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Ultrastructure of Mutual Adhesion and Multinuclearity of Erythroblasts in a Patient with Dyserythropoietic Anemia

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Morphological changes of erythroblasts were observed in the bone marrow of a 29-year-old female patient with dyserythropoietic anemia by light and electron microscopy.

The most remarkable observation in this patient was the existence of erythroblastic junctions which had never been described in such patients. Multinuclearity of erythroblasts was also observed. Light microscopically, most of the cell junctions looked like vacuoles. Electron microscopically, both cell membranes were attached in parallel at the cell junctions, and protruded into the cytoplasm or ran in almost a straight line. The type of the junction was considered to be adhesion. All changes were restricted to the erythroblastic series.

Ineffective erythropoiesis which caused anemia in this patient might be due to these morphological abnormalities of the erythroblasts.

(**Key Words: Ultrastructure, Abnormal Erythroblasts**)

INTRODUCTION

Blood cells which originate from mesenchymal cells migrate into the blood vessels and peripheral tissues and usually do not have cytoplasmic connections between them. Under certain circumstances, however, conjugations are observed among blood cells alone or among blood cells and endodermal cells (1, 2, 5, 6, 7).

This is a case report of a patient with anemia, associated with mutual conjugation and multinuclearity of erythroblasts in the bone marrow. Erythroblastic conjugation has never been found in such patients.

This report deals with particular findings in the cells of the erythroblastic series. The possible relation between the abnormal morphology of erythroblasts and dyserythropoietic anemia is discussed.

CASE REPORT

The patient, a 29-year-old housewife, was admitted to the hospital in January, 1971 because of anemia. She was a pale, moderately developed, slightly obese woman. Hematological studies on admission were as follows; hemoglobin: 44%, hematcrit: 24%, red blood cell count: 263×10^4 per cmm and white blood cell count: 5, 900 per cmm, with a normal differential count. The reticulocyte count was less than 1‰. Platelet count was

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26. 3×10^4 per cmm. A bone marrow specimen revealed moderate hypercellularity and marked erythroid hyperplasia. Other elements were normal. More than two basophilic or polychromatic erythroblasts conjugated mutually (Fig.1). May-Giemsa's, Sudan Black and PAS stains of the conjugated region, which looked like a vacuole, were all negative. There were a few cell-conjugations between young and mature erythroblasts. There were many multinucleated erythroblasts which had several nuclei. Most of these cells were in the stage of orthochromatic erythroblasts. Repeated bone marrow aspirations demonstrated that conjugated erythroblasts were 40 to 140 and multinucleated erythroblasts were 120 to 220 per 10^3 mononucleated erythroblasts respectively. The number of these abnormal erythroblasts decreased and improved after blood transfusion.

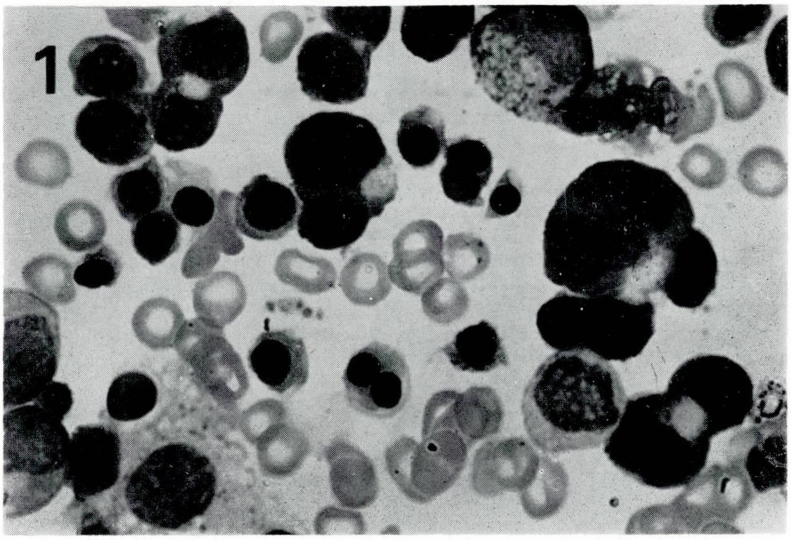

Fig. 1 Light microscopically the erythroblastic junction looks like vacuoles. X 3, 000

ULTRASTRUCTURE OF ERYTHROBLASTS

1. Material and Methods

Bone marrow specimens were fixed immediately in 2. 5% glutaraldehyde for 2 hours followed by 1% osmium tetroxide fixation for 1 hour. Dehydration was carried out in an ascending series of ethanol and propylene oxide, and the tissue was embedded in epon. Thin sections were stained successfully with uranyl acetate and lead citrate.

2. Results

A. Conjugated erythroblasts

Electron microscopically, the membrane of two adjacent cells were attached tightly together at the erythroblastic junction, and the cytoplasms of both cells were clearly defined (Fig. 2, 3). The interdigitation of the cell membrane was so complex that membrane tracing could not be completely pursued. High magnification of the conjunction revealed that the distance between both membranes was relatively constant at about 180Å. The electron density of the intermembranous space was higher than that of the cytoplasm (Fig. 4). The junctional apparatus such as zonula adherens, macula adherens, zonula occludens, macula occludens and virus-like particles were not observed along with the cell membrane. Cell membranes were observed between erythroblasts which were considered to be multinucleated cells by light microscopy (Fig. 2). Most membranes ran in almost a straight line, but a few membranes curved rather complicatedly. Sometimes only a small portion of the cell surface was conjugated, and the other part of the cell surface was in loose contact (Fig. 5), the latter was considered to be the initial stage of intercellular conjugation. Occasionally, rather complicated cell junctions were seen and one cell was in the stage of mitosis (Fig. 6). This phenomenon suggests that conjugated cells still maintain the ability of cell division. Autoradiography using ^{3}H-thymidine and ^{3}H-uridine revealed that conjugated erythroblasts synthesized nucleic acids but the degree of the synthesis in such cells was less than that in mononuclear erythroblasts.

B. Nucleus

Multinucleated erythroblasts were mostly orthochromatic erythroblasts. Partial obscurity of the nuclear envelope and disintegration of peripheral nuclear chromatin were observed(Fig. 7). Certain cells demonstrated two nuclei attached each other over a wide area of the nuclear envelope, thus forming a curious shape (Fig. 8). Disintegration of nuclei which were isolated by budding were seen in a few mononuclear erythroblasts (Fig. 9). No nucleolus abnormalities were observed.

C. Organelles

Many round and a few deformed mitochondria with irregular cristae were seen. The number of mitochondria decreased during the process of maturation. The endoplasmic reticulum was not well developed. Occasionally, microtubuli were seen. Conjugated erythroblasts were rich in atypical mitochondria and Golgi apparatus.

The morphological changes described above were restricted to the erythroblastic series and were not observed in other cell series. In addition, the intercellular junction was seen only between erythroblasts and never observed between erythroblasts and other blood cells.

DISCUSSION

The morphological classification of the cell association employed in this study is as follows : (1) free cells which do not have a direct relationship to each other ; (2) discontinuous connection in which both cell membranes are formed between adjacent cells ; and (3) continuous connection in which cell membranes disappear and both cells showed similar morphology.

Usually, erythroblasts which are precursors of erythrocytes have no mutual association in the bone marrow. Fukuda et al. (1) reported that desmosome-like attachments connecting erythroblasts to hepatocytes were seen in the liver of human fetuses. Desmosomes have been found in other mesenchymal cells (2, 5, 6). However, connection between erythroblasts in the bone marrow has never been reported. This discontinuous connection was neither cell contact nor cell fusion because of the close contact without fusion. The morphology of the connection clearly indicated that this connection was due to adhesion. The factor which caused this adhesion remains unknown. The type of the connection can be classified as partial, straight, rather complex and markedly complex conjunction, and there were sometimes connections between erythroblasts with quite different stages of growth. Therefore, it is suggested that the connection occured after cell division.

On the other hand, erythroblasts are mutually conjugated under specific conditions. Toister et al. (7) reported that Parainfluenza myxovirus could induce the formation of conjugated chick erythrocytes by the fusion of single cells. In this patient, virus-like particules were not observed in the region of cell connection and the type of the connection was not fusion. Therefore, it is not likely that the conjugated erythroblasts were induced by virus.

The second characteristic finding in this case was the presence of many erythroblasts with two or several nuclei. Multinucleated erythroblasts are most often seen in congenital dyserythropoietic anemia which was first reported by Wolf et al. (8) internationally and by Kosaka et al. (4) in Japan. Recently, Heimpel et al. (3) also reported the morphological changes of erythroblasts in this disease. In this case, degenerative changes of nuclei of erythroblasts were observed but such changes were not seen in the family of the patient. Therefore, this patient is not considered to have been suffering from congenital dyserythropoietic anemia.

The diseases in which multinucleated erythroblasts are often observed are megaloblastic anemia, erythremia and erythroleukemia. Since the PAS stain of the erythroblasts of this patient was weakly positive by several percent, there was a possibility that she was suffering from erythremia.

Is there any relationship between conjugated and multinucleated erythroblasts ? There are no reports indicating that multinucleated erythroblasts were formed from conjugated ones. Therefore, it seems that these abnormal erythroblasts are generated by quite different causes.

It is a very interesting fact that these abnormal erythroblasts decreased in number after blood transfusions associated with the improvement of anemia. In this patient, examinations have shown a remarkably ineffective erythropoiesis which caused severe anemia Sideroblastic anemia, thalassemia and abnormal hemoglobinemia accompany ineffective erythropoiesis but the possibility of these diseases in this patient were excluded from clinical data. It is concluded that the ineffective erythropoiesis in this patient might be due to the morphological abnormalities of erythroblasts. Such morphological changes may be useful in evaluating the prognosis of patients with dyserythropoietic anemia.

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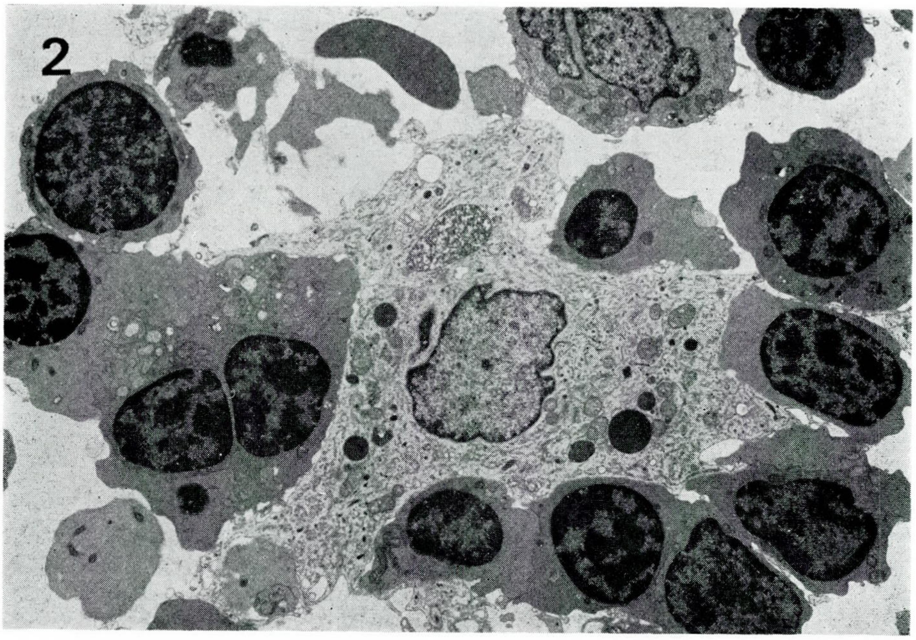

Fig. 2 Many conjugated erythroblasts. The contact cell membranes run in almost a straight line. X 4, 000

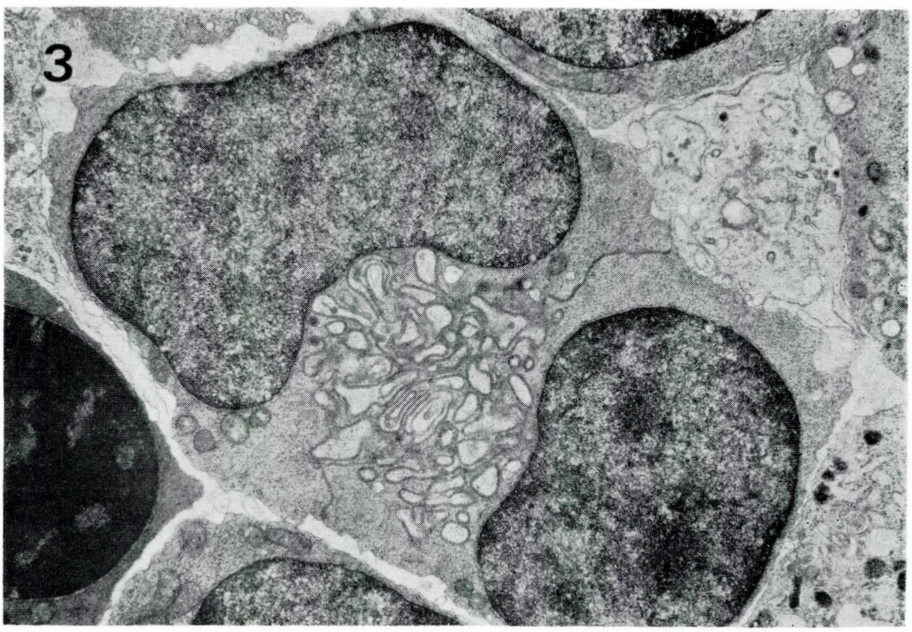

Fig. 3 In erythroblastic junctions, both cell membranes are seen to run in parallel and protrude into the cytoplasm. X 10, 000

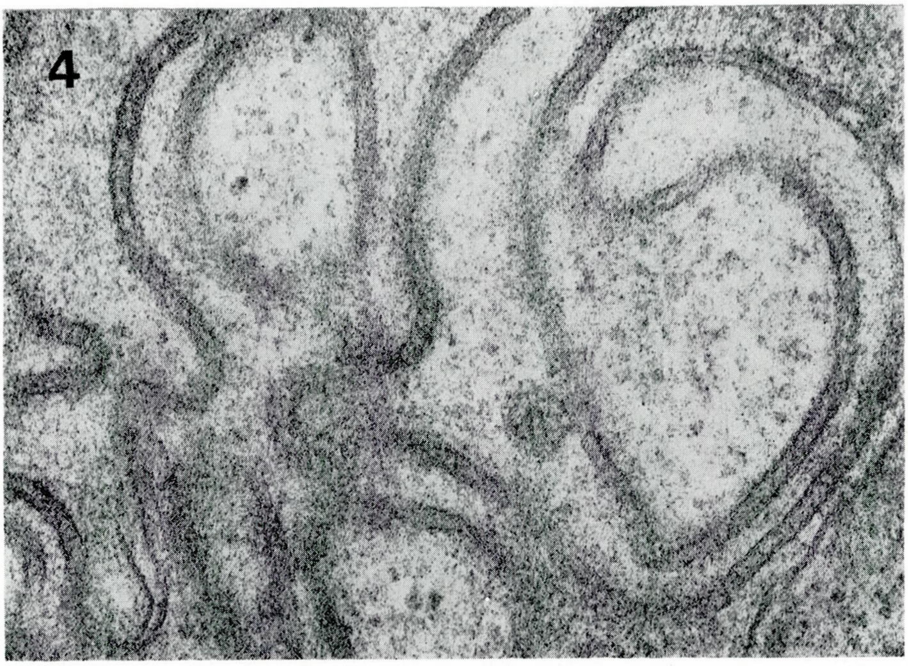

Fig. 4 Region of erythroblastic junction. The space between the membranes is about 180Å in width. X 80, 000

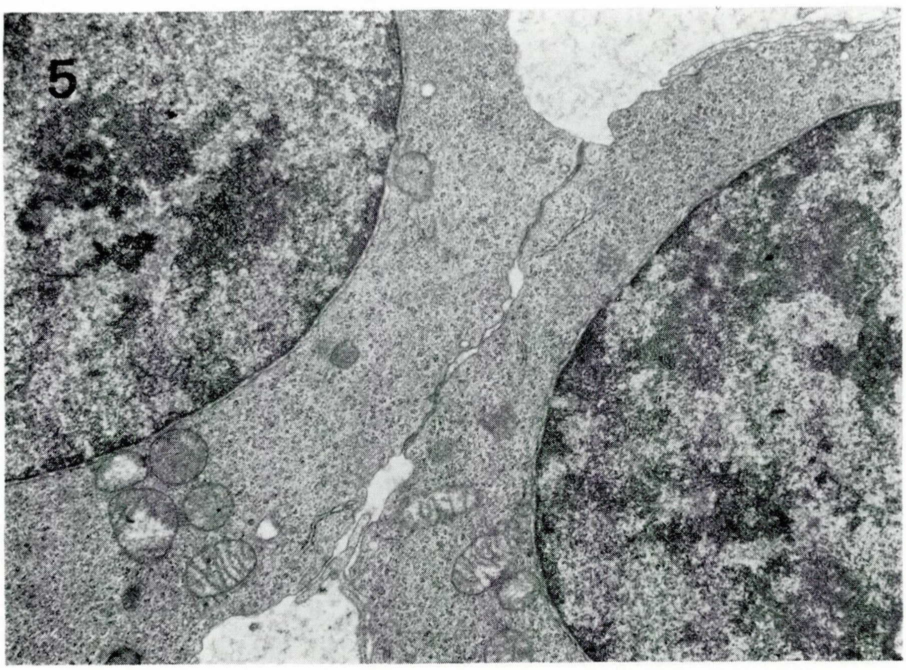

Fig. 5 Erythroblasts. Junction is observed in part of the cell membranes. X 15, 000

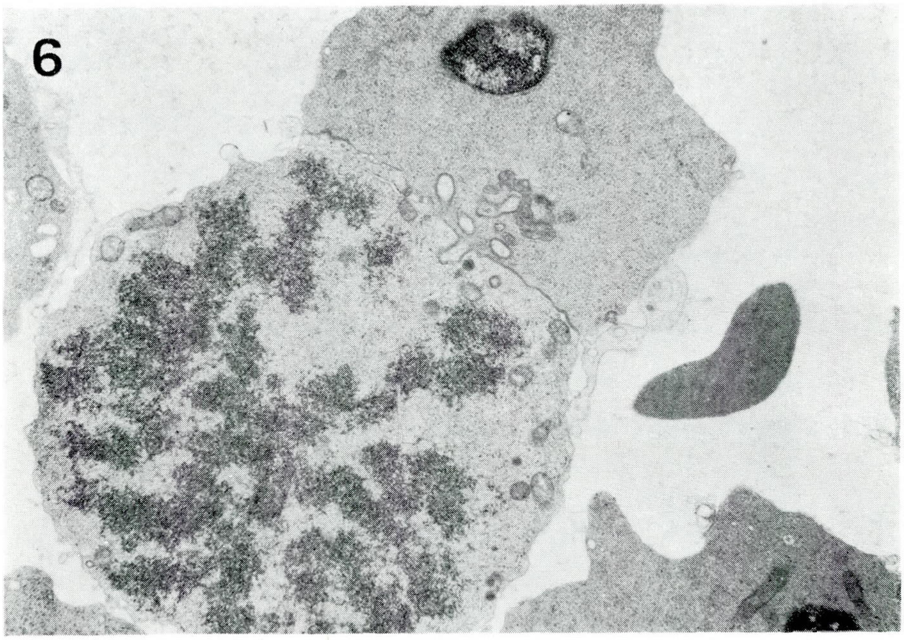

Fig. 6 Relatively complicated erythroblastic junction. One erythroblast is in the stage of mitosis. X 10, 000

Fig. 7 Multinucleated erythroblast. Notice the degeneration of the nuclei. X 15, 000

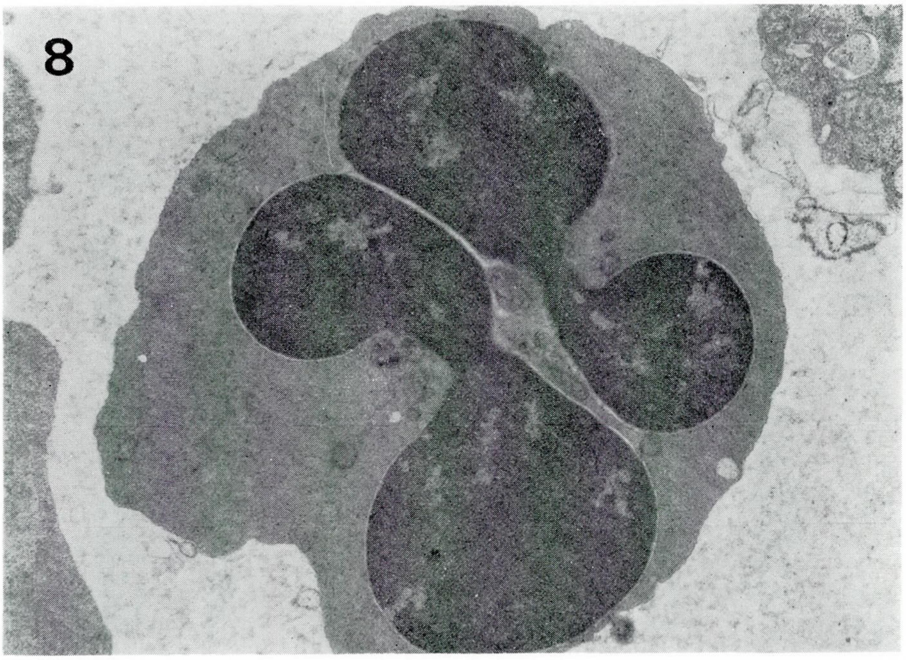

Fig. 8 Contact of two nuclei in an erythroblast. Curious deformity of both nuclei. X 15, 000

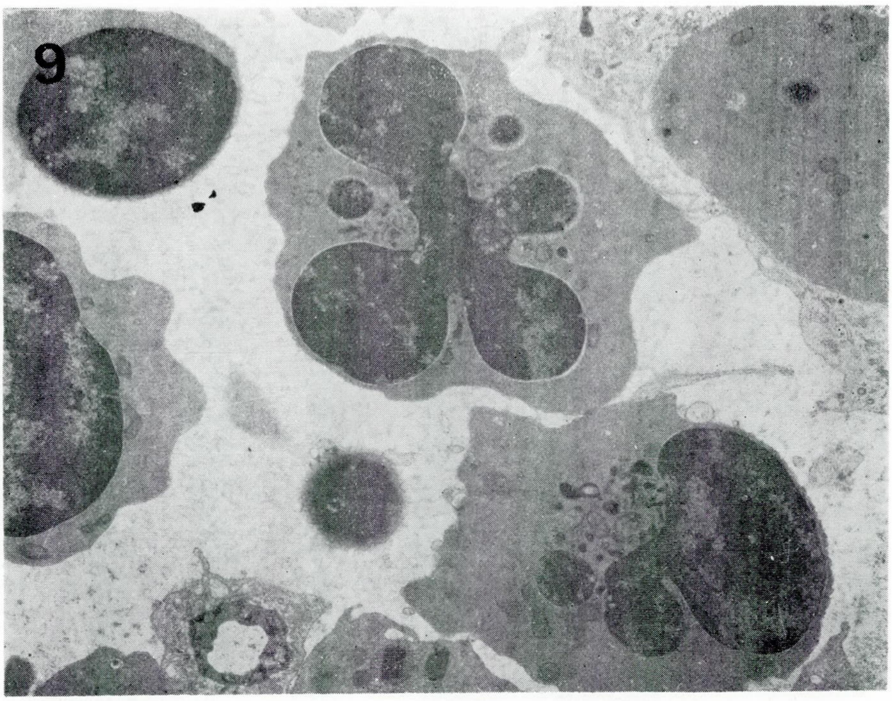

Fig. 9 Erythroblasts. Many protrusions of the nucleus and disintegration of the nuclear chromatin. X 10, 000