

Bone marrow lipofuscin

K. G. A. CLARK AND W. M. DAVIDSON

From the Department of Haematology, King's College Hospital Medical School, London

SYNOPSIS Lipofuscins are commonly present in the macrophages of the marrow. In unstained preparations they may be confused with haemosiderin, but they are readily distinguished by fluorescence microscopy.

In contrast to the belief that lipofuscins are a manifestation of senility, no age dependence has been demonstrated.

Exceptionally large amounts have been found in illnesses accompanied by fever and leucocytosis, in keeping with the concept of their formation from insoluble remains of ingested cell fragments. It is probable that the 'sea-blue histiocytes', described in the literature, are macrophages laden with strikingly uniform granules of lipofuscin.

The microscopical study of the iron in the marrow is a valuable procedure in the differential diagnosis of anaemia (Rath and Finch, 1948; Davidson and Jennison, 1952). Stainable iron may be found in the macrophages in both diffuse and granular forms. However, macrophages often contain another pigment which does not give a positive Prussian blue reaction. This paper records observations upon the character, distribution, and possible significance of this pigment.

Materials and Methods

All the smears used were from routine marrow biopsies. To obtain consistent results, mechanical staining machines (Davidson, Bareham, Kitchen, and Pegg, 1958) were used for the Jenner-Giemsa method. The Prussian blue preparations were counter-stained for five minutes in 0.01% aqueous safranin. Other staining procedures were carried out according to the descriptions given by Lillie (1965).

Smears for fluorescence microscopy were fixed for five minutes in absolute methanol and then washed with several changes of chloroform to remove readily extractable lipids containing potentially fluorescent materials. In selected cases, the smears were exposed to a variety of solvents; chloroform, N hydrochloric acid, 5% sodium bicarbonate, pyridine, acetone, methanol, and amyl alcohol, to test the solubility of the autofluorescent pigment. The preparations were mounted in buffered glycerol,

and examined by means of a Zeiss photomicroscope equipped for fluorescence, phase contrast, and bright field microscopy. Illumination for fluorescence microscopy was provided by an HBO 200 mercury vapour lamp. The preparations were classified according to the amount of fluorescent pigment observed: grade I, trace only; grade II, light accumulation; grade III, heavy deposits; and grade IV, gross amounts. Marrow lacking stainable iron but containing much intracellular autofluorescent pigment was fixed in buffered glutaraldehyde, post-fixed in osmium tetroxide, dehydrated, and embedded in epoxy resin. Sections 80 to 100 nm thick were stained with lead citrate for electron microscopy.

Results

When examined by bright field and phase contrast microscopy, most preparations showed particulate, pale gold pigment. Much of this pigment lay within macrophages and varied in appearance from granules of less than 1 μm to blocks several μm in diameter. Upon excitation by light of approximately 365 nm wavelength the pigment showed yellow autofluorescence which was not removed even by prolonged extraction with solvents (Fig. 1). Thirty minutes' exposure to light from the mercury vapour lamp failed to produce appreciable fading and unfixed material retained the ability to fluoresce for at least several months. During storage leucocytes and their precursors were found to develop a silvery fluorescence, probably as a result of atmospheric

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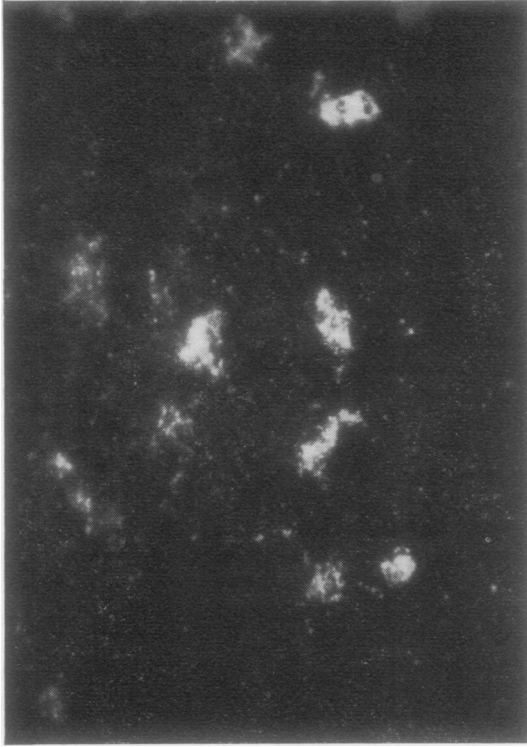


Fig. 1 *Autofluorescent macrophages. Bone marrow, × 260.*

oxidation of lipids, but this artefact could be distinguished easily by its distribution and low intensity.

The autofluorescent pigment stained weakly with Nile blue in 1% sulphuric acid, dark brown with Sudan black B, and was acid fast and weakly PAS positive. It did not give a positive reaction for iron, but took up the safranin counterstain. Ferric ferri-cyanide reduction was not convincingly demonstrated.

Electron microscopy of marrow containing much autofluorescent pigment, but lacking storage iron, demonstrated osmiophilic bodies of varying opacity. These corresponded in size and distribution to the pigment particles observed by fluorescence microscopy. Some showed the remains of surrounding membranes, suggesting a lysosomal localization of the pigment (Fig. 2).

Autofluorescent pigment was found in varying quantities in all the samples from a series of 32 patients from whom adequate marrow was obtained (Table). The mean age of the 17 patients showing grades III and IV was 63.8 years (range 6 to 94 years) and that of 15 patients with grades I or II was 54 years (range 16 to 83 years). The difference is not significant ($P > 0.2$).

Two patients showed grade IV fluorescent pigment. One was admitted with chest pain, developed fever, jaundice, and a leucocytosis. He died soon after and at necropsy the chief findings were extensive atheroma, a dissecting aneurysm, and a large

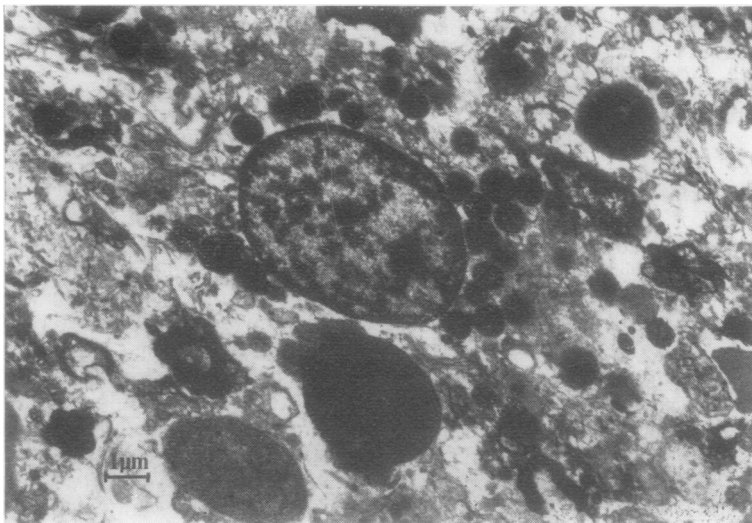


Fig. 2 *Electron micrograph showing osmiophilic bodies, of varying size and electron density, inside a macrophage from marrow lacking stainable iron.*

Diagnosis	Grade			
	I	II	III	IV
Megaloblastic anaemia	4	—	3	—
Chronic renal failure	2	1	—	—
Iron deficiency	1	1	5	—
Chronic myeloid leukaemia/ruptured gallbladder	—	—	—	1
Aneurysm, cholangitis, abscess	—	—	—	1
Glandular fever	1	—	—	—
Agranulocytosis	—	—	1	—
Rheumatoid arthritis and iron deficiency	—	—	1	—
Marrow hypoplasia	—	—	2	—
Monocytosis (preleukaemic)	—	1	—	—
Acute lymphatic leukaemia	1	—	1	—
Acute myeloid leukaemia	1	—	—	—
Familial degeneration of central nervous system	—	—	1	—
Normal marrow	—	1	1	—
Idiopathic thrombocytopenia	1	—	—	—

Table Grading of autofluorescence of marrow samples from 32 patients

intraabdominal abscess secondary to cholangitis. The second patient suffered from chronic myeloid leukaemia. He was admitted with abdominal pain and jaundice and died a few days later. A ruptured gall-bladder was found at necropsy.

Discussion

Golden yellow refractile granules in the marrow have been interpreted as haemosiderin (Rath and Finch, 1948; Coleman, Stevens, and Finch, 1955; Bothwell and Finch, 1962). However it has now been shown that they may be composed of pigment which, in contrast to haemosiderin, contains no iron and is autofluorescent. These pigment granules are found in most, if not all, marrow samples and show the staining reactions, stable autofluorescence, and insolubility of that class of pigments known as the lipofuscins (Strehler, 1964). They are particularly well defined in iron deficiency, but tend to be overshadowed when there is excessive iron storage.

The granules of lipofuscin found in neurons, liver cells, and cardiac muscle have been called 'residual bodies', conglomerates of materials which have resisted lysosomal digestion. It is not surprising, therefore, that their composition is complex and they may contain acid-fast, PAS-positive lipid and reducing substances (Essner and Novikoff, 1960). Accumulation of such granules in the tissues has been taken to reflect cellular aging (Strehler, Mark, Mildvan, and Gee, 1959; Reichel, 1968; Reichel, Hollander, Clark, and Strehler, 1968), but this is doubtful for they can be demonstrated in the liver cells of neonates (Goldfischer and Bernstein, 1969). It also seems unlikely that the marrow lipofuscin is associated with aging of the individual. The mean

age of those with grade III or IV marrow pigment was not significantly different from those with grade I or II. It is suggested that the lipofuscin in the macrophages of the marrow is the indigestible end product of their phagocytic activity, and is increased when cell destruction is excessive. This concept is supported by the massive quantities found in the two patients with particularly severe sepsis (Table). However, in both these patients disease involved the liver and bile ducts. Endicott and Lillie (1944) and Lillie (1965) recorded the occurrence of lipofuscins in the liver, spleen, lymph nodes, lungs, and phagocytes of the bone marrow of rats suffering from experimental dietary cirrhosis. Although none of the 15 patients showing grade III pigment was affected by primary liver disorders the numbers were small and this may still be a significant factor.

Liver disease has also been a prominent feature in the clinical histories of patients described as having 'sea-blue histiocytes'. Silverstein, Young, ReMine, and Pease (1964) and Silverstein, Ellefson, and Ahern (1970) presented evidence for the existence of a lipid storage disease which they named 'the syndrome of the sea-blue histiocyte'. They collected eight case histories from the literature and added a description of a similar patient of their own. All nine had splenomegaly; six had hepatomegaly, two died of cirrhosis, and some were or had been jaundiced. Purpura or bleeding were features of the disorder in seven and four were known to have been thrombocytopenic. In the marrow there were histiocytes containing basophilic granules which took up Sudan black stain. These were interpreted as pathological storage cells, but they were not studied by fluorescence microscopy. In one case the extractable liver lipids showed miscellaneous abnormalities.

The existence of this syndrome has been questioned by Kattlove, Gaynor, Spivack, and Gottfreid (1970), who rejected the suggestion that 'sea-blue histiocytes' are peculiar to any specific syndrome. They interpreted the inclusions as the remains of phagocytosed material. Certainly the morphological and staining properties attributed to the cells are those of lipofuscins. Dr S. Ardeman very kindly made available unstained liver sections and Jenner-Giemsma stained marrow slides from his patient with 'sea-blue histiocytes' (Ardeman and Lewis, 1972), who also had liver disease and splenomegaly. The pigment in the Kupffer cells and hepatocytes was strongly autofluorescent and visually indistinguishable from lipofuscin by this technique. The particles of pigment within the macrophages of the marrow were strikingly uniform. The majority were between 1 and 2 μm in diameter, with occasional larger aggregates.

While the striking microscopical appearances

certainly suggest that this is a distinct entity, no corroborative evidence has been produced to show that it is a specific storage disorder. Alternatively, the appearance of the granules could indicate a lysosomal disturbance reminiscent of the Chediak Higashi anomaly of the neutrophil leucocytes. However, the possibility that sea-blue histiocytes are the extreme result of lipofuscin accumulation secondary to liver or other disease has not been completely excluded.

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