

# Sea Blue Histiocytosis Associated with Hyperlipoproteinemia Type IIb

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Sea-blue or ceroid histiocytosis is a storage phenomena associated with a variety of conditions especially abnormal lipid metabolism and particularly hyperlipoproteinemia. It is characterized by histiocytic proliferation in the bone marrow and spleen, containing sea-blue inclusions by Romanovsky stain. The present case is a 1½ year-old Korean boy who had marked enlargement of the spleen which was eventually removed. Aspirates of the bone marrow and histology of the spleen disclosed an enormous proliferation of histiocytes containing numerous cytoplasmic inclusions which stained sea-blue with Wright stain, was strongly positive to PAS and weakly positive to oil red-O and Sudan black B in frozen and in paraffin embedded section. Ultrastructurally histiocytes were markedly hypertrophic and contained numerous cytoplasmic inclusions which showed three distinct types and conglomeration of all three types, presumably representing age or maturation steps of the inclusions. The early type consisted of a high electron dense core or deposits within a low electron dense matrix, evolving into homogeneous moderately electron dense inclusion and finally a well developed finger print-like internal structure. Analysis of the plasma lipid disclosed type IIb hyperlipoproteinemia. Types of hyperlipoproteinemia previously reported in association with sea-blue histiocytosis were type I, III, IV and V, and this is the first case of type IIb hyperlipoproteinemia.

**Key Words:** Sea-blue Histiocytosis, Hyperlipoproteinemia Type IIb

The syndrome of sea-blue histiocytosis was coined by Silverstein *et al* (1970) to describe the morphologically distinct histiocyte in the bone marrow and spleen together with splenomegaly. Its outstanding feature is the presence

of deep-blue staining granular inclusions in the cytoplasm of the histiocytes with Romanovsky stain. Because of the histochemical characteristics of these granules, the syndrome is also called ceroid histiocytosis. Although several cases of sea-blue histiocytosis have been reported without associated disease (Ardeman and Lewis, 1972; Reidbord *et al*, 1972; Levin *et al*, 1974; Golde *et al*, 1975, Varela-Duran *et al*, 1980), this syndrome has been reported in association with a wide variety of hematologic and metabolic

Received August 10, 1983

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disorders (Dosik *et al*, 1972; Jacobsen *et al*, 1972; Rosen *et al*, 1974; Robinowitz *et al*, 1975; Swaiman *et al*, 1975; Tadmor *et al*, 1976). Hyperlipidemia is one of the frequently associated metabolic disorders and several such cases have been reported (Fredrickson and Lees, 1965; Ferrans *et al*, 1971; Rywlin *et al*, 1971; Castoldi *et al*, 1974; Parker *et al*, 1976).

The present case showed characteristic features of sea-blue histiocytosis associated with plasma lipid abnormalities. Histochemical, ultrastructural and plasma lipid patterns are described.

## CASE HISTORY AND COURSE

A 1½ year-old male patient visited Severance Hospital of Yonsei Univ. College of Med. for the evaluation of distended abdomen. He was the second baby of two siblings. Physical examination at 2 month disclosed markedly enlarged spleen, 10cm below the left lower costal margin.

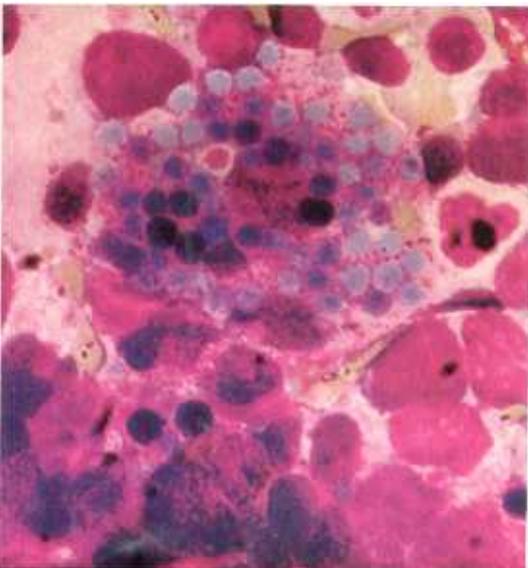


Fig. 1. The bone marrow smear showing histiocyte containing varying sized granules that stain deep-blue with Wright-Giemsa stain. Wright-Giemsa, x1,000.

Hematologic examination revealed anemia and thrombocytopenia (Hemoglobin 8.6gm/dl, Platelet count 54,000/mm<sup>3</sup>). Other laboratory tests including serology were normal. Four months later bone marrow aspiration revealed proliferation of histiocytes containing numerous deep blue staining granules by Wright stain (Fig. 1). A liver biopsy was taken at that time, which revealed no abnormalities. A cervical lymph node biopsy was also taken, which was diagnosed as sinus histiocytosis at that time. Fourteen months later a splenectomy was performed because of respiratory difficulty. Postoperative course was uneventful. At present, the patient is three years old and growing well without any specific problems.

## PATHOLOGIC FINDINGS

### 1. Gross Findings

The spleen was markedly enlarged and weighed 1,200 gm. The external surface was smooth but tense (Fig. 2). Cut surface revealed a diffusely homogeneous dark red appearance without discernable Malpighian corpuscles.

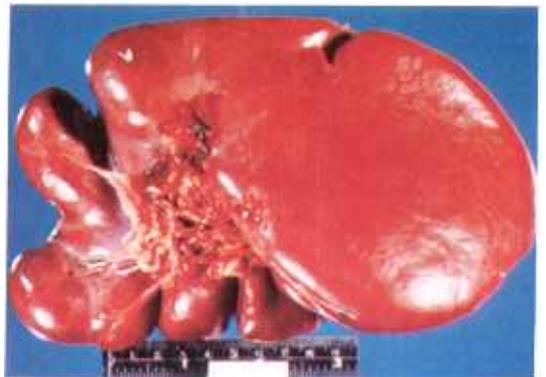


Fig. 2. Gross photograph of the spleen showing marked enlargement with tense external surface.

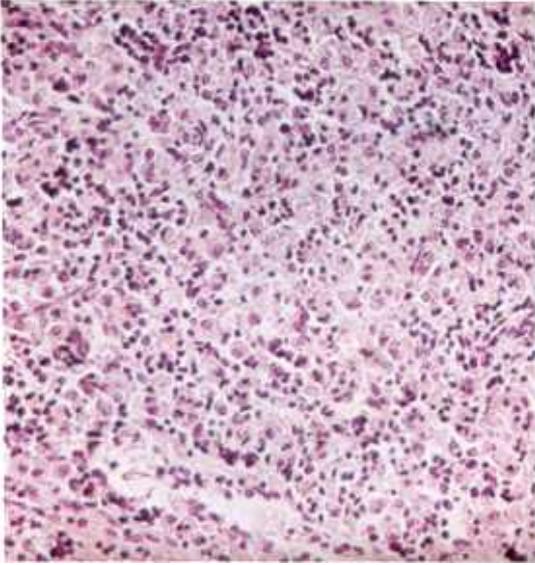


Fig. 3. Light microscopic features of the splenic sinusoids showing diffusely, filled with large aggregates of hypertrophied histiocytes. H&E, x250

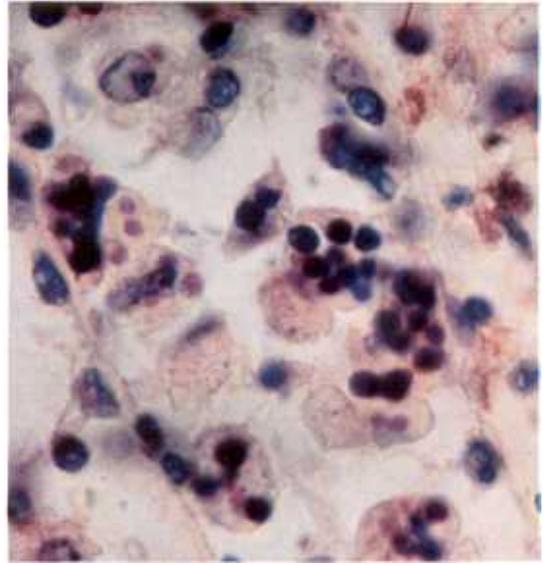


Fig. 4. The splenic histiocytes showing many sea-blue granules. Giemsa, x1,000.

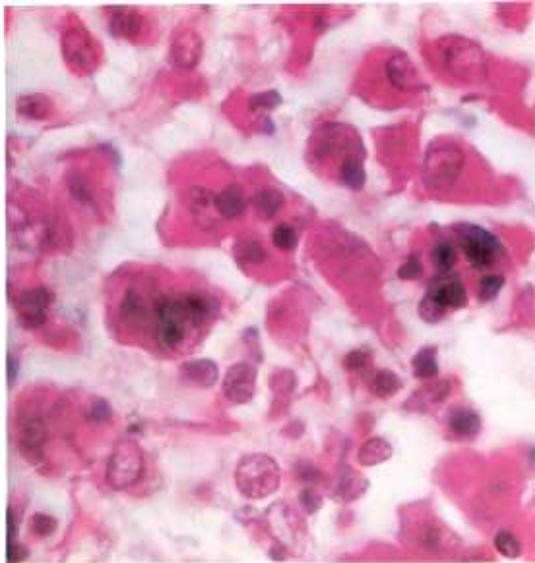


Fig. 5. The granules of the splenic histiocytes showing strong PAS positive reaction. PAS, x1,000.

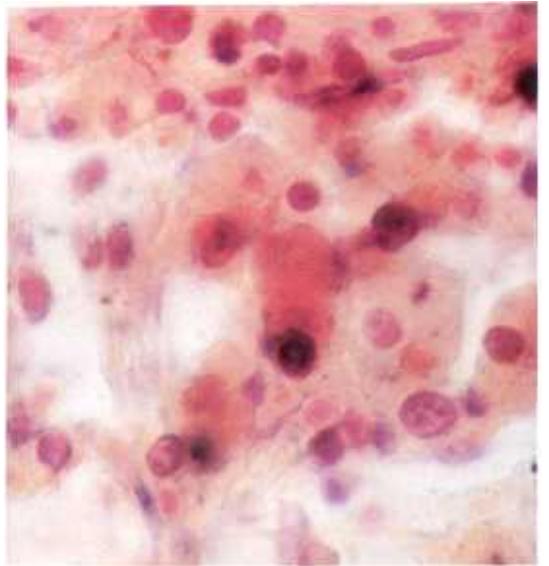


Fig. 6. Splenic histiocytes showing weakly positive reaction to oil red-O stain. oil red-O, x1,000.

## 2. Light Microscopic Findings

The entire splenic tissue was replaced by hyperplastic red pulp. The sinusoids were dif-

fusely filled with large aggregates of hypertrophied histiocytes, which were of variable size, ranging from 20 to 70 $\mu$  in diameter, with peripherally displaced nucleus and prominent

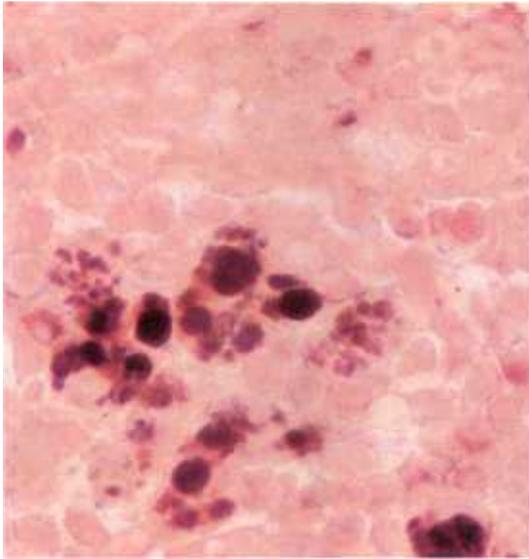


Fig. 7. Light microscopic features of the cervical lymph node showing the proliferation of some histiocytes in the interfollicular space, H&E, x400.

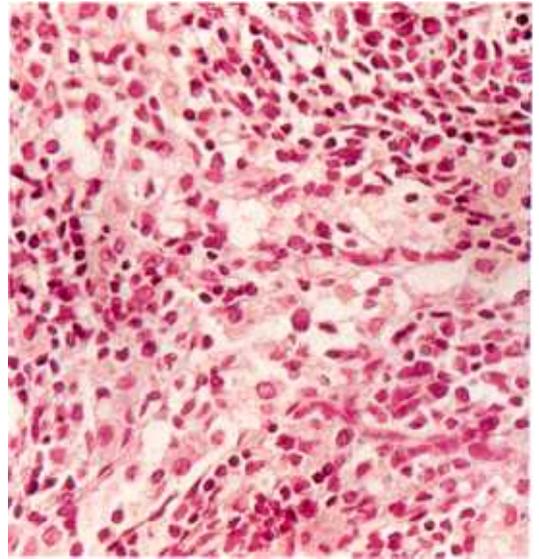


Fig. 8. The granules of lymph node histiocytes showing positive reaction to sudan black B stain in paraffin embedded section, sudan black B, x1,000.

nucleolus (Fig. 3). The cytoplasm contained many granules of variable size (Fig. 4). These granules gave a strong positive reaction to PAS before and after diastase digestion (Fig. 5) and weakly positive to oil red-O stain in frozen (Fig. 6) and in paraffin embedded section. However, iron and Ziehl-Neelsen stain were negative. The sections of cervical lymph node which was reported as sinus histiocytosis were carefully reevaluated. The same histiocytes were proliferated in the interfollicular space (Fig. 7) and they showed same histochemical reactions, but the sudan black B stain in paraffin embedded sections showed more strong positive reaction than the splenic histiocytes (Fig. 8).

### 3. Electron Microscopic Findings

Electron microscopic observation of the spleen showed marked proliferation of histiocytes (macrophages) in the sinusoids of the red pulp. The histiocytes were markedly enlarged with abundant cytoplasm which was packed

with numerous inclusion bodies pushing the nucleus aside (Fig. 9). The inclusion bodies were single membrane bound and varied in size from 1.2 to 3.4 $\mu$  in diameter. They were mostly round to oval and occasionally large and irregular due to conglomeration of a few inclusions. Each inclusion body showed various degrees of electron density and internal structure. According to the degree of electron density and the development of internal structures, the inclusion bodies could be divided into four different types. Type I consisted of a central core of highly electron dense amorphous deposit surrounded by a halo zone of low electron dense matrix or scattered high electron dense deposits within a low electron dense matrix (Fig. 10). Type II, the most common type, consisted of a moderately electron dense homogeneous matrix with many vacuoles and an occasional high electron dense deposit (Fig. 9). Type III showed a highly developed internal structure characterized by concentric lamella-

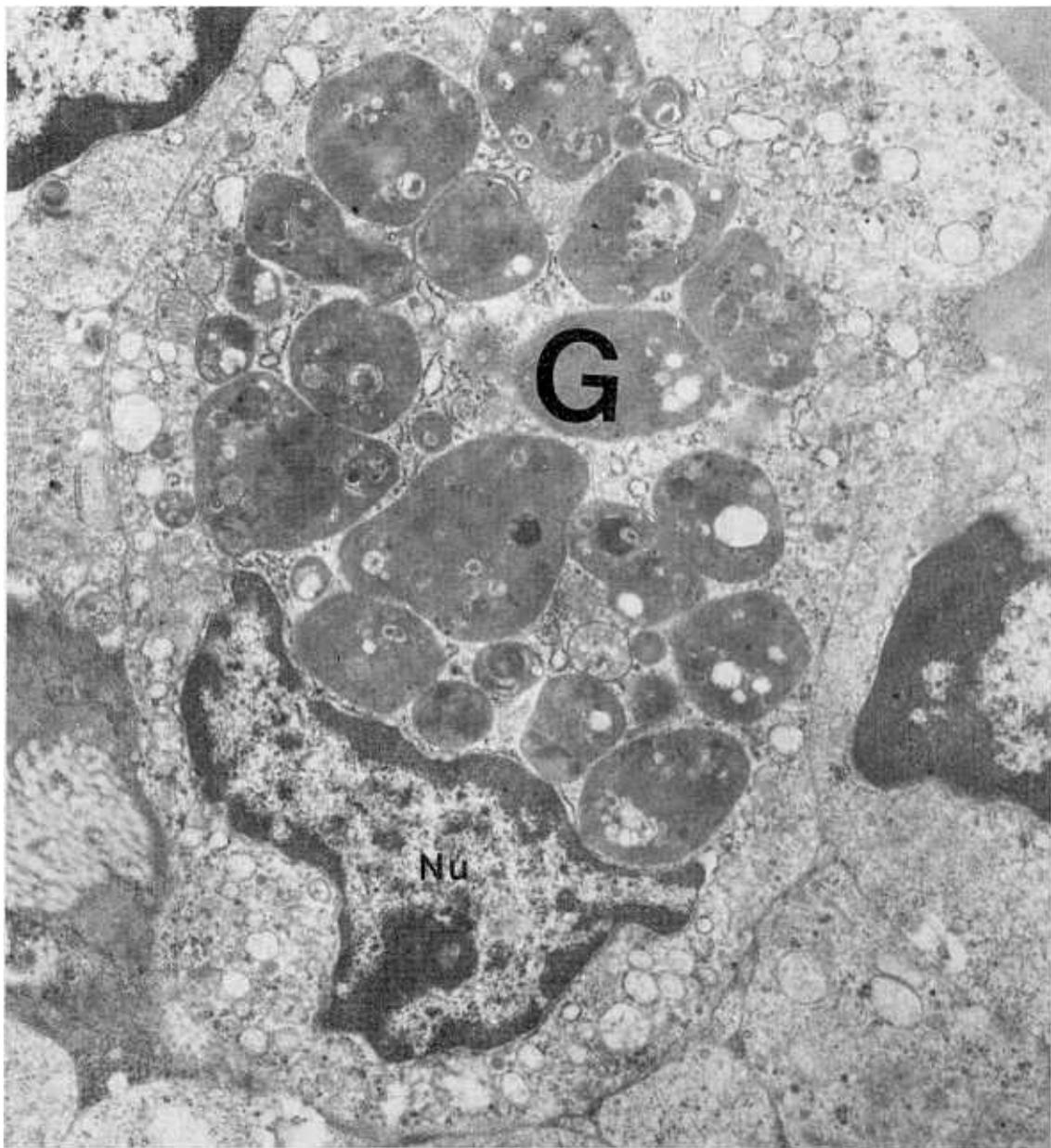


Fig. 9. Electronmicrophotograph of a splenic histiocyte (center) containing numerous and variable sized electron dense granules (G) with frequent vacuolation. (type II inclusion). Original magnification x12,500.

tion with a periodicity of 45 to 50Å within a homogeneous moderately electron dense matrix, giving a finger print appearance (Fig. 11). Type IV consisted of a mixture of above three features, probably due to conglomeration of different types of inclusion bodies or transition from

type II to III (Fig. 12). It appeared that these different types of inclusion bodies represent the ages of inclusions, probably beginning from type I and evolving into type II, IV and III in sequence. No internal structures to suggest protozoa or histoplasma were noted.

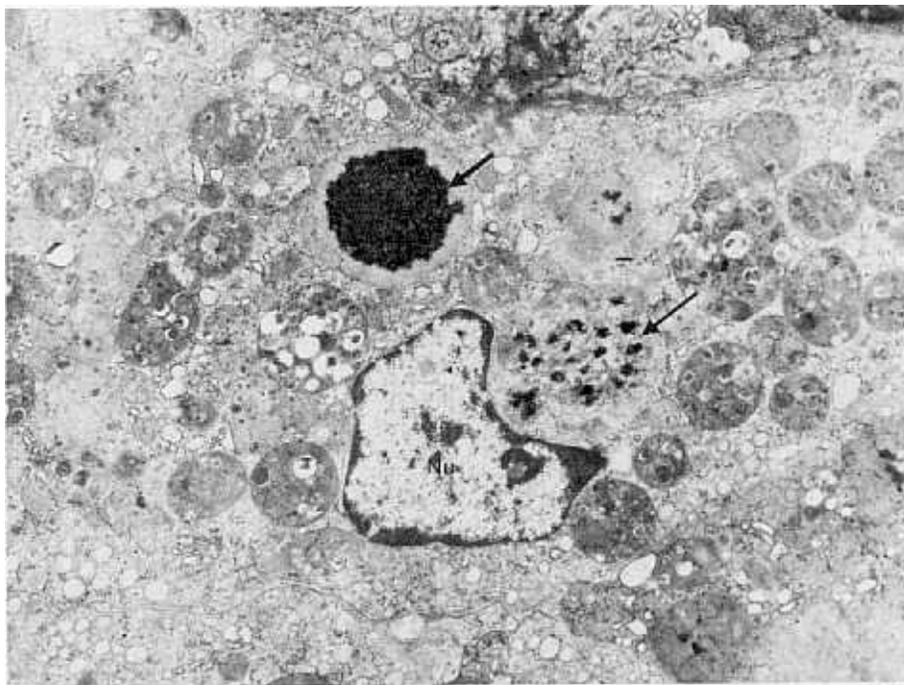


Fig. 10. Electronmicrophotograph of a splenic histiocyte containing many inclusions among which two show highly electron dense central core (thick arrow) or scattered dots within low electron dense matrix (thin arrow). (type I inclusion). Original magnification,  $\times 12,500$

## PLASMA LIPID ANALYSIS

Six months after splenectomy, plasma lipid analysis of the patient and his family were carried out, and the results are shown in table 1. The patient's plasma showed an increased level of cholesterol and triglyceride in comparison to the other family members and to normal values of other Korean children. Lipoprotein electrophoresis of the patient's plasma revealed

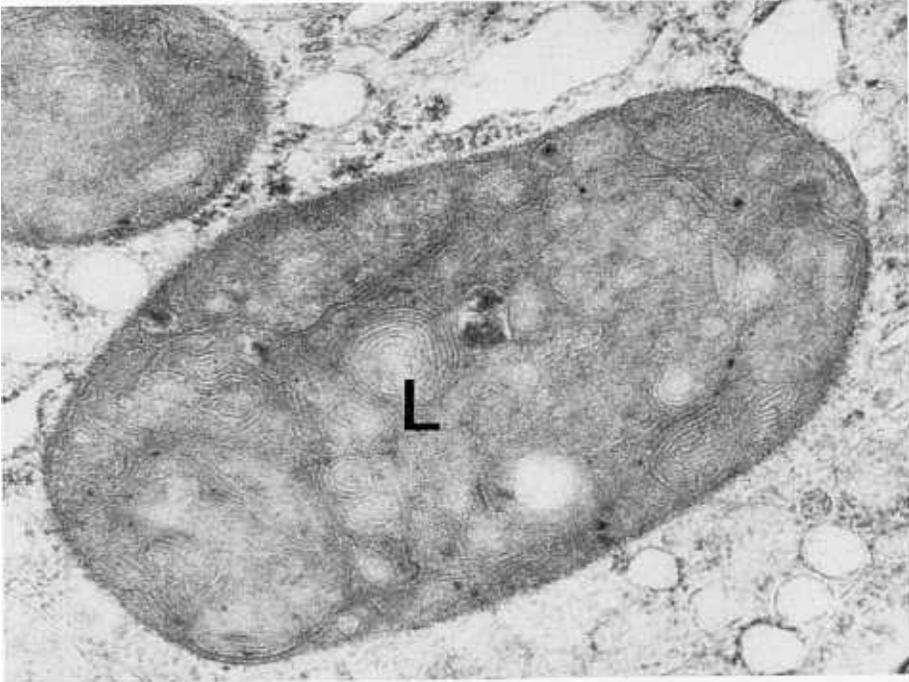
a marked elevation of beta and prebeta-lipoprotein fractions, which is consistent with type IIb hyperlipoproteinemia. Other members of the family had normal electrophoretic patterns for lipoprotein.

## COMMENT

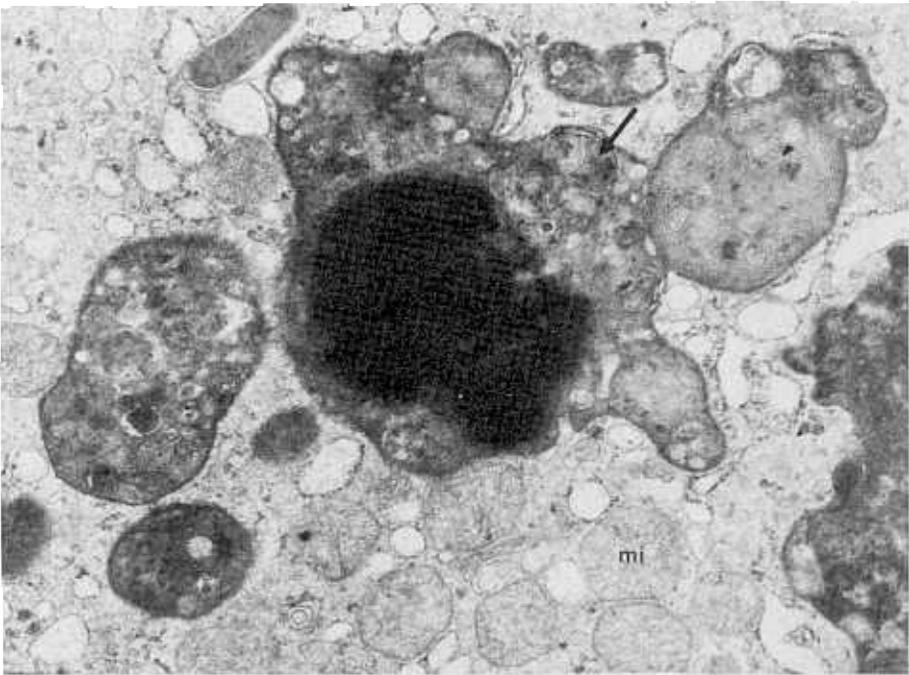
It is known that sea-blue histiocytosis is usually associated with hematologic and metabolic disorders. Although this syndrome is

Table 1. Plasma lipid values of the patient and his family

	Age (year)	Sex	Total lipid	Cholesterol (mg%)	Triglyceride (mg%)	Lipoprotein (mg%)		
						Alpha	Beta	Prebeta
Patient	1.5	M	521	196	112	78	328	115
Father	31	M	325	140	49	67	200	68
Mother	29	F	411	161	88	78	247	86
Brother	6	M	344	151	55	72	209	64



**Fig. 11.** Electronmicrophotograph of a inclusion showing a concentric lamellar inclusion (L) within a homogeneous electron dense matrix. (type III inclusion). Original magnification, x60,000.



**Fig. 12.** Electronmicrophotograph of a splenic histiocyte showing large irregular shaped inclusion (arrow) by conglomeration of type I, II and III inclusions. (type IV inclusion). Original magnification, x30,000.

closely related to the abnormality of lipid metabolism, the direct association with plasma lipid abnormality is not so common as expected. The lipid abnormalities associated with sea-blue histiocytosis include hypercholesterolemia (Rosen *et al*, 1974), hyperlipoproteinemia (Fredrickson and Lees, 1965; Ferrans *et al*, 1971; Rywlin *et al*, 1971; Castoldi *et al*, 1974; Parker *et al*, 1976), and lecithin cholesterol acyl transferase deficiency (Jacobsen *et al*, 1972). The previously reported hyperlipoproteinemia associated with sea-blue histiocytosis are type I (Ferrans *et al*, 1971), type III (Fredrickson and Lees, 1965; Parker *et al*, 1976), type IV (Castoldi *et al*, 1974) and type V (Rywlin *et al*, 1971). The present report is the first case of sea-blue histiocytosis associated with type IIb hyperlipoproteinemia. The normal plasma values of the total cholesterol and triglyceride are  $172.2 \pm 21.5$  mg% and  $98.4 \pm 28.7$  mg%, respectively, in the Korean adult. Although the normal plasma lipid values of Korean children, which should be lower than the adult, have not been clearly documented, those of this patient were higher than the upper limit of the normal Korean adult values.

The characteristic sea-blue histiocyte is a large macrophage that is packed with deep-blue or blue-green granules when stained with Romanovsky stain (Silverstein *et al*, 1970; Reidbord *et al*, 1972; Varela-Duran *et al*, 1980). Histochemically the blue staining granules were both lipid and glycoprotein in nature. The result of histochemical and tissue lipid analysis by Silverstein *et al* (1964, 1970) suggested that the storage substance to be a glycolipid or phospholipid. In 1971, Rywlin and associates observed sea-blue histiocytes in the spleen of a patient with idiopathic thrombocytopenia and type V hyperlipoproteinemia. Histochemical examination gave autofluorescence, positive reaction of PAS before and after diastase diges-

tion and acid fastness. Based on these findings they concluded that the sea-blue granules were made of ceroid. Ceroid is a pale yellow to dark brown pigment that results from the peroxidation and polymerization of an unsaturated lipid (Hartroft and Porta, 1965; Siakotos *et al*, 1970; Swaiman *et al*, 1975). Histochemically it reacts with fat stains such as oil red O and sudan black. As the ceroid matures it shows autofluorescence, PAS positivity and acid fastness (Hartroft and Porta, 1965; Rywlin *et al*, 1971). The negative acid fastness in our case is considered to be related to the age of the ceroid.

Ultrastructurally the most constant and prominent finding is the large numbers of membrane linked lamellar inclusions arranged with a periodicity of approximately 45 Å (Reidbord *et al*, 1976; Golde *et al*, 1975; Parker *et al*, 1976; Varela-Duran *et al*, 1980). As in our case, varying types of electron dense granules were found by others (Ferrans *et al*, 1971; Rywlin *et al*, 1971; Reidbord *et al*, 1972; Parker *et al*, 1976). In this case variable types of lamellar inclusions and electron dense homogeneous matrices were found and some of them were conglomerated in the same granule. These findings suggest that these different types of inclusions represent the different maturation stage of ceroid, probably from the electron dense deposit to the lamellar inclusion in sequence. Numerous studies indicate that the lamellar configurations seen in the lipid inclusions represent a physicochemical phenomenon rather than degenerative alterations of cellular organelles such as mitochondria or lysosomes (Revel *et al*, 1958; Uzman, 1958; Stoeckenius, 1962; Lynn and Terry, 1964). Some authors thought that these lamellar configurations are the result from spatial orientation of water and lipid complex molecules (Lynn and Terry, 1964; Golde *et al*, 1975). Moreover, Hovig and Gjone (1973) found abnormal lamellated particles in the plasma of

a patient with lecithin cholesterol acyl transferase deficiency. In conclusion the sea-blue histiocytosis is associated with various types of hyperlipoproteinemia and the sea-blue granules are a storage complex of lipid molecules brought about by phagocytosis or other possible mechanisms instead of being a degenerating process of macrophage.

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