the immature to the mature. The findings indicate that as reported before[14] in the table 2-a, 2-b, immature cells resemble fibroblasts in size and shape but are different from them in cytoplasmic structure. In this respect, the immature cells described by the author coincide with the stromal cells originating from undifferentiated mesenchymal cells as reported by De Santo.[5]

Therefore, the author is of the opinion that the undifferentiated fibrocytic cells should be regarded as the origin of foam cells, as proposed by Larmon.[24] It is thought that under some factors and conditions, the primitive mesenchymal cells differentiate to many types of cell through their development, one of them is a differentiation to foam cell. Among other related diseases, the researches are most advanced in the formation of atheromata in the aorta and in their lipid-cholesterol metabolism. However, also in these subjects there are three different opinions concerning the origin of foam cell:

(1) The origin of foam cells is the smooth muscle cells of undifferentiated cells or tunica media.
(2) Its origin is the lymphocyte or the monocyte in blood.
(3) Foam cells are differentiated from endothelial cells themselves (Friedman[8] Still,[33]).

In 1958, Uehlinger[35] discussed in his report the pathogenesis of this disease. He stated that the cytogenesis of these lesions should be clarified in the near future by biochemical investigation including electron microscopic examination and radioisotopic study etc.. After his report, much brilliant work was performed and some of them are as follows: Pollack[27] tried to identify the origin of foam cells as the endothelial cells and macrophages by means of tissue culture; by using electron microscope, Buck[2] observed the foam cells in the tunica media of experimental atherosclerosis; Geer[10] suggested that monocytes and lymphocytes migrated from blood stream and endothelial cells, as well as smooth muscle cells in human aortic wall can be all differentiated into foam cells. Simonton,[31] who used radioisotope could not determine its origin. In experimental animals fed on cholesterol-diets, Wilson[39] succeeded in making xanthomata similar to human xanthomata by using a small skin clip. Accordingly he proposed a fibroblastic origin for the foam cells.

Recently, one of author's co-workers, Kumon[22] has been done experimental studies about this subject, (by modified Wilson's feeding experiment) and he obtained the findings of xanthomatous changes in the rabbit synovial membrane. He presented the foam cells in his materials similar those in PVS. It was his opinion that the undifferentiated mesenchymal cells almost will be capable to develop into the foam cells under the abnormal circumstance of lipid-cholesterol imbalance.

Many other theories have been proposed concerning the origin of foam cells, but opinions are quite different. There are many reports which try to establish some relation between the foam cells and systemic lipid-metabolic disturbance (Fletcher[7]).

In the present cases, the proliferation of this type of cell was always accompanied with the elevation of tissue-cholesterol values,[17] while cholesterol values in blood was within normal limit. Mittelmeier[25] is in agreement with the author's finding and has stated that the foam cells were found without elevation of cholesterol value in blood. Fletcher[7] reported that in case of xanthoma in skin, the lipid and cholesterol value in blood and in tissues both increase almost in parallel. But xanthomata of skin must be discriminated from the subject disease. In these cases it should not be denied that there is local disturbance of lipid metabolism in affected tissue, which has some relationship to foam cells.

The electron microscopic observation of foam cell showed many findings relating to lipid metabolism. In the past the only report made is Imaeda's on xanthoma multiplex tuberosum[18] in the field of dermatology. His findings are similar to those by the author; Ve-granules corresponding to his vacuoles. But he concluded that the localization of cholesterol and its ester was unknown. As regards the foam cell in PVS, we were the first to carry the electron microscopic observation.[17] Of course, no reports have been made to clarify the differentiation of foam cells. As already described, author's findings indicated that the P-ch granules are polymorphic in structure through various stages of maturation. Based on this, the author is wondering whether such morphological changes are due to the biosynthesis of lipid-cholesterol in the cell.

The observation of cholesterol crystal itself under the electron microscope was reported by Carsten and Merker.[3] They observed this crystal in a form of a lancet having a length up to 2μ and a width up to 0.5μ in cells of adrenal cortex of hypophysectomized rat and human corpus lutea. Seifert[30] observed needle-like crystals in the aortic wall of rabbit. But both of these crystals were electron-lucent. There is no other report that cholesterol crystal was observed in foam cell.

As previously stated, the author considered that the electron-lucent area, 0.01μ in width and 2μ in length observed in P-ch granules in the foam cell, contained cholesterol crystal which were dissolved out by organic solvent during dehydration and viewed as an electron-lucent area. The reasons for this presumption are:

(1) By cholesterol-staining method, the cytoplasm of foam cell as well as crystals of matrix are stained green in frozen section[17] and shows double refraction by polarizing microscope (Fig. 3-c), while in paraffin section the crystals of

matrix are not stained green. (dissolution by alcohol).

(2) The same is observed as that in Carsten's adrenocortical cells or in corpus lutea cells.[3]

(3) Pure cholesterol crystals[14] observed with the electron microscope are the same in width and shape as the abovementioned figure in foam cells.

(4) According to the chemical analysis of tissues[17] performed by the author elevation of tissue-cholesterol value was found in company with the foam cell proliferation. Therefore, it is impossible to associate the figure with other crystals.

If the crystal figure in P-ch granules of foam cells is actually cholesterol one, it would be the first presentation in these cells of the subject disease and will give priority to this cytogenetic study.

Also the large extracellular electron-lucent figure forming a mass should be regarded as cholesterol crystals precipitated into the intercellular matrix. It has been said that the cholesterol in the human body is mostly taken from diet and that the biosynthesis of cholesterol is held mainly in liver, from acetic acid as its source, under the action of water-souluble coenzyme, through mebabulonic acid and squalene to cholesterol. In disaccordance with this theory, however, cholesterol was observed by the author in the abovementioned foam cells, and this fact forces us to presume that cholesterol is synthesized even in the synovial and bursal tissue which belong to RES.

Of course, such figures are not observed in normal and other affected tissue. Therefore, the presence of cholesterol crystal and ester in cells suggests some morbid changes in the tissue, for example, local disturbance of lipid metabolism. Meanwhile, Jaffé[19,20] remarked that such cholesterol was found in the florid stage and was not considered to be the cause of the affection.

Under these circumstances, more advanced study would be required regarding P-ch granules and Ve-granules classified by the author.

P-ch granules present a myelin-figure in some sections. This figure is the phospholipid described by Stoeckenius.[34] This material is capable of reducing osmic acid and forming a lamellar structure. Recently, this was thought to be a kind of lysosomes which was named as the residual body.[32] Bases on the fact that cholesterol and phospholipid was contained in them, the reason for this naming of P-ch granules is evident. However, people must consider the possibility that these P-ch granules, Ve-granules and intermediate granules are from the by-products through the process of intracellular digestion (lysosomal activity).[32] In spite of a lysosome demonstrates polymorphism, depending upon the cell-type and cellular activity, Ve-granules were named for their origin on the presumption that they contain cholesterol ester, according to the following facts: These granules occupied the entire cytoplasm of large mature cells, and were dissolved out by alcoholic dehydration and became electron-lucent. They are sudanophilic and stained green by cholesterol-staining and in addition show double refraction by polarizing microscope but do not show crystal appearance. As already described, both type of granules have close structural relation with each other, P-ch granules are gradually developing to Ve-granules while increasing their size. In other words, it may be considered certain that phospholipid, and other lipids in addition to cholesterol,

are also accumulated in these granules accompanied with lysosomal activity.

Regarding biosynthesis of these substance, Wilson[39] examined the synthesizing ability in two cases of xanthoma multiplex tuberosum where high cholesterol value in blood was recorded, by using respective radioisotopes (cholesterol 14-C^{14}, $Na_2HP^{32}O^4$), phospholipid and fatty acid were synthesized in xanthoma conspicuously and cholesterol was synthesized little. He reported in this case that cholesterol was synthesized rather in epidermis than in skin but mostly came from blood stream. His verification of the fact that cholesterol and phospholipid are synthesized, though not much, in these cells is really unique and deserves serious attention from all those concerned.

What is then the source materials of cholesterol synthesis? If cholesterol is really synthesized in the affected tissue, its source must be searched after. Earlier De Santo et al.[5] presumed that the origin was hemosiderin or hemoglobin. This idea was based on hemorrhage. But there are actually some cases where no hemorrhage is observed. Therefore, hemosiderin and hemoglobin cannot be regarded as the source of local accumulation of cholesterol.

In the present paper, this problem can be answered merely by presumption. Probably cholesterol is produced from a part of the P-ch granules or from lipid which has been taken into the cell by endocytosis in a form of chylomicron (A granule of 0.3–1.5μ in diameter, composed of triglyceride (80%) and phospholipid, cholesterol, protein; Wasserman[38]) as suggested by Geer[10] and accumulated in cytoplasm as an end-product.

After all, in view of the structural and morphological change of P-ch granules and Ve-granules as well as the process of cell differentiation, the author presumes that cholesterol is accumulated in the cell under the action of cytoplasmic enzyme such as lysosome. It is impossible that cholesterol is synthesized by transformation of protein or carbohydrate, considering the function and ability of immature cells where cholesterol crystal is observed.

The present study also suggests two aspects of this diseases: some cases where inflammatory changes was predominant and some other cases which impress a tumor. And such findings were the cause of confusion in pathogenetical study of this disease. Even Jaffé's inflammatory theory is not a well-founded one but a vague opinion. Nevertheless, his naming is today accepted among orthopedists and pathologist without much conviction. The author is of opinion that the inflammation is a secondary sign attributable to the connective tissue reaction, caused by local disturbance in lipid metabolism. The hemorrhage which is one of the cardinal signs is due to the fragility of new vascularization produced as a result of abnormal lipid-cholesterol accumulation. The histological appearances are modified by this hemorrhage and the discharge of cholesterol out of the cells. In other words, the inflammtory change may be considered as the results of such "circulus vitiosus". The hemorrhage has no direct relation with the cause of this disease. It is obvious from the results of biochemical investigation reported in our published literature[19] and from the histological findings obtained from the cases of hemophilic arthropathy.[21] Furthermore, the foam cell described above were not observed in the

authors' extensive resarches on the synovial tissue in osteoarthritis and rheumatoid arthritis. Therefore the abovementioned findings can never support the inflammatory theory.

The present clinical, histological and biochemical studies on this subject may be summarized as follows:

(1) There is a tendency of reccurence.

(2) The tumorous development is closely related to the existence of appearance of fibroblasts, immature and mature foam cells. In case of reccurent or long-standing PVS and PVB, foam cells have a diameter of more than 20μ appear in a cluster, and collagen fibers are proliferated.

(3) There are always a number of foam cells observed in affected tissue which has a high elevation of lipid and cholesterol value. In cytoplasm of the foam cells, P-ch granules and Ve-granules show the polymorphic and structural changes as the cells mature and the findings which suggest a biosynthesis of lipids and cholesterol.

(4) There is no evidence which indicates any systemic disturbance of lipid metabolism, as in the case of xanthoma.

(5) There are no such atypia, mitosis, or abnormality of nucleus as observed in genuine tumor.

These findings indicate that the subject disease has a fairly noticeable aspect of a tumor. This disease should be regarded rather as the disturbance of lipid metabolism caused by abnormality of mesenchymal cells. To the special tissue reaction is added a vigorous fibroblastic activity in synovial tissue. From these studies, the author impressed that this disease should be given the name "foam cell granuloma". In this disease, the foam cells play a great role.

SUMMARY

The author has presented in this paper 18 cases of the subject disease comprising 14 cases of PVS and 4 cases of PVB (pigmented villonodular synovitis and bursitis), with the age of patients ranging from 18 months to 71 years. The light microscopic, electron microscopic and biochemical studies on the extirpated synovial tissue and bursa were undertaken to verify and discuss the pathogenesis of pigmented villonodular synovitis and bursitis.

(1) Cholesterol value in blood was normal, but in eight cases out of nine where cholesterol value was measured in affected tissue, the value of cholesterol in tissue is highly elevated.

(2) Foam cells are various in numbers, depending upon the site of tissue sectioned and upon the stage of disease. Tissues containing many foam cells show high value of cholesterol; the elevation being in parallel with the number of foam cell.

(3) It has been found that the foam cells are in a series of maturity. The origin of these cells is not macrophage but undifferentiated mesenchymal cells.

(4) Discrimination was made between P-ch granules and Ve-granules which were observed in the cytoplasm of foam cells. The former are observed mostly

in immature and intermediate type of cells, showing changes morphologically as they are maturing. In these granules are observed cholesterol crystals having a minumum width of 0.05μ and phospholipid. This phospholipid appears in large cells having a diameter of 20μ. The latter is supposed to contain cholesterol ester.

(5) The foam cells are presumed to be the cells in which the biosynthesis of phospholipid and cholesterol is occuring, but the source material of this product remains unknown.

(6) The inflammation is a secondary mesenchymal reaction and the joint hemorrhage is attributable to the fragility of new vascularization caused by local disturbance of lipid metabolism.

The subject disease should be called "foam cell granuloma" which shows noticeable tumor-like aspects and causes local disturbance of lipid-cholesterol metabolism.

ACKNOWLEGMENTS

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EXPLANATION OF THE FIGURES

Fig. 1. The synovial villi consist mainly of F-type of lining cells (SL) which include numerous dense siderosomes and scattered hemosiderin particles (Ss) in their cytoplasm accompanied with abundant rough surfaced endoplasmic reticulum.

Fig. 2. High-power magnification of Fig. 1. A single membrane-bounded siderosomes (Ss) and scattered hemosiderin particles in cytoplasm are well identified.

Fig. 3. The light and polarizing microscopic pitctures of individual cells appeared in PVS.
A) Giant cells, macrophage, fibroblastic cells are seen at the surface area.
B) In nodules, where are a cluster of foam cells, rich vascularizations are observed.
C) Using the polarizing microscope, cytoplasmic inclusions in foam cells show the typical double refraction which prove cholesterol crystal and its ester.

Fig. 4. The endothelial cells (Ec) of newly formed blood vessels have fine stellate cytoplasmic process, which interdigitate to form luminal tubules (Lm).
The pinocytotic granules and lysosomes are scant and lipid granules are never found in perivascular cells.

Fig. 5. The giant cells are variable in size, with the number of nuclei ranging from several to more than ten.
A lot of free ribosomes and numerous mitochondria are found in their cytoplasm. Neither siderosomes nor lipid granules are observed.

Fig. 6. A) Electron microscopic picture of immature type of foam cells which are spindle-shaped or oval. Well-developed rough-surfaced endoplasmic reticulum, lipid droplet and so-called P-ch granules are observed in cytoplasm. This cell and undifferentiated mesenchymal cells used to be surrounded by coarse collagen fibers.
B) The mature type of foam cells which show more than 20μ (P-ch↑↑) and vesicles (Ve) in cytoplasm. These cells contact with each other by interdigitation of cytoplasmic process (CP).

Fig. 7. A) Intermediate type of foam cells. A lot of crystal-like extraction in so-called P-ch granules are observed.
B) Usually, the isolated immature type of foam cells are surrounded by dense collagen fibers. They have a few P-ch granules, Ve-granules and large swollen mitochondria with fewer cristae.

Fig. 8. A) The picture showing the immature (IMF) and mature type (MF) of foam cells. Despite of dehydrating process, some of contents in granules are well preserved.
B) High-power magnification of the so-called P-ch granules. The electron-lucent areas in granules show rectangular, diamond-shaped or needle-shaped or needle-like form. Such crystals which are thought to be extracted, have a minum width of 0.05μ but mostly a width of 0.1μ and sometimes of 0.2–0.6μ. Their maximum length is 2μ but depending upon the direction of section.
C) High-power magnification of the so-called Ve-granules. These electron-lucent Ve-granules usually are enveloped by a single membrane, and few homogenous, electron-dense substance in these granules can be observed. Some of the granules fused together and became larger.

Fig. 9. In immature type of foam cells, the nucleus is large as compared with the cytoplasm in amount and has a renal shape or 2–3 indentations. So-called P-ch granules can be seen even in this type of cells. However, other organelles are few. This cell should be distingunished from younger type of fibroblastic cells.

Fig. 10. Active filroblastic cells which show both well-developed rough surfaced endoplasmic reticulum and Golgi apparatus. Sometimes less dense, homogenous lipid-droplet is included in cytoplasm.

Fig. 11. The extracellular cholesterol cleft observed under the light microscope shows electron-lucent areas of more than 2–3μ in width, which is recognized as corresponding to the light microscopic findings. This cleft is surrounded wth giant cell or macrophages as seen in this picture.

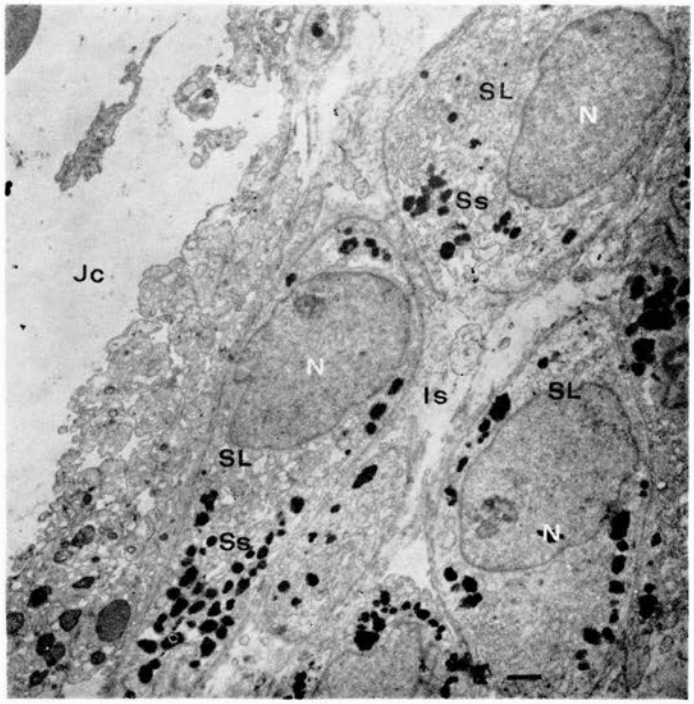
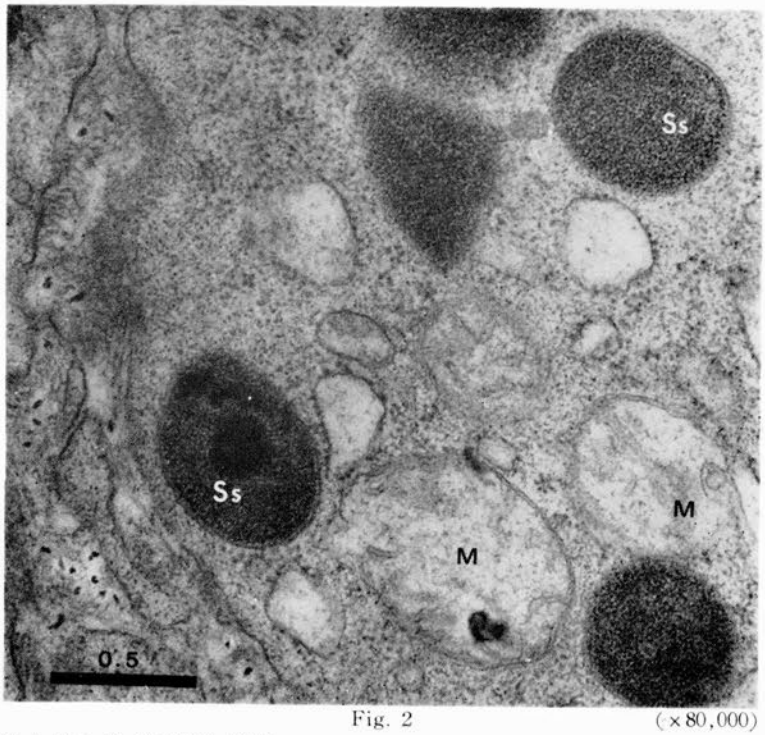


Fig. 1 (×8,000)



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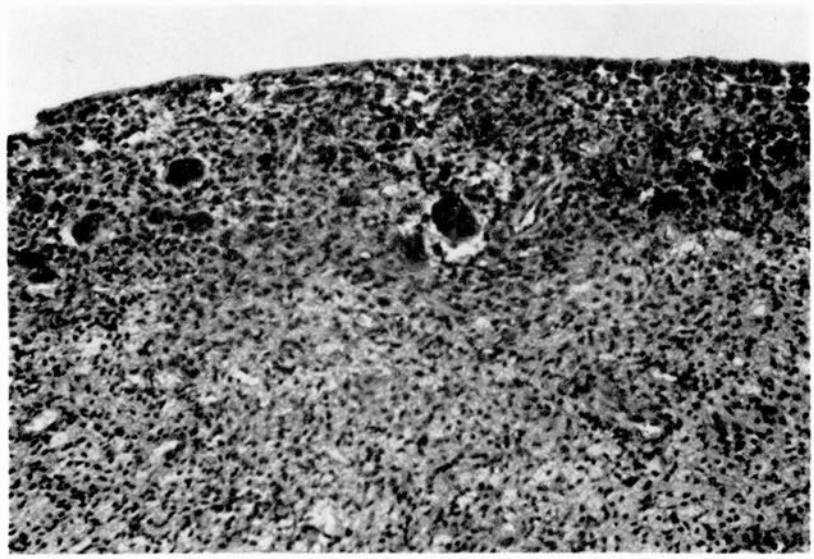


Fig. 3-A (×200)

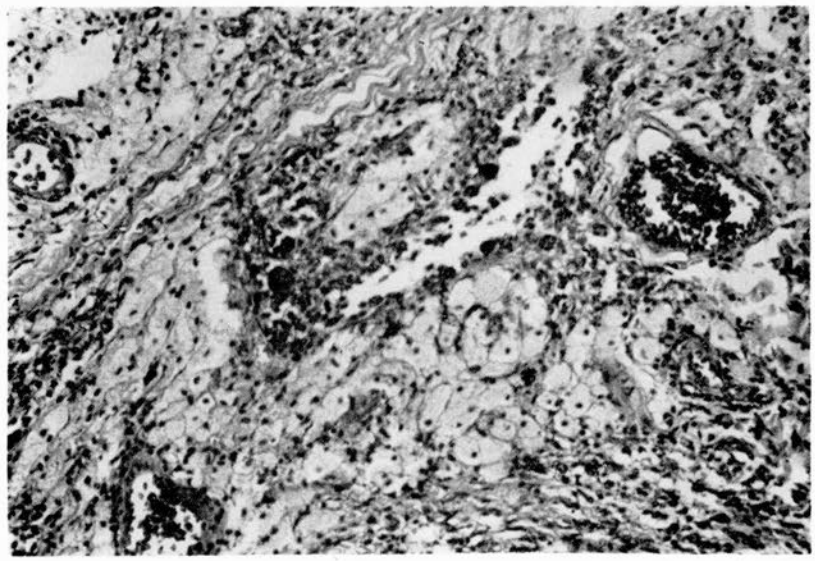


Fig. 3-B (×200)

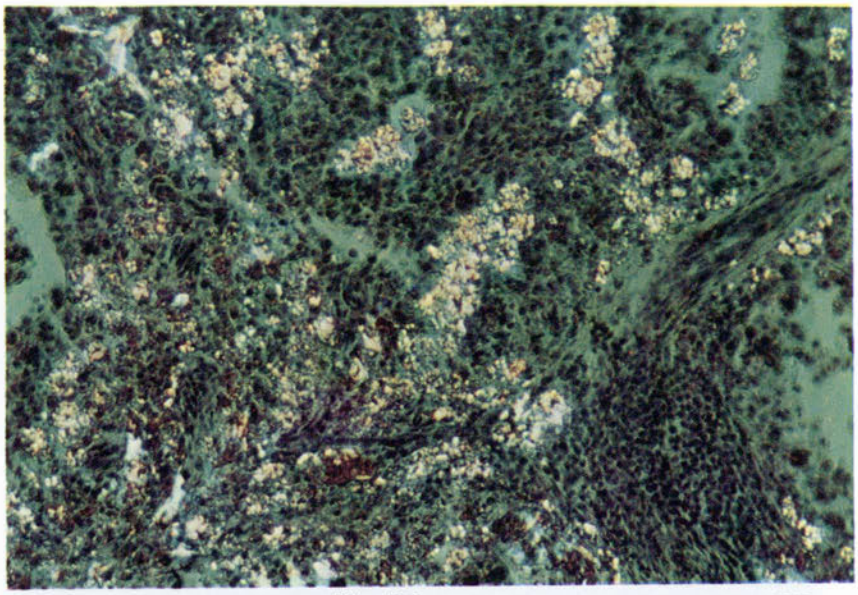
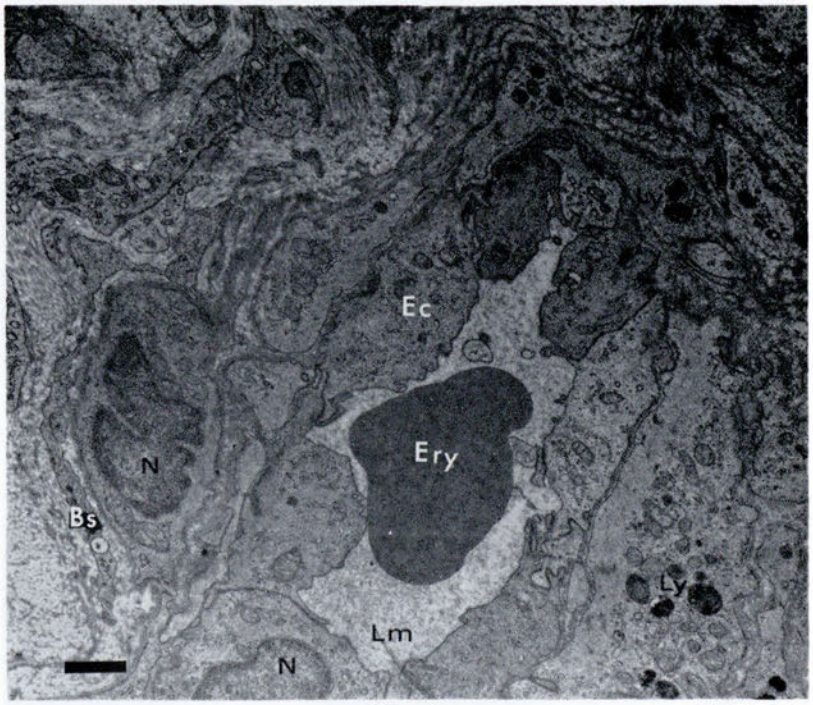


Fig. 3-C (×200)



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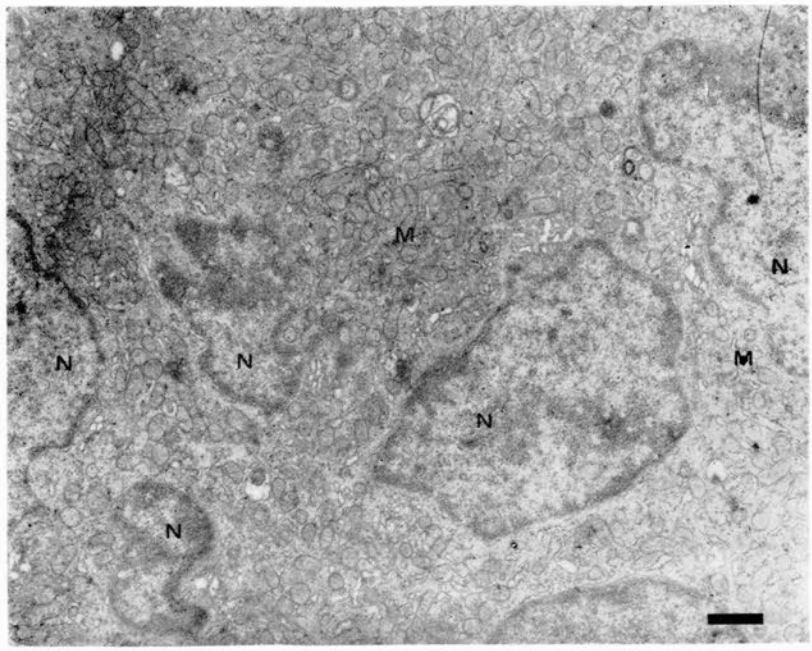

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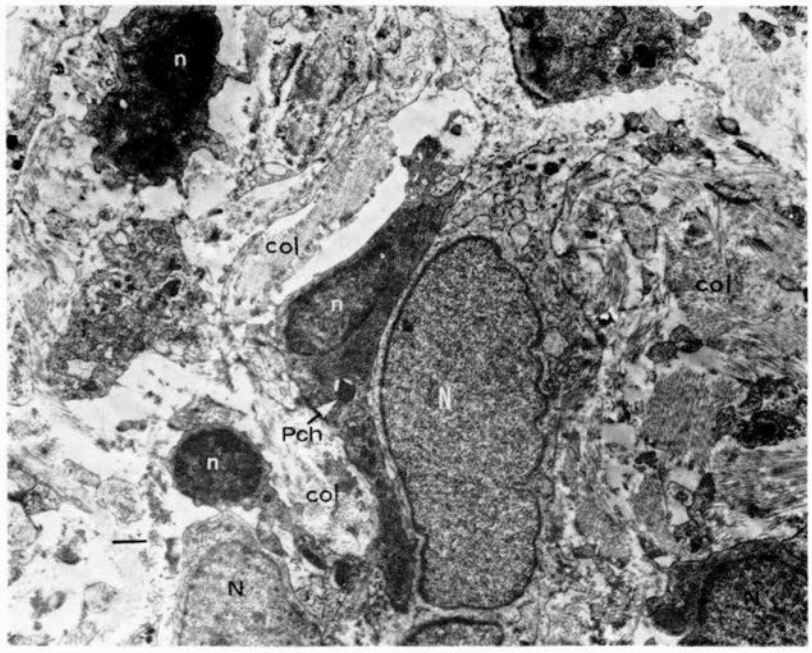

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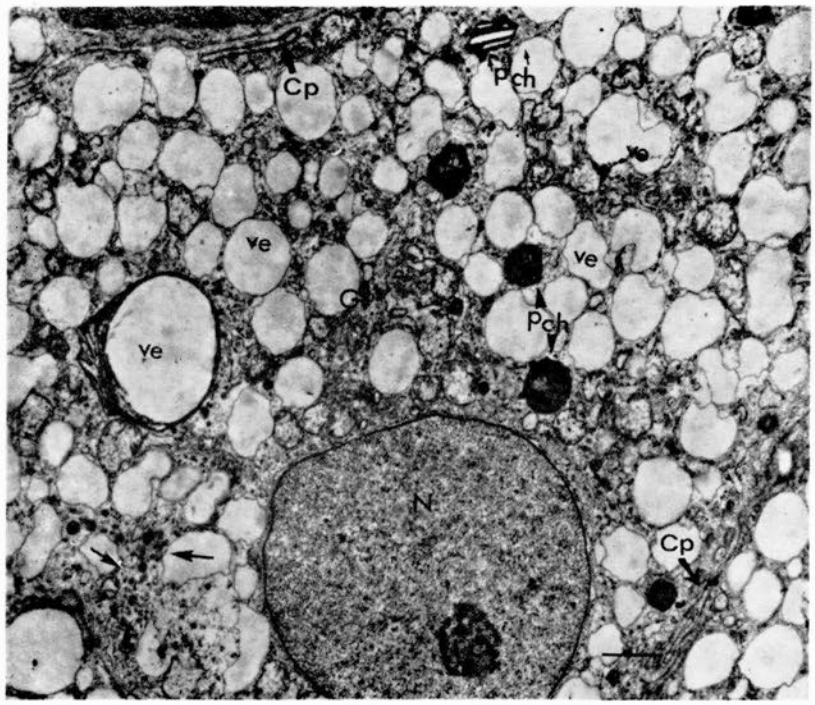

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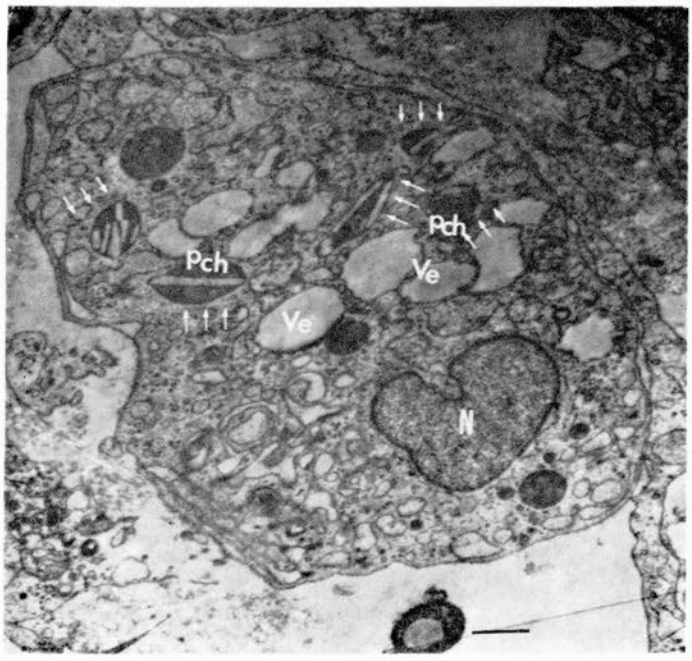

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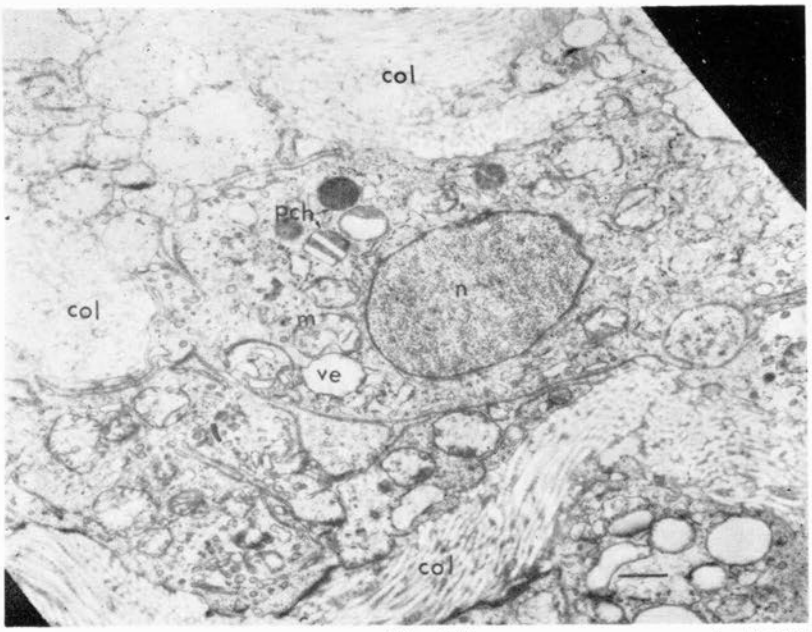

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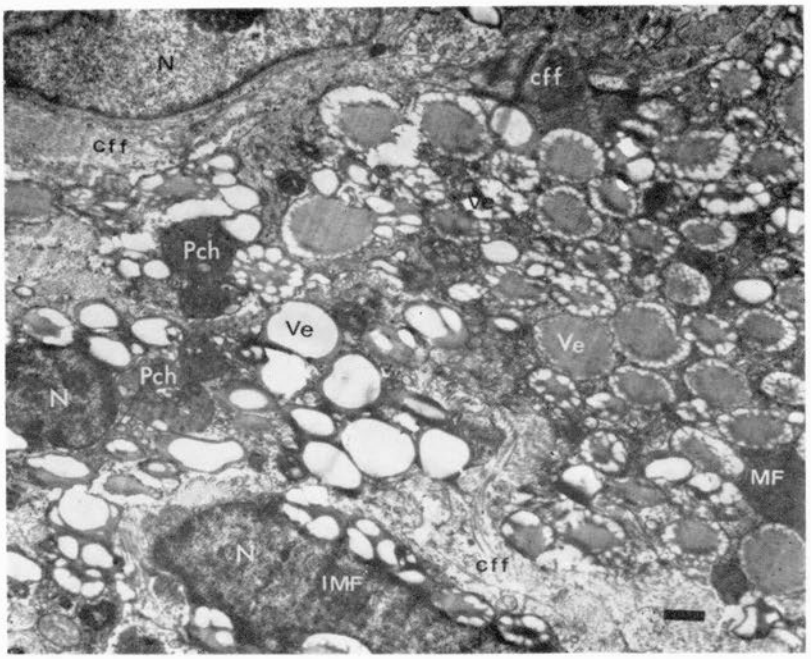


Fig. 8-A (×12,000)

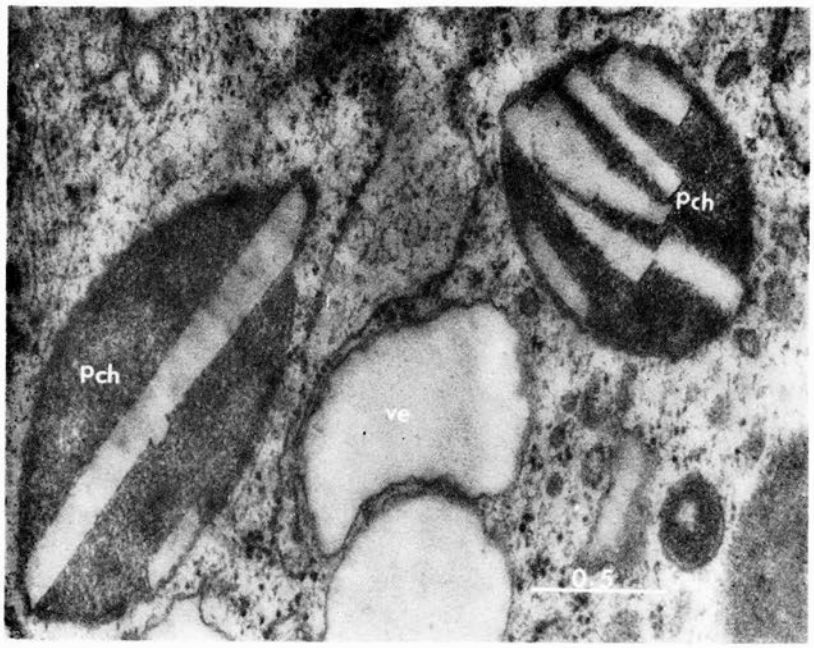
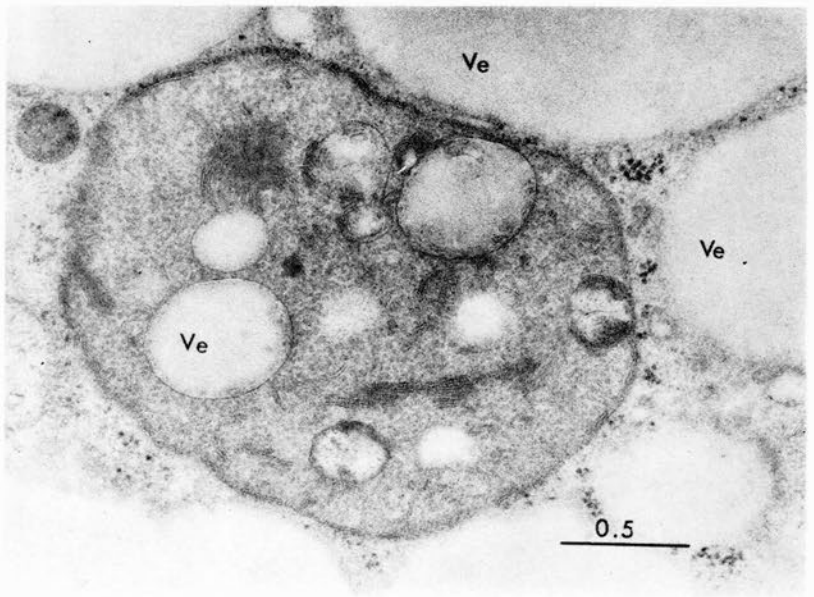


Fig. 8-B (×80,000)



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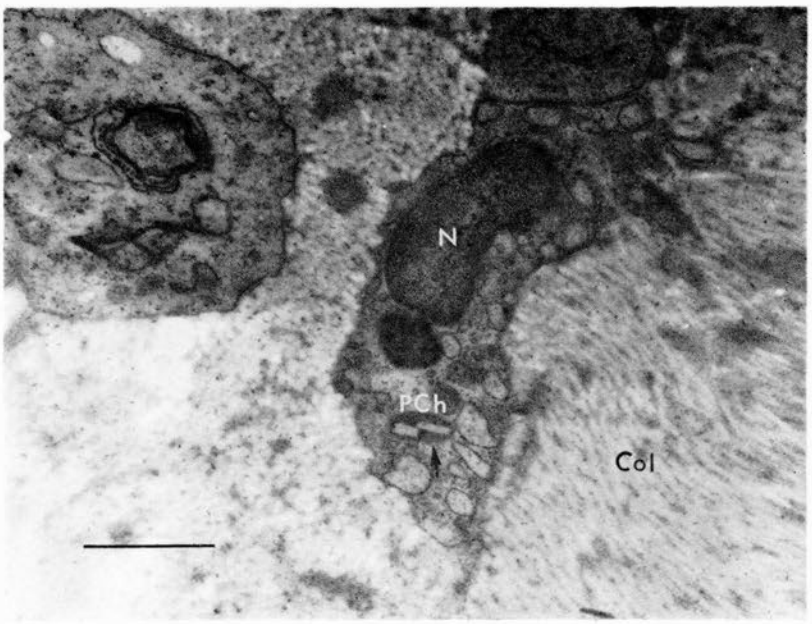

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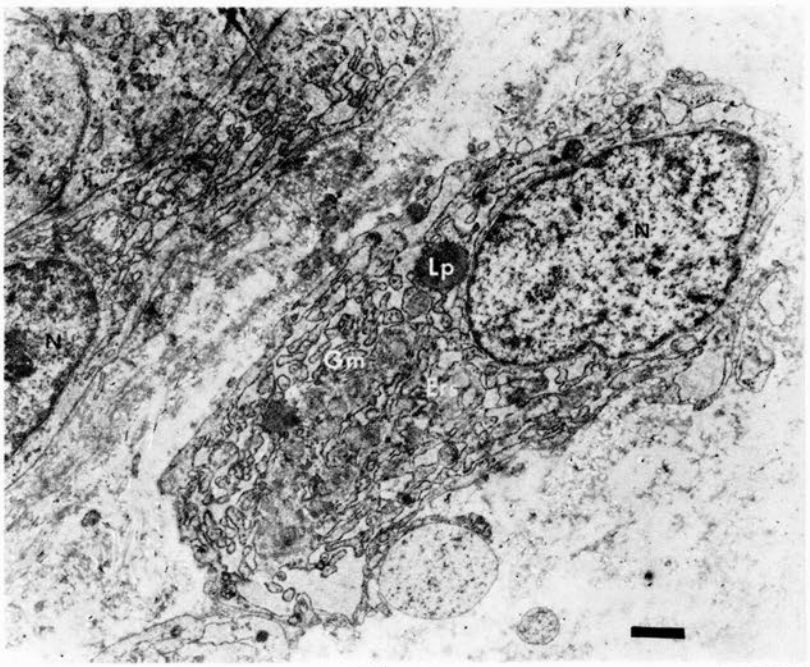

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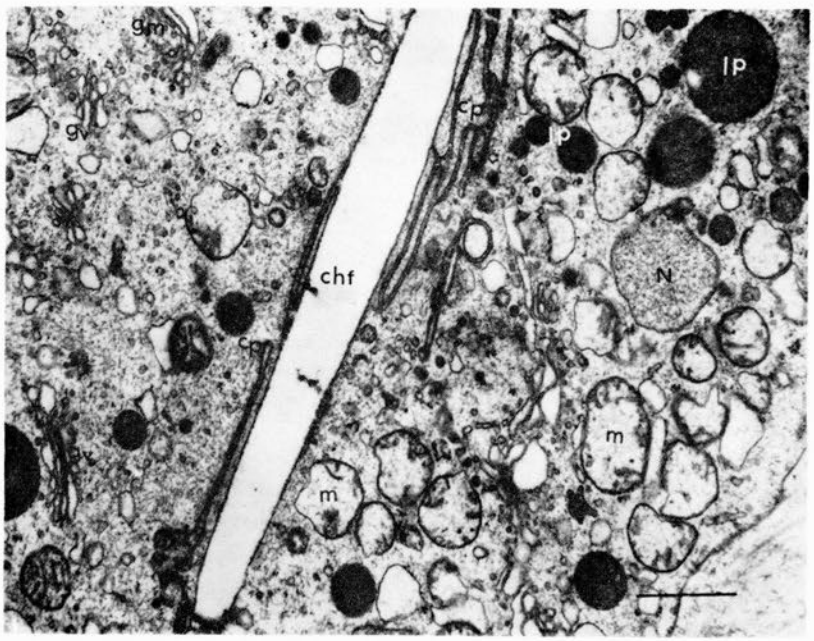

Fig. 11 (×28,000)