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# Electronmicroscopic and Biochemical Observations on Erythroid Cells in Congenital Dyserythropoietic Anemia Type II

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*Summary.* Erythroid cells in a patient with dyserythropoietic anemia (CDA) were examined for biochemical, serologic and ultrastructural alterations. The activity of hexokinase and 6-phosphogluconate dehydrogenase was increased and the acidified serum test was positive. These observations are consistent with CDA type II (CDA II), a diagnosis that has been said to depend on a positive acidified serum test. Electronmicroscopic analysis of the erythroid cells of the present patient indicated multinuclearity, karyorrhexis, continuity between cisternae of endoplasmic reticulum and the nuclear envelope at sites where the latter would show a complete break, extrusion of intracellular material and organelles, and excessive smooth endoplasmic reticulum. These observations may be considered the ultrastructural hallmark of CDA II. Most of them have also been made in four of five examined patients with positive acidified serum test whereas the fifth patient was reported to have markedly dissimilar erythroid ultrastructure. It appears, therefore, that diagnostic classification of CDA by the acidified serum test may not always coincide with that achieved by electronmicroscopy.

## Introduction

To date, congenital dyserythropoietic anemia type II (CDA II) has been described in approximately forty patients (Verwilghen *et al.*, 1973). In every instance, the diagnosis was based on clinical, light microscopic and serologic criteria.Abno rmal erythroid ultrastructure was initially noted simultaneously in two independent laboratories (Heimpel *et al.*, 1970; Hug *et al.*, 1970) and this abnormality was first illustrated in 1971 by electronphotomicrographs of erythroid cells from a girl with CDA II (Hug *et al.*, 1971). Three additional publications have since appeared (Hug *et al.*, 1972; Wong *et al.*, 1972; Breton-Gorius *et al.*, 1973) with illustrations that are adequate for an assessment of the unusual fine structure in CDA II. The present report of a fifth case is added because the patient's erythroid cells exhibit some ultrastructural findings not previously described. Their detailed description should facilitate the derivation of a morphologic classification for CDA. The degree of coincidence can then be determined that may exist between the classification based on morphologic findings and that based on clinical, serologic or biochemical observations. The use of morphologic versus serologic criteria may not always result in the identical classification; at any rate, this will have to be our suspicion after comparison of the findings by Keyserlingk *et al.* (1970) and Meuret *et al.* (1970) with our own observation.

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## Patient and Method

The patient is a 23 year old married woman who had been described by Schärer *et al.* (1965) as the third recorded patient with the clinical features of CDA II. Mild anemia had first been recognized when she was six years old. The liver was minimally enlarged. There was splenomegaly prior to splenectomy done in 1957. On skull x-ray, there was widening of the diploic space and atrophy of the outer table with generalized radial striation; these radiographic findings persist. Despite recurrent episodes of benign jaundice the patient leads a normal working life and requires no therapy.

The acidified serum test was done as described by Crookston *et al.* (1969). Dr. J. H. Crookston kindly supplied anti-i and anti-I serum for the serologic examination of the red cells.

The determination of enzymatic activities and of metabolic intermediates in erythrocytes was according to standard methods (Beutler and Yeh, 1963; Grignani and Löhr, 1960; Jütting *et al.*, 1965). The preparation of bone marrow specimens for electronmicroscopy has been described (Hug *et al.*, 1972). The ultrastructural findings were compared with those of the reported patient with CDA II (Hug *et al.*, 1971, 1972) as well as with thirty-two bone marrow specimens from children with the following conditions (number of patients in parentheses): type II glycogenosis (8), other types of glycogen storage disease (7), Tay-Sachs disease (2), leukemia (3), Gaucher disease (1), Hurler disease (2), mucosulfatidosis (1), Down syndome (1), undiagnosed (7).

Table 1. Analysis of peripheral blood cells (All determinations are done on erythrocytes except for the last entry of the table)

| | Patient | | Normal |
|---|---|---|---|
| | 1969 | 1972 | |
| Acidified serum test with group compatible, homologous serum | lysis | lysis | No lysis |
| Agglutination with anti-i serum | 1:1000 | 1:1000 | 1:100 (cord RBC) |
| Agglutination with anti-I serum | 1:5000 | 1:5000 | 1:1000 (adult RBC) |
| Reduced glutathione (mg/100 ml RBC) | 82 | 104 | 50–95 |
| 2,3-diphosphoglycerate ($\mu$moles/$10^{11}$ RBC) | | 47 | 20–48 |
| ATP ($\mu$moles/$10^{11}$ RBC) | 25.6 | 18.8 | 10.5–21.8 |
| ADP ($\mu$moles/$10^{11}$ RBC) | 2.08 | 2.04 | 1.28–2.06 |
| AMP ($\mu$moles/$10^{11}$ RBC) | 0.49 | 0.31 | 0.29–0.47 |
| Osmotic fragility (% NaCl) | 0.26–0.55 | | 0.28–0.55 |
| Acetylphenylhydrazine | No enhanced formation of inclusion bodies | | |
| Glutathione reductase (IU) | 11 | 10 | 7–14 |
| Hexokinase (IU)[a] | 3.2 | 2.9 | 0.8–2.0 |
| 6-phosphogluconate dehydrogenase (IU)[a] | 28 | 25 | 10–16 |
| Hemoglobin (g-%)[a] | 7–10 | | 13 |
| Reticulocyte (%)[a] | 1–3 | | 0.9 |
| Erythrocyte mean corpuscular volume ($\mu m^3$)[a] | $109 \pm 9$ | | 86 |
| Leucocyte alkaline phosphatase (U)[a] | 223 | | 10–110 |

[a] The last six entries exhibit abnormal values.

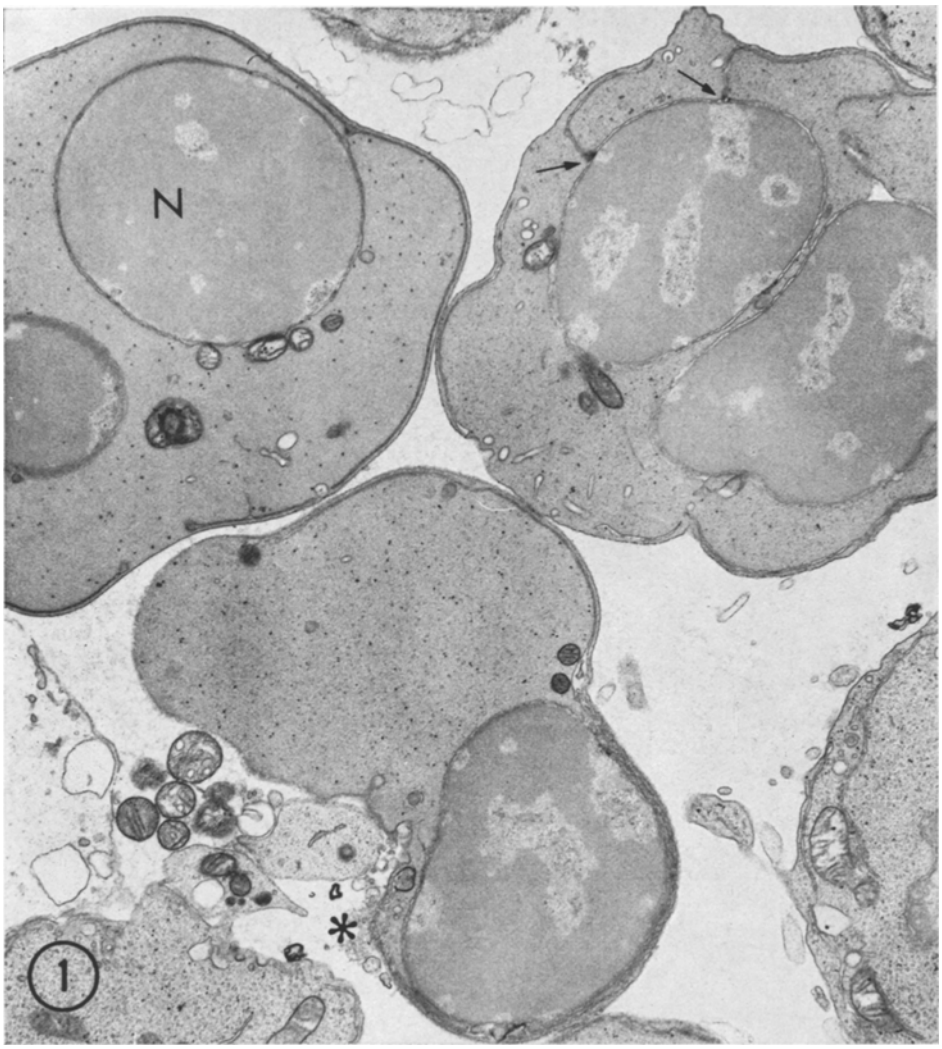


Fig. 1. Three late erythroblasts, with abnormal cisternae of endoplasmic reticulum that show continuity with the perinuclear space (arrow). Two of the cells are binuclear, the third one is in the process of nuclear extrusion. The asterik indicates a site of extrusion of granular intracellular material, possibly of nuclear origin. *N* nucleus (× 7000)

## Results

### *Biochemical Examination*

Biochemical and serologic data are summarized in Table 1. Agglutination of the patient's red cells occurred with anti-I serum at a titer five times higher than that observed with normal adult erythrocytes, and with anti-i serum the titer was ten times higher than that required for the agglutination of normal newborn erythrocytes. The patient's red cells were not lysed by her own serum; however, her erythrocytes lysed when they were exposed to acidified serum of her

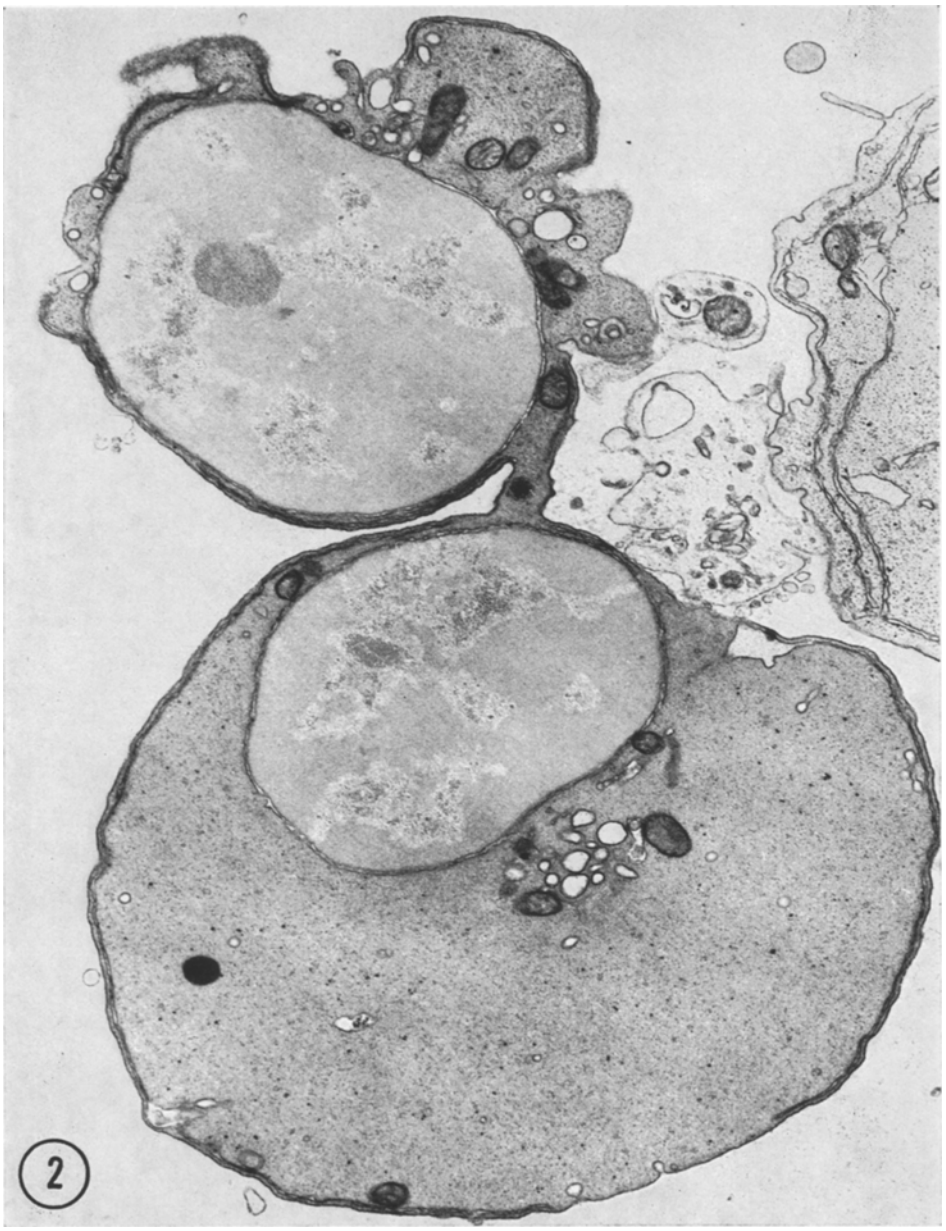


Fig. 2. Binucleated erythroblast that appears to "pinch off" one nucleus. (× 8000)

blood group but obtained from another individual (positive or abnormal acidified serum test).

Activities of hexokinase and 6-phosphogluconate dehydrogenase were increased in the patient's red cells. Activities of the following enzymes were normal:

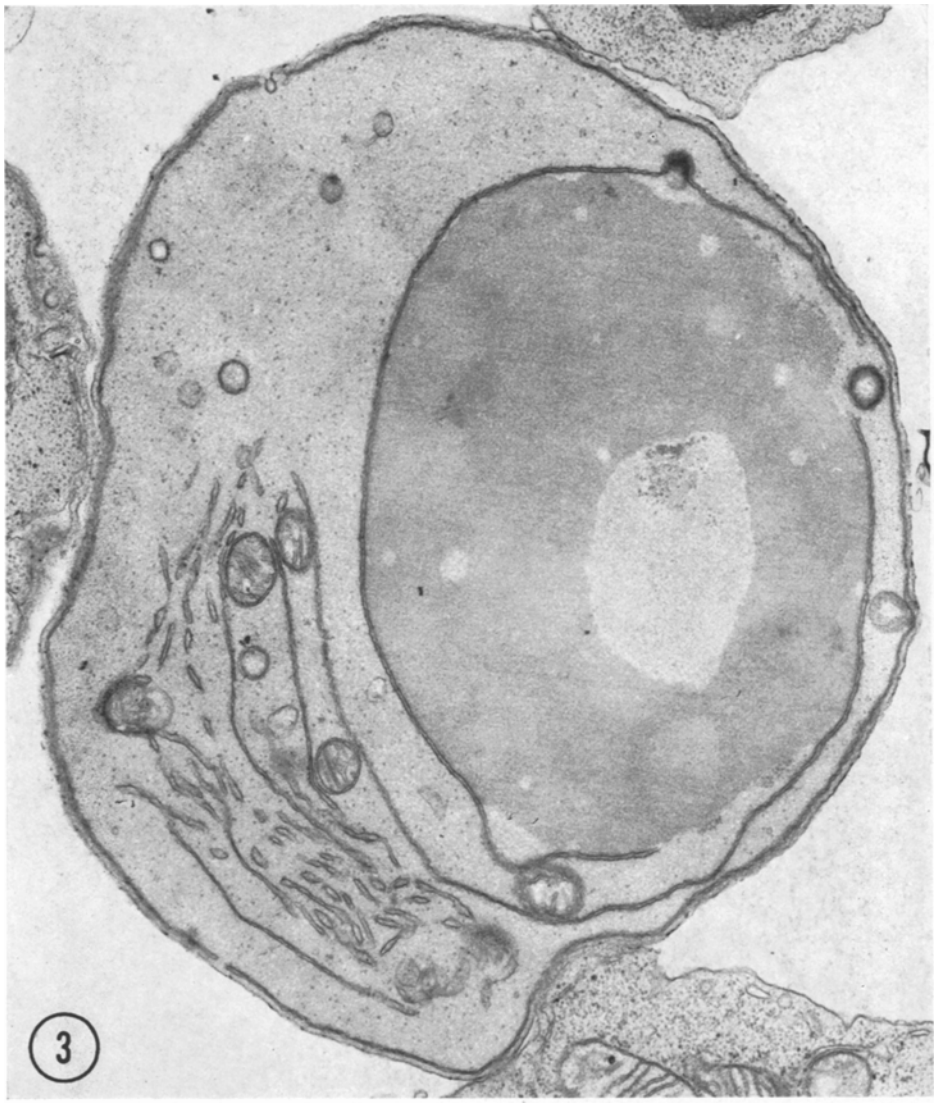


Fig. 3. Erythroblast with stacks of cisternae that seem to "unwind" from the nuclear envelope resulting in a gap of the envelope (× 14000)

glutathione reductase, pyruvate kinase, glucose-6-phosphate dehydrogenase, phosphohexose isomerase, phosphoglycerate kinase. Concentrations of the following red cell metabolites were normal: reduced glutathione, AMP, ADP, ATP, 2,3-diphosphoglycerate. Methemoglobin was not detected. Hemoglobin thermal stability was normal. Acetylphenylhydrazine did not promote enhanced formation of inclusion bodies.

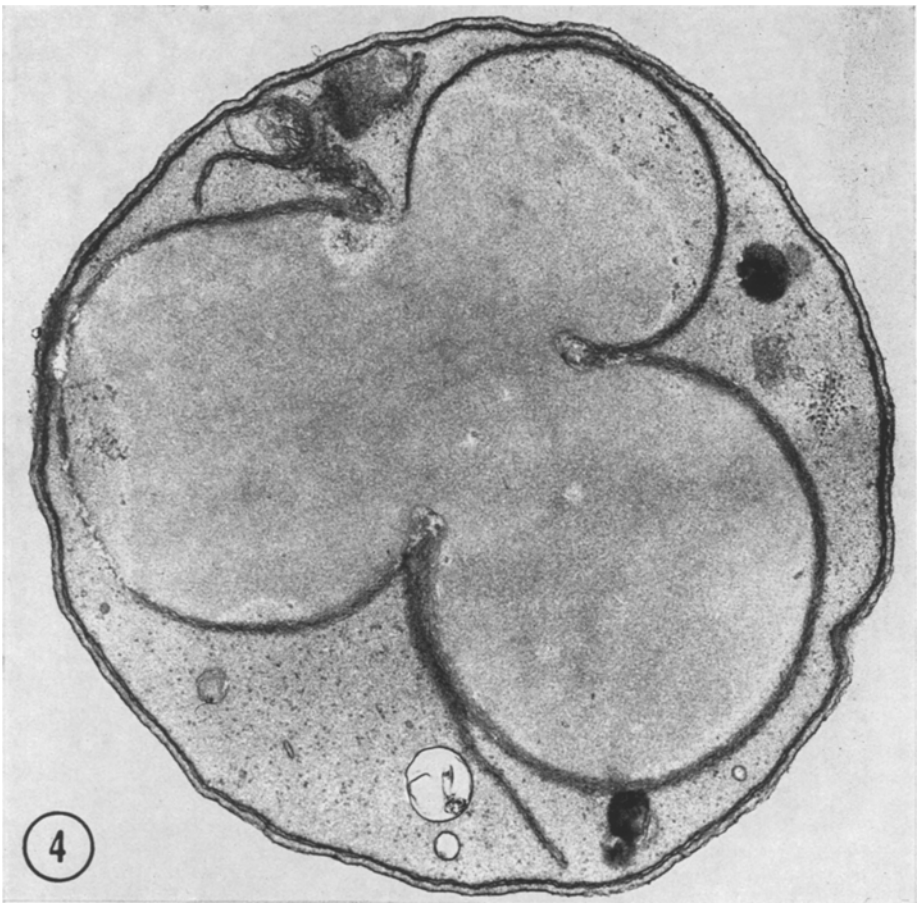


Fig. 4. This late erythroblast has one continuous peripheral cisterna and three abnormal nuclear protrusions in the form of a clover leaf. (× 10000)

*Electron Microscopic Examination*

For the following description of the ultrastructural abnormalities and in the interest of pictorial economy, we shall refer to illustrations of previous publications (Hug *et al.*, 1971, 1972) when observations in the present patient are similar to our previous findings.

No abnormality of platelets or myeloid cells was detected. The usual sequential stages of erythroid maturation as seen in controls were also observed in the patient's marrow. These stages included proerythroblasts in mitosis, erythroblasts, nuclear extrusion and mature erythrocytes. Representative cells for all of these progressive stages of maturation contained excessive cytoplasmic membranes arranged as cisternae of smooth surfaced endoplasmic reticulum (Hug *et al.*, 1971). Such cisternae could not be seen in control erythroblasts that typically contained the single nucleus within hemoglobin surrounded by the plasma membrane. All of

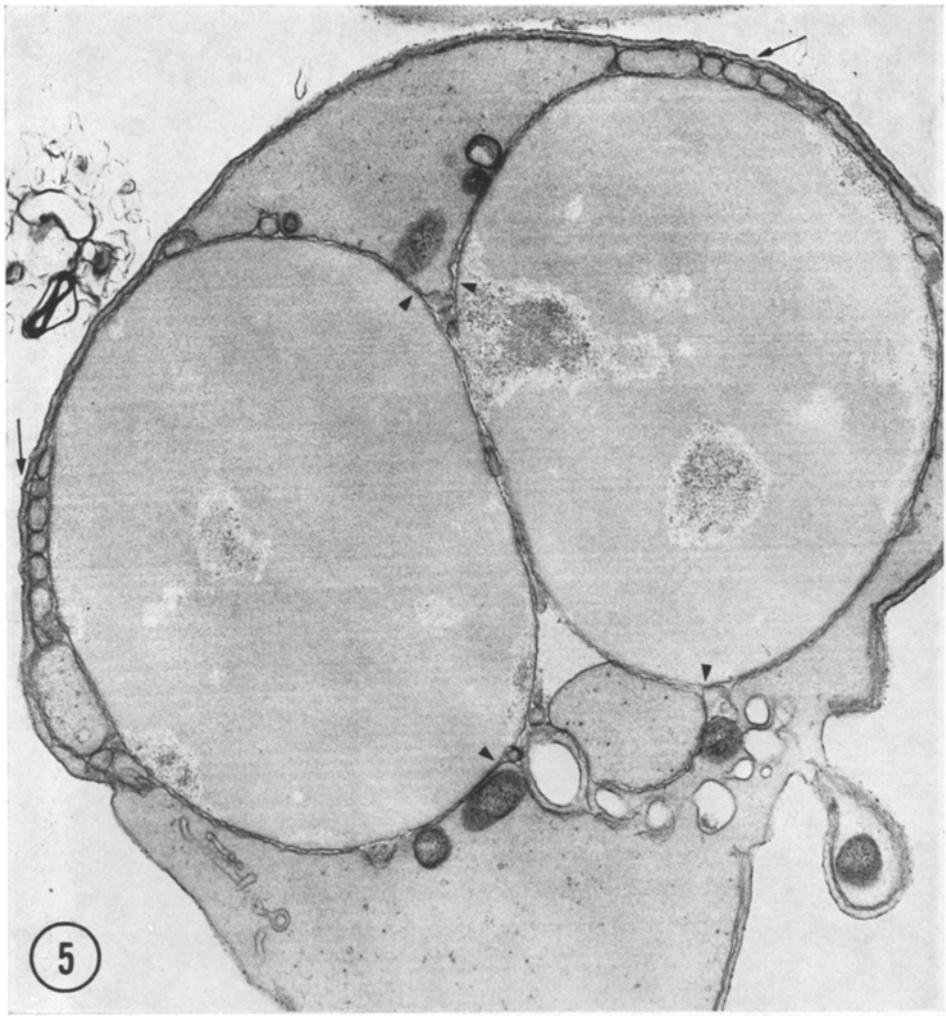


Fig. 5. Binucleated erythroblast with peripheral cisterna that contains groups of round and oblong cytoplasmic profiles (arrows). The two nuclei share the outer membrane of their envelopes. Arrow heads indicate sites where the outer membrane leaves one nuclear circumference and begins to course towards the other. ($\times$ 13000)

the observed nucleated erythroid cells of the patient had abnormal cisternae. Frequently, they were separated by a narrow rim of hemoglobin from the plasma membrane and followed its entire circumference. Within the abnormal cisternae, there were frequent round or oblong profiles apparently of cytoplasm surrounded by a membrane (Figs. 5—8). The cisternae were continuous with the perinuclear space (Figs. 1, 3, 5–7, 9 and Hug *et al.*, 1971). About two per cent of mature erythrocytes showed remnants of these cisternae (Hug *et al.*, 1971). There were bizarre "plate" formations in a few of the patient's mature erythrocytes (Fig. 10).

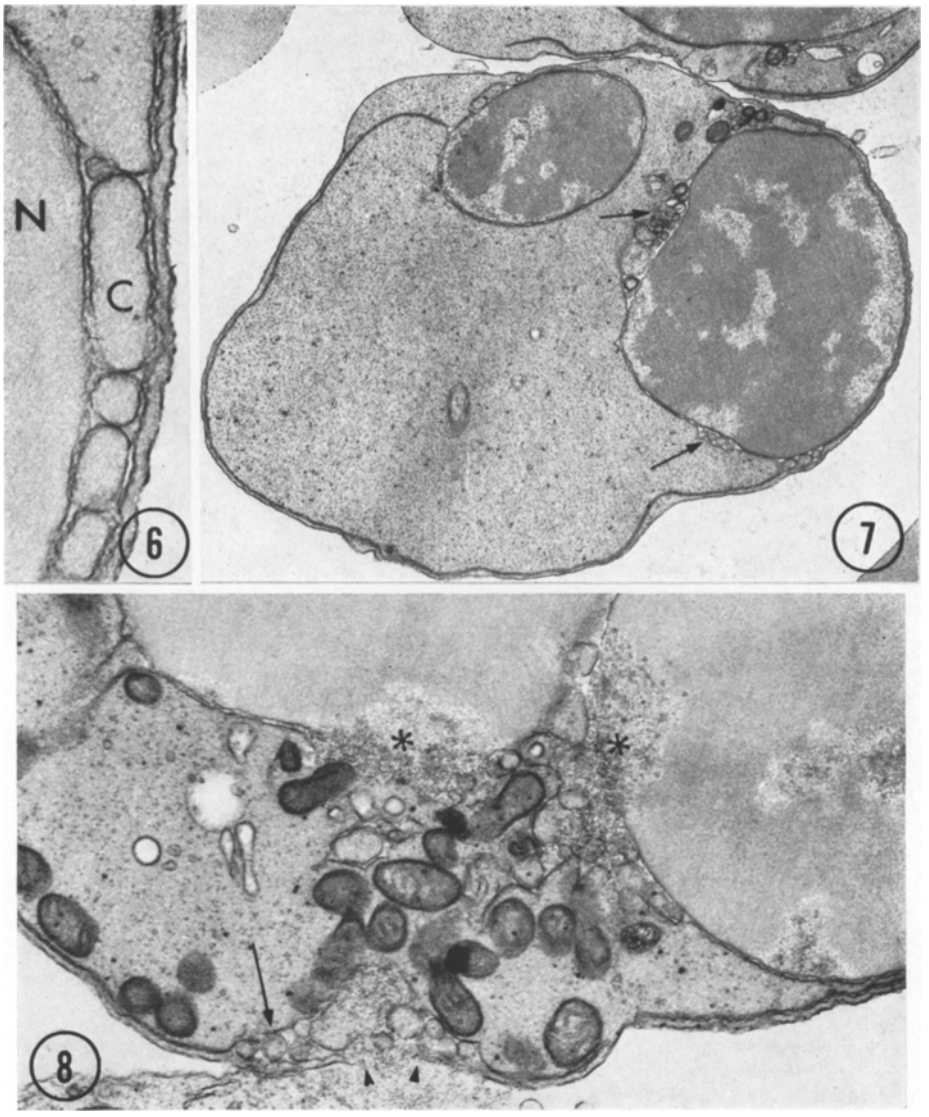


Fig. 6. This detail of an erythroblast documents the continuity between perinuclear space and peripheral cisternae. Within the cisterna, there are round and oblong cytoplasmic profiles. *N* nucleus, *C* cytoplasm. (× 18000)

Fig. 7. This erythroblast has two nuclei that are surrounded by the same continuous peripheral cisterna. There are groups of round cytoplasmic profiles (arrows). (× 8000)

Fig. 8. Detail of binucleated erythroblast that indicates disintegrating nuclear envelopes, exit of nuclear material into the cytoplasm (asterik), and abundance of intracellular organelles such as mitochondria crowded against the plasma membrane that has ruptured (arrowhead). In the underlying cisterna, there are groups of cytoplasmic profiles (arrow). (× 16000)

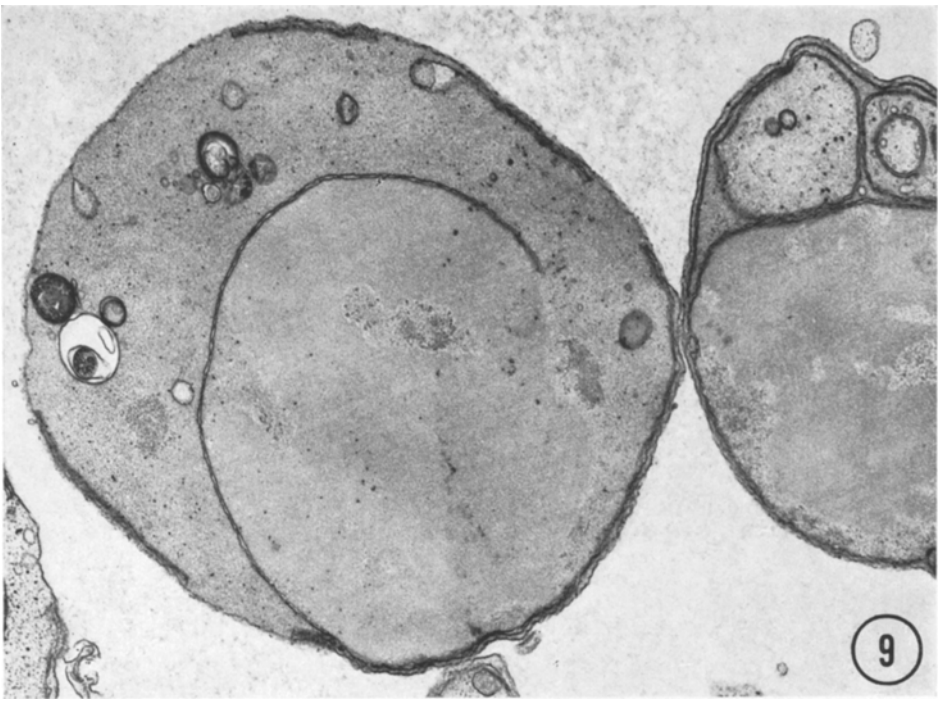


Fig. 9. Two abnormal erythroblasts one of which exhibits partial loss of nuclear membranes resulting in continuity between nuclear and cytoplasmic material ($\times$ 10000)

There were erythroblasts with completely separated nuclei their perinuclear spaces connected by one cisterna (Fig. 7). Occasional cells had nuclei with multiple protrussions giving them a clover leaf appearance (Fig. 4). There were breaks and extended gaps in nuclear envelopes that seemed to unwind into cisternae of endoplasmic reticulum (Figs. 3, 9). As a consequence, there was continuation between cytoplasm and nuclear content (Figs. 3, 9). The latter contained blebs of granular material (Figs. 1–6, 8, 9, 11). Material of similar appearance was expelled from some cells (Figs. 1, 8, 11–13) as were mitochondria (Fig. 11). Erythroblasts containing cisternae went through the process of nuclear extrusion (Fig. 1 and Hug *et al.*, 1972); binucleated cells were thus reduced to mononuclear erythroblasts (Fig. 2). Bone marrow macrophages of the patient were frequently packed with erythroblasts (Fig. 14 and Hug *et al.*, 1972).

There were no cisternae or other unusual morphologic findings in erythroid cells of controls.

## Discussion

Clinical, biochemical and serologic features of forty patients with CDA II on record in the literature have been reviewed by Verwilghen *et al.* (1973). These authors accepted the diagnosis of CDA II if the acidified serum test was positive in an individual with ineffective erythropoiesis and erythroblastic multinuclearity.

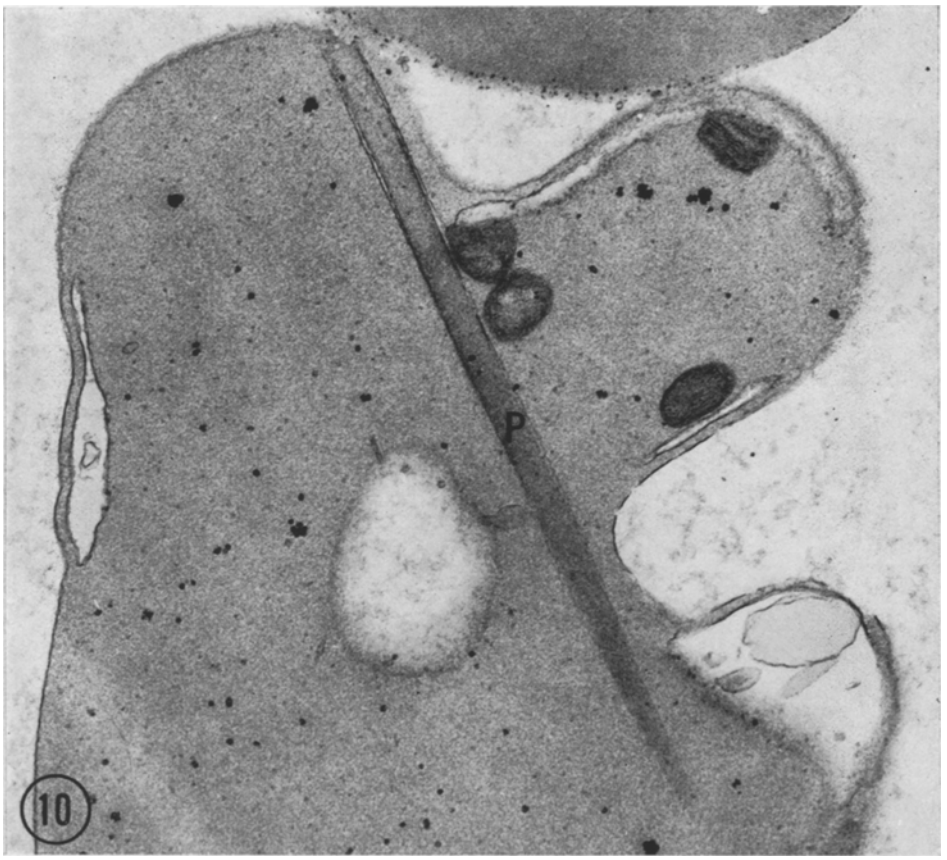


Fig. 10. Unusually shaped mature erythrocyte with cisternae and cytoplasmic "plate" formation (*P*). (× 14000)

These features have been observed in our patient. The forty recorded patients had the following clinical findings with the indicated frequency: recurrent benign jaundice 90%; anemia 87%; splenomegaly 74%; hepatomegaly 44%; gall stones 20%; hepatic cirrhosis 18%; abnormal x-ray of the skull 15%. Most patients tolerated the disease without difficulty or therapy, although a few had transfusions in childhood. Splenectomy was done in eleven patients including the present one. The most severe cases of CDA II occurred among the patients eventually splenectomized. Two of the latter died, one with demonstrated septicemia. Out patient did not have hepatic cirrhosis or gallstones, and she had only minimal hepatomegaly. Despite the presence of the other clinical features mentioned above, she has never been incapacitated by her disease.

Biochemical findings in erythrocytes of CDA II have been presented by Valentine *et al.* (1972). These authors demonstrated increased activity of hexokinase and 6-phosphogluconate dehydrogenase, but normal activity of other glycolytic enzymes. Similar results were obtained in our patient.

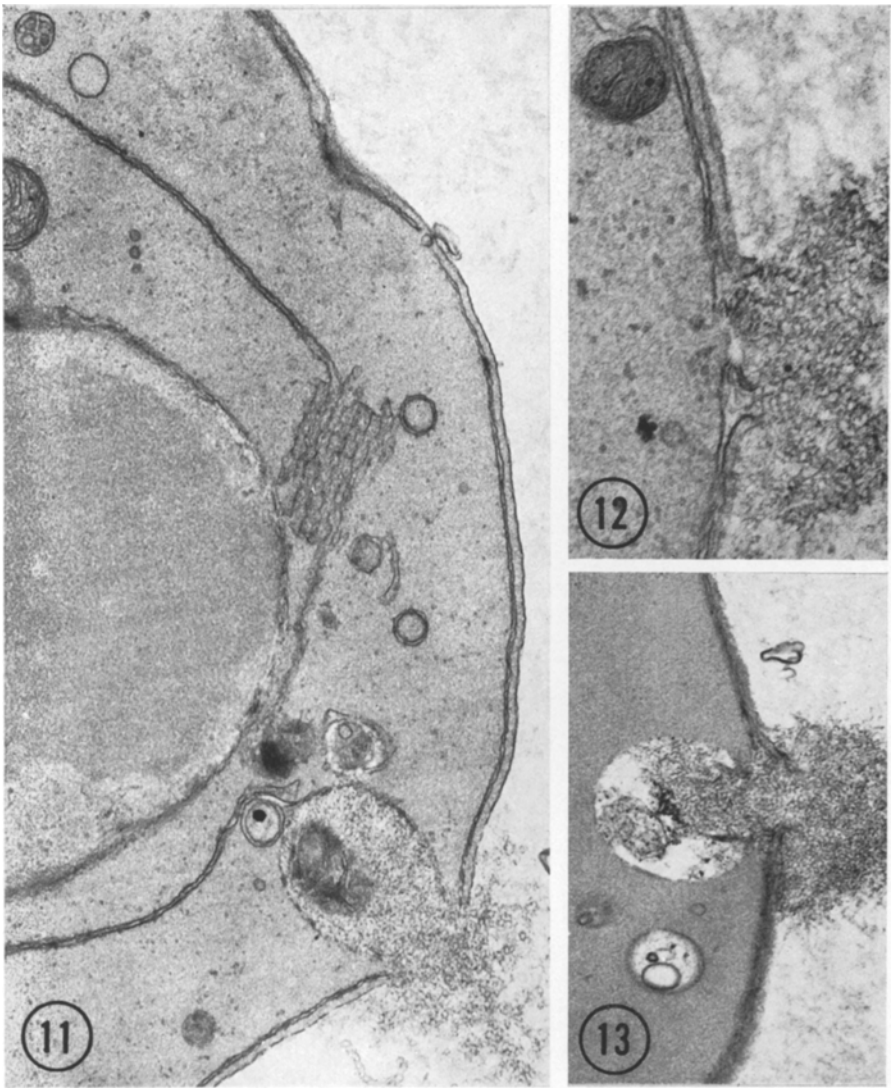


Fig. 11. Extrusion of ( ? nuclear) material from a cavity that also contains a mitochondrion. ($\times$ 16000)

Fig. 12. Rupture of plasma membrane ( ? and of peripheral cisterna) with extrusion of intracellular ( ? nuclear) material. ($\times$ 16000)

Fig. 13. Gap in plasma membrane through which material is expelled that may be nuclear in origin. ($\times$ 16000)

The first patients with CDA II, as diagnosed by a positive acidified serum test, in whom abnormal erythroid ultrastructure had been mentioned were those of Heimpel *et al.* (1970) and Hug *et al.* (1970). The abnormality of the latter patient was illustrated (Hug *et al.*, 1971, 1972; Wong *et al.*, 1972) and this pictorial

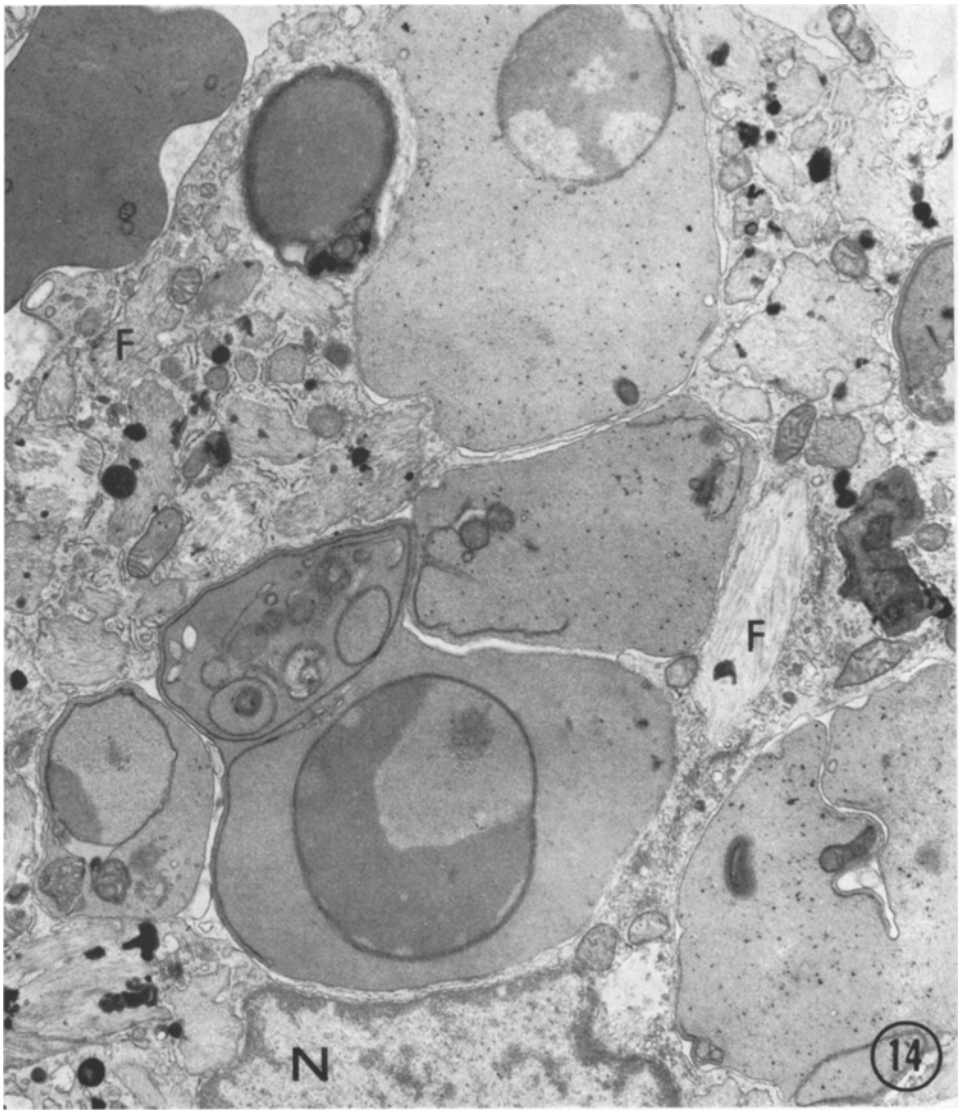


Fig. 14. Detail of bone marrow macrophage (*N* nucleus) that contains at least six late erythroblasts all of which exhibit abnormal cisternae. Frequently such macrophages also contain fibrillar material (*F*). (× 6000)

documentation exhibited morphologic aberrations since recorded in two additional individuals (Breton-Gorius *et al.*, 1973) and in the present patient. Electronmicrographs and their descriptions in three other articles on CDA II (Verwilghen *et al.*, 1971, 1973; Van Dorpe *et al.*, 1973) are not sufficiently detailed to allow comparison with the findings in the five patients mentioned above.

The salient ultrastructural features of erythroid cells as gleaned from illustrations and descriptions of the five recorded patients are multinuclearity, bizarre protrusions and karyorrhexis, gaps in the nuclear envelope that "unwinds" into cisternae of endoplasmic reticulum, gaps in the plasma membrane through which intracellular material such as mitochondria and nuclear debris is expelled, and excessive cisternae of smooth endoplasmic reticulum where there should be none.

Erythroblastic multinuclearity is normally seen in many species of animals (Bloom *et al.*, 1970), but it is rare in man (Berman, 1947). It has been observed in heritable conditions of turkey (Bloom *et al.*, 1970) and of man; in the latter, it was found in congenital dyserythropoietic anemia type I (Meuret *et al.*, 1970; Keyserlingk *et al.*, 1970; Heimpel *et al.*, 1971; Lewis *et al.*, 1972) and type II (CDA I and II). In addition, erythroblastic multinuclearity has been encountered in Vitamin E-deficient pigs (Nafstad and Nafstad, 1968).

To our knowledge, the total of the erythroid abnormalities summarized above has been illustrated only in electron micrographs of bone marrow specimens of the five listed patients with CDA II. One may speculate, therefore, that these abnormalities are a morphologic hallmark of CDA II. This interpretation would gain credence if patients with other types of dyserythropoiesis did not show similar ultrastructural abnormalities.

To data, the absence of excessive cisternae of endoplasmic reticulum has been documented in CDA I and in a newly described form of dyserythropoiesis (Weatherall *et al.*, 1973). However, the patient with CDA I described by Meuret *et al.* (1970) had a positive acidified serum test which according to Verwilghen *et al.* (1973) is the decisive serologic feature for a diagnosis of CDA II. The erythroid ultrastructure of the same patient, studied carefully by Keyserlingk *et al.* (1970) did not exhibit abnormal endoplasmic reticulum or any of the other abnormalities described above save those related to the nucleus. One may reasonably predict the converse situation, namely the existence of patients with a negative acidified serum test but similar ultrastructural findings as described in the present report and a bone marrow specimen of such a patient has indeed been examined recently by one of us (G.H.). At any rate serologic criteria for diagnosis may not always coincide with diagnostic ultrastructural characteristics. Hereof arise some nosological problems; more importantly, the occasional lack of correlation may suggest that the reported serologic and ultrastructural changes are perhaps not related causally.

The observation of cisternae in erythrocytes of the present patient indicated the maturation of at least some abnormal erythroblasts. Persistent endoplasmic reticulum is thus compatible with complete erythroid maturataon. This observation does not disprove the postulate of two erythroid populations, one of which, the "normal" one, would provide most of the mature erythrocytes (Wong *et al.*, 1972). However, the presence of excessive cisternae in all observed erythroblasts of our present patient mitigates against this postulate as do the observed serologic abnormalities of the peripheral erythrocytes.

Expulsion of intracellular organelles such as mitochondria through gaps of the plasma membrane has been described as a step during erythroid maturation (Simpson and Kling, 1968). In the present patient pictures suggestive of this

mechanism can be obtained easily. Their significance is unclear and deserves further study.

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