Virchows Arch. A Path. Anat. and Histol. 374, 239–247 (1977)

Virchows Archiv A

A Histochemical Study of Bone Marrow Hypoplasia in Anorexia Nervosa

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Summary. The bone marrow in patients with anorexia nervosa is commonly hypoplastic with transformation of marrow fat. The normal fat cells which appear clear and open in the marrow are surrounded by an amorphous, gelatinous material, thought to represent an increase in the ordinary acid mucopolysaccharide ground substance of the bone marrow. Since this lesion has a similar appearance grossly and microscopically to the lesion of serous fat atrophy found in cachectic patients, we have compared the histochemical properties of this amorphous material in a bone marrow from a patient with anorexia nervosa and from cachectic patients with epicardial serous fat atrophy and with the background substance in hypoplastic marrows. Both this fat-associated deposition in the bone marrow and serous fat atrophy were found to be predominantly a hyaluronic acid mucopolysaccharide. In contrast, the background substance contained a less acid mucopolysaccharide. The increase in bone marrow acid mucopolysaccharide in anorexia nervosa may represent a serous fat atrophy change rather than an increase in ground substance.

Key words: Bone marrow hypoplasia – Anorexia nervosa – Histochemical study – Acid mucopolysaccharide.

Introduction

Hypoplasia of the bone marrow is a common lesion associated with the severe emaciation of anorexia nervosa. The hypocellularity observed is accompanied by deposition of a gelatinous pink material in sections stained with hematoxylin and eosin, thought to be an acid mucopolysaccharide.

We recently observed this material in the bone marrow of a young woman suffering from severe anorexia nervosa as well as in patients cachectic due to

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neoplasia, small intestinal malabsorption, and Parkinson's disease. Since the lesion resembled the lesion of serous atrophy of fat found in cachectic patients, we conducted a histochemical study to identify the gelatinous pink staining substance.

Materials and Methods

Patients Studied

Patient 1. The patient with anorexia nervosa is a 33 year old white woman with a history of frequent hospitalizations for emotional disturbance and weight loss with amenorrhea since age 16–18. Her weight on the current admission was 31.8 kg, with a weight one year previously of 25.5 kg. Her past history includes a lumbar sympathectomy in 1970 and 1971 for slow healing ulcers in the right leg and a diagnosis of Raynaud's phenomenon in her hands in 1973. Physical examination demonstrated a severely ill, cachectic white female. Examination of head, ears, eyes, nose and throat was unremarkable. Chest was clear to percussion and auscultation. Heart was of normal size with a grade I/VI systolic ejection murmur at the apex. Abdomen was scaphoid, no masses and no organomegaly. Central nervous system examination revealed a slightly agitated and restless patient with a flat affect. Motor examination showed good strength. Deep tendon reflexes were normal, cranial nerves and gait were normal. The clinical impression was anorexia nervosa with severe cachexia.

Laboratory data included a white blood count of 2400 leukocytes/ml^3 and the differential showed 38 segmented neutrophils, 2 band neutrophils, 55 lymphocytes and 5 monocytes. The hemoglobin was 11.9 g/dl, hematocrit 35%, with a mean cell volume of 85 μm^3, mean corpuscular hemoglobin of 30 μg, and a mean corpuscular hemoglobin concentration of 34%. Acanthocytes were present. Platelet count was 203,000/ml^3. The reticulocyte count was 0.9%. The prothrombin time: patient 11.8 s with a control of 10.4 s. The sedimentation rate (Westergren) was 3 mm/min. Serum chemistry values were: glucose 75 mg/dl, blood urea nitrogen 12 mg/dl, creatinine 0.5 mg/dl, bicarbonate 26 mEq/l, chloride 95 mEq/l, sodium 134 mEq/l, potassium 4.1 mEq/l, calcium 9.4 mg/dl, phosphate 3.7 mg/dl, direct bilirubin 0.2 mg/dl, total bilirubin 0.7 mg/dl. The aspartate aminotransferase was 18 IU/ml and the lactate dehydrogenase was 142 IU/ml. The creatine phosphokinase was 104 IU/ml. Iron was 74 μg/dl with an iron binding capacity of 279 μg/dl. Total tetraiodothyronine was 6.4 μg/dl. Cortisol on both morning and afternoon specimens was 21.0 μg/dl. Folic acid was 9.8 mg/dl, and Vitamin B12 was 780 pg/ml. The chest X-ray was normal except for some minimal left posterior costophrenic angle pleural thickening. The electrocardiogram showed a sinus bradycardia and low voltage. There was a probable left axis deviation and left anterior hemiblock. Thus, the patient was felt to have leukopenia and anemia secondary to the cachexia of anorexia nervosa.

Patient 2. A 31 year old man weighing 53.6 kg, with recurrent Hodgkin's disease, stage III-As, mixed cellularity, received previous total nodal irradiation. bone marrow from the left posterior iliac crest was normal, while that from the right side was hypoplastic with fat cells surrounded by pink gelatinous material.

Patient 3. This is a 76 year old woman with severe Parkinson's disease weighing 31.4 kg who expired after an operation for acute intestinal obstruction. Bone marrow at autopsy was normocellular with a focal area of gelatinous material surrounding the fat cells.

Patient 4. This is a 63 year old woman weighing 27.3 kg with an eight year history of malabsorption secondary to post-gastrectomy. Autopsy revealed generalized serous fat atrophy, particularly prominent in the epicardial fat.

Other patients include three patients with hypoplastic marrows, secondary to chemotherapy of acute leukemia or Hodgkin's disease. Normal marrow from a patient with a metastatic adenocarcinoma was also studied.

Histochemical Techniques

Sections of bone marrow biopsies and/or clot sections and epicardial fat were stained with hematoxylin-eosin, periodic acid Schiff stain, reticulin stain, and Alcian Blue stain at pH 0.4, 1.0, 1.7, 2.5, and 4.0 (Luna, 1968). Amyloid was stained by Highman's Congo Red method (Putt, 1972). Reticulin was quantitated as described by Bauermeister (1971), 0 to 2+ being normal. Treatment with testicular hyaluronidase was performed according to Zugibe (1970). Neuraminidase digestion was accomplished by incubating deparaffinized sections overnight at 37° C in a solution containing 1 unit of purified *Clostridium perfringens* neuraminidase (Worthington Biochemical Corporation) per 10 ml of 0.1 M sodium acetate–0.4 M calcium chloride buffer, pH 7.4. In the case of both hyaluronidase and neuraminidase, duplicate sections were incubated overnight with buffer alone, and both control and enzyme treated slides were stained with Alcian Blue, pH 2.5.

Results

Bone marrow biopsy from the patient with anorexia nervosa (Patient 1) is shown in Figure 1. The marrow is markedly hypoplastic with slightly decreased open spaces of fat cells surrounded by an amorphous, gelatin-like material. Similar material surrounding the fat is seen in the patient with Hodgkin's disease

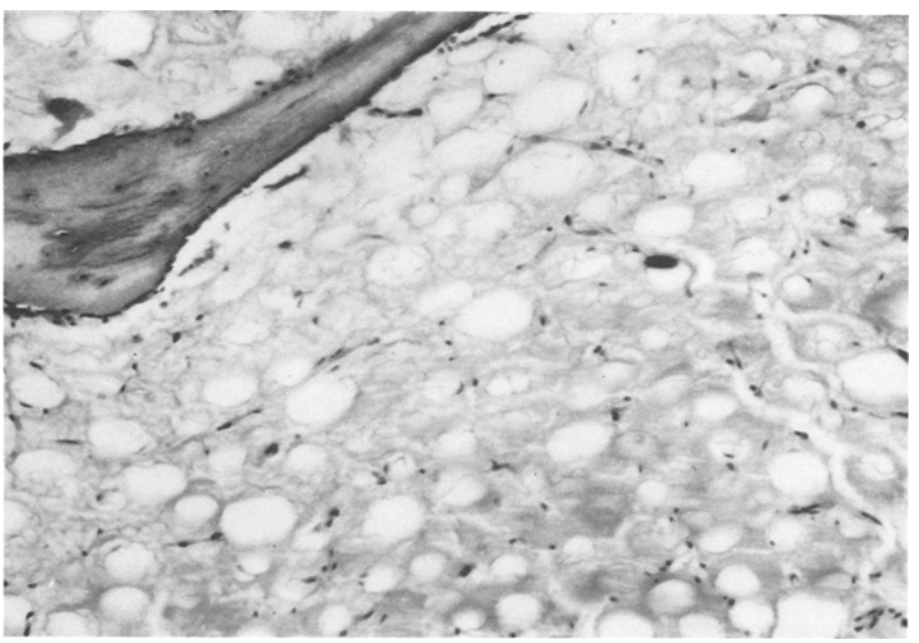

Fig. 1. Bone marrow biopsy from patient with anorexia nervosa, demonstrating hypoplasia and amorphous, gelatinous material surrounding the open fat spaces, hematoxylin and eosin stain, ×100

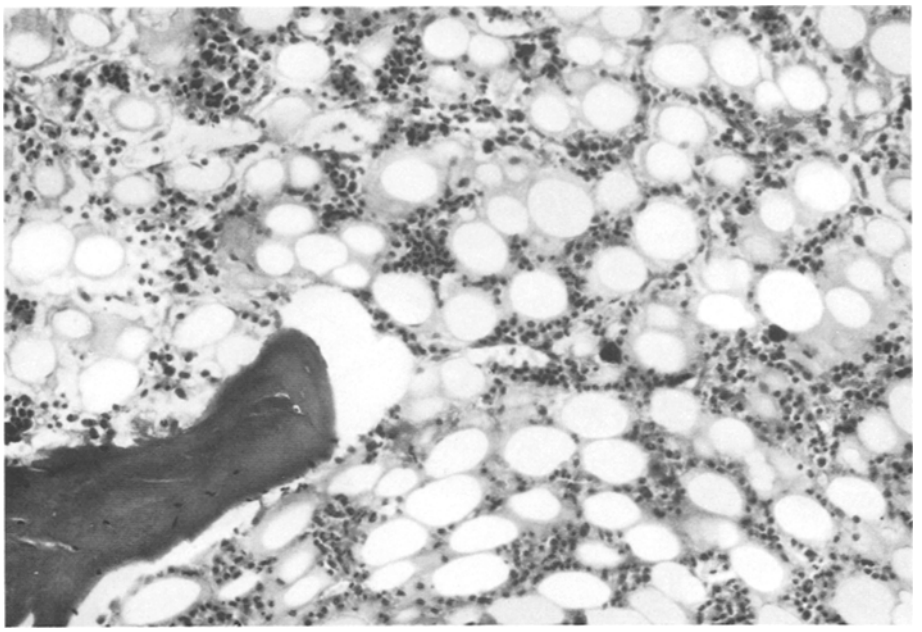

Fig. 2. Bone marrow biopsy from patient with Stage IIIA-s Hodgkin's disease, showing hypoplasia and deposition of amorphous, gelatinous material, hematoxylin and eosin stain, ×100

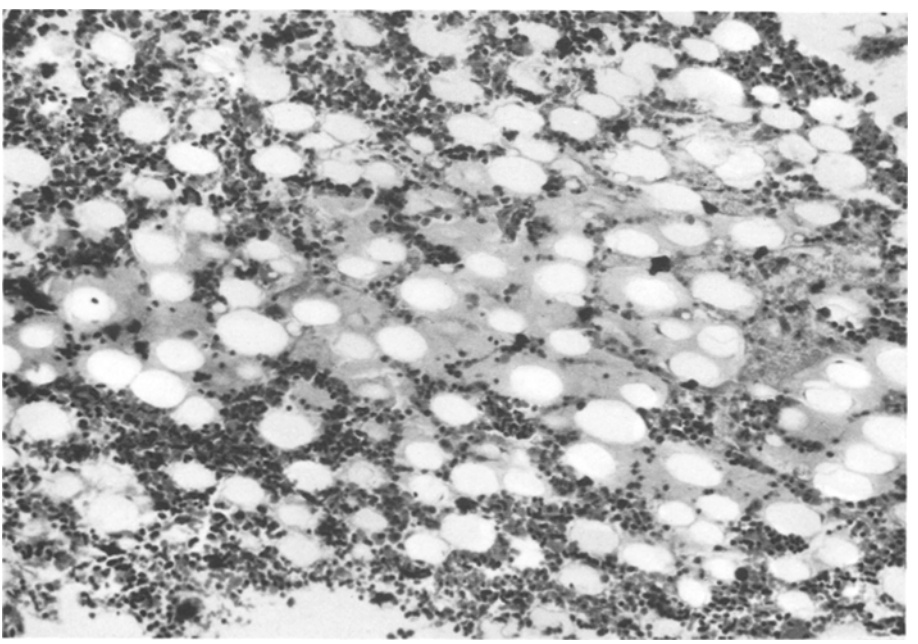

Fig. 3. Section of bone marrow from autopsy of chronically ill patient with Parkinson's disease, showing focal area of gelatinous material, hematoxylin and eosin stain, ×100

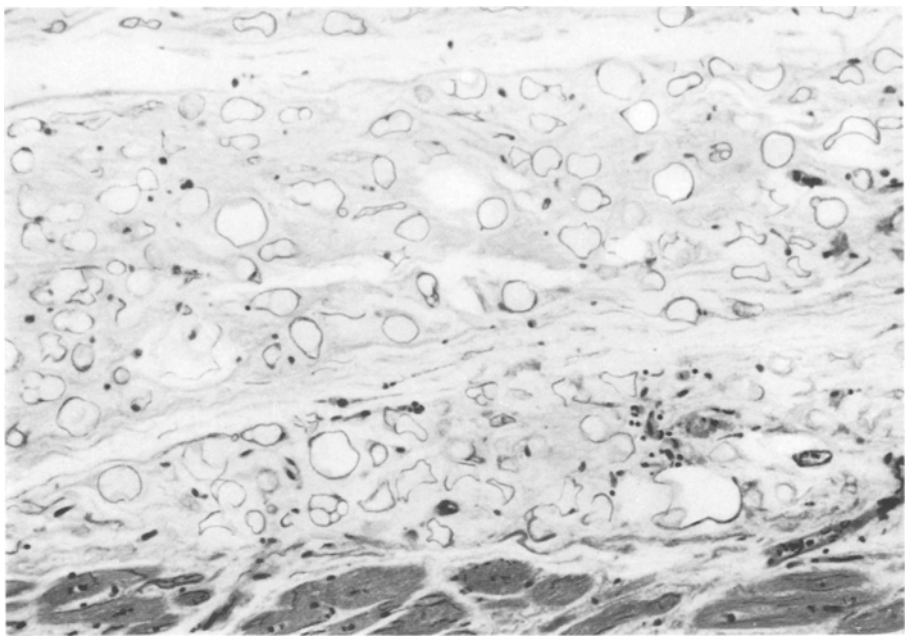

Fig. 4. Section of heart and pericardial fat showing serous atrophy change from a patient with malabsorption secondary to partial gastrectomy, hematoxylin and eosin stain, ×100

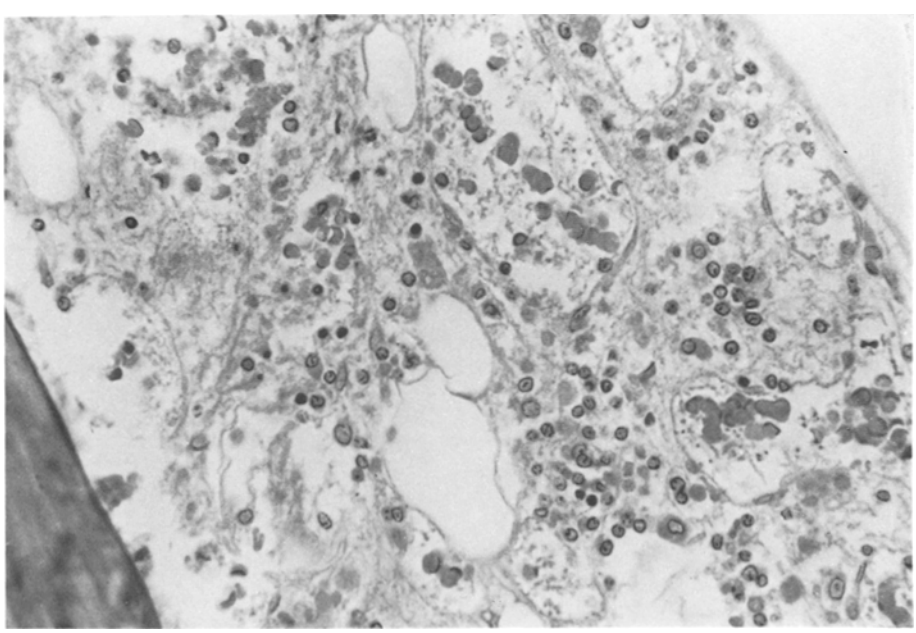

Fig. 5. Section of bone marrow biopsy showing hypoplastic marrow from a patient receiving chemotherapy for acute leukemia, hematoxylin and eosin stain, ×250. Increase in background material does not show the dense, gelatinous appearance observed in the 4 preceding figures

Table 1. Characteristic of Alcian Blue-staining background in bone marrows

Case	Diagnosis	Alcian Blue staining material	Alcian Blue					Hyaluronidase treatment[a]	Neuraminidase treatment[a]	PAS	Trichrome	Congo red	Amt. reticulin
			0.4	1.0	1.7	2.5	4.0						
1	Anorexia nervosa	Large amount, amorphous, gelatinous, fat-associated	−	+	+	+	+	↓↓	sl ↓	±	−	−	±
2	Hodgkin's disease	Large amount, amorphous, gelatinous, fat-associated	−	+	+	+	+	↓↓	↓	+	−	−	±
3[b]	Chronic Parkinsonism, acute intestinal obstruction	Small focus or amorphous, gelatinous, fat-associated	−	+	+	+	+	↓↓	sl ↓	±	−	−	±
4[b,c]	Postgastrectomy malabsorption	Epicardial serous fat atrophy	−	+	+	+	+	↓↓	↓	+	−	−	1+
Hypoplastic marrows (3)	Chemotherapy for leukemia, Hodgkin's disease	Increase in strands of background stroma	−	−	±	+	+	No change[d]	QNS[e]	++	−	−	2+
Normal marrow	Metastatic adenocarcinoma	Minute amount in strands	−	−	−	±	±	Not performed	Not performed	±	−	Not performed	±

[a] Effect of enzyme on Alcian Blue staining at pH 2.5
[b] Autopsy material
[c] Specimen of epicardial fat
[d] No change on 2/3 marrows, decrease in 1 marrow
[e] Quantity of tissue insufficient for reaction

(Patient 2) in Figure 2, and in the elderly cachectic patient with Parkinson's disease (Patient 3) in Figure 3. Note the similarity of this material to serous fat atrophy of epicardial fat in Figure 4 seen in the cachectic patient with malabsorption (Patient 4). The gelatinous appearance of this material clearly differs from the more fibrillar appearance of the increased ground substance that is usually observed in hypoplastic marrows, as illustrated in Figure 5.

Table 1 compares the staining characteristics of this amorphous substance in the bone marrows with the epicardial serous fat atrophy. All stain pink with hematoxylin and eosin, do not stain with Masson's trichrome or Congo Red, do not show increased reticulin, are faintly positive with PAS, and stain with Alcian Blue at pH 4.0, 2.5, 1.7, and 1.0, but not at 0.4, suggesting that the material is an acid mucopolysaccharide. The lack of staining at extreme acid pH suggests that the material does not contain chondroitin sulfate. Treatment overnight with testicular hyaluronidase (which will remove hyaluronic acid and chondroitin A and C) removed nearly all the Alcian Blue staining material at pH 2.5. Neuraminidase treatment slightly decreased the intensity of Alcian Blue stain at pH 2.5. Thus, the amorphous material in bone marrows and in serous fat atrophy is an acid mucopolysaccharide consisting predominantly of hyaluronic acid.

In contrast, the increased "ground substance" in the 3 hypoplastic marrows did not have a gelatinous appearance, and consisted of scattered strands of weakly Alcian Blue positive material, which was also strongly PAS positive. Alcian Blue staining was equivocal at pH 1.7 and absent at pH 1.0 and 0.4. The staining was not so significantly affected by hyaluronidase digestion in two of the three marrows, but was markedly decreased in the third. Thus, this background substance may contain neutral as well as acid mucopolysaccharides. Normal marrow showed only minute strands of Alcian Blue staining material in the stroma surrounding the open spaces of fat cells, similar in characteristics to that seen in the hypoplastic marrow.

Discussion

An amorphous, gelatinous material has previously been associated with bone marrow hypoplasia in eight patients with anorexia nervosa (Mant and Faragher, 1972; Pearson, 1967). Reversal of hypoplasia and reduction in this material were readily obtained in the patients who improved clinically (Pearson, 1967). This material was associated with fat depletion in one series of patients (Pearson, 1967), but seen surrounding abundant open spaces of fat cells by another investigator (Mant and Faragher, 1972). Both investigators felt that this material represented an increase in the ordinary acid mucopolysaccharide ground substance of the bone marrow, perhaps replacing depleted fat as a support structure.

Similarly, hypoplasia of bone marrow has been observed in starved man and animals, occasionally accompanied by focal or widespread "gelatinous degeneration" of the bone marrow (Keys et al., 1950). Wintrobe (1961) has briefly mentioned that "gelatinous marrow composed of albuminoid substance" may

be observed in the starvation marrows. Likewise, cachectic individuals may show a generalized gelatinous, edematous appearance of fat grossly on autopsy referred to as serous fat atrophy.

Our results suggest that the amorphous, gelatinous material seen in the bone marrow of a patient with anorexia nervosa and two other cachectic patients is histochemically similar to serous fat atrophy. Thus, this bone marrow change may represent part of a generalized lesion of fat in cachectic individuals. This change may be focal in a given area, as seen in Patient 3, or be present in one anatomical area of marrow while absent in others, as seen in Patient 2. Whether this "gelatinous degeneration" of fat is the cause of marrow hypocellularity in these patients is not known. Although the more usual accompaniment of marrow hypoplasia in both cachectic and noncachectic patients is an increase in ground substance, this change differs both in appearance and histochemistry from the fat-associated gelatinous material seen in anorexia nervosa, and, rarely, in other cachectic patients.

Of interest in our patient with anorexia nervosa was the large number of spur cells present in the peripheral blood smear. This finding is a typical one in anorexia nervosa and is thought to relate to a reduction in plasma betalipoprotein levels (Mant and Faragher, 1972). This morphologic lesion is also seen in abetalipoproteinemia. Another finding in the marrows of some patients with anorexia nervosa is histiocytes containing blue-green granules on Romanowsky stain, thought to represent ceroid (Mant and Faragher, 1972) although our patient did not demonstrate this abnormality.

Leukopenia, usually with neutropenia and a relative lymphocytosis, is a frequent occurrence in anorexia nervosa, usually attributed to marrow hypoplasia. Thrombocytopenia is occasionally observed, and erythroid hypoactivity has been demonstrated in one patient by ferrokinetic studies (Pearson, 1967). In spite of marrow hypofunction, even when coupled with the occurrence of iron and folate depletion, anemia is only seen in 30% of patients with anorexia nervosa and is usually mild (Seidensticker and Tzagournes, 1968). The finding of normal marrow from the left iliac crest and hypoplastic marrow with deposition of acid mucopolysaccharide in fat from the right iliac crest in patient 2 suggests that the marrow dysfunction may not be generalized. This patchy hematopoiesis would account for the maintenance of normal peripheral counts despite demonstrated marrow hypoplasia, and the reduced ability of both starved patients and patients with anorexia nervosa to mount a leukocyte response to infection (Pearson, 1967).

Conclusion

Hypoplasia in the bone marrow of patients with anorexia nervosa is accompanied by amorphous acid mucopolysaccharide material surrounding the fat cells. Histochemically, this lesion appears to be part of the process of generalized serous atrophy of fat that occurs in longstanding malnutrition.

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Received January 20, 1977