Scanning electron-microscopic and light-optics investigations of erythrocytes in toxic anaemia

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ABSTRACT Detailed assessment of the peculiarities of erythrocyte morphology by applying scanning electron microscopy and light-optics methods gives valuable information on the age characteristics, functional failures of the erythrocyte, pathogenesis of anaemia, and also diagnosis. In toxic anaemia caused by the chronic effect of lead and chlorobenzene there is accumulation of aging erythrocytes in blood that are spherical with rough and folded surfaces, fragmented with protuberances, processes, crypt-like hollows, and holes. In lead intoxication there is increased destruction of erythrocytes; anaemia caused by chlorobenzene is mainly due to a decreased entry of the young forms of the erythrocyte into the blood. Light-optics investigation combined with scanning electron microscopy allows a quantitative calculation of erythrocytes with abnormalities of form and surface. This may be used as an additional diagnostic test as part of the haematological examination of patients with anaemia.

The study of the morphological features of cellular elements has great importance for assessing their functional state, vitality, and kinetics. The possibilities, however, of red blood cell investigations both by light and transmission electron microscopy are limited because of the loss of the greater part of the specialised structures of these mature cells. The cytometric investigations that have found greatest application in haematological practice have turned out to be those giving the most information in the assessment of age and red blood cell vitality. In recent years the introduction of scanning electron microscopy into cytological investigation has given an opportunity of obtaining important additional information on the shape and surface of erythrocytes.

The normal erythrocyte surface is smooth. With different forms of anaemia small elevations, folds, micropores, perforations, and different kinds of protuberances appear on the surface.¹⁻⁶ A great variety of shapes of erythrocyte also occur both in the normal cell and especially in pathological cells.⁷⁻¹¹

We describe the peculiarities of shape, size, and surface of erythrocytes in toxic anaemia, studied by means of scanning electron microscopy, in combi-

nation with light-optics morphological and cytometric methods.

Materials and methods

Two groups of patients with anaemia were examined: 10 men with long exposure to lead and 10 women who had had contact with a complex of organic solvents. The men (aged 25-50) had worked as founders, braziers, moulders, and assemblers at a copper foundry for 10-20 years. Three had mild lead intoxication, while the rest had it more seriously. There were abnormalities of porphyrin metabolism, moderate changes in the blood, polyneuritis, and increased excretion of lead. In men with intoxication the medium degree there was anaemia, polyneuritis, and, in some cases, lead colic.

The women with anaemia (aged 34-50) were producing electroinsulating materials and were in contact with a wide range of toxic agents (chlorobenzene, ethylglycol, cyclohexane, solvents, and other substances) for 10-20 years. The main toxic factor among them was chlorobenzene; its concentration sometimes exceeded the permissible level by a factor of eight. The same investigations were carried out in control groups of 10 men and 10 women of similar ages. Peripheral blood in both control groups

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Results of light-optics investigations of erythrocytes in controls and in patients with toxic anaemia

	Men		Women	
	Controls	Lead intoxication	Controls	Chlorobenzene intoxication
Erythrocytes $\times 10^{12}/l$	4.531 $\pm$ 0.065	3.621 $\pm$ 0.075	4.251 $\pm$ 0.078	3.330 $\pm$ 0.070
Haemoglobin (g/dl)	15.3 $\pm$ 0.22	10.9 $\pm$ 0.30	12.8 $\pm$ 0.29	9.8 $\pm$ 0.31
Colour index	0.95 $\pm$ 0.007	0.86 $\pm$ 0.006	0.93 $\pm$ 0.006	0.90 $\pm$ 0.006
Reticulocytes (%)	7.2 $\pm$ 1.3	24.5 $\pm$ 5.0	7.6 $\pm$ 1.6	8.2 $\pm$ 1.7
Punctate basophilia (%)	6.8 $\pm$ 2.1	121.2 $\pm$ 23.2	7.9 $\pm$ 2.6	12.4 $\pm$ 1.8
Medium diameter (μm)	7.6 $\pm$ 0.07	7.9 $\pm$ 0.06	7.7 $\pm$ 0.06	7.3 $\pm$ 0.008
Volume (μm^3)	97.0 $\pm$ 1.3	100.0 $\pm$ 5.2	97.7 $\pm$ 1.2	104.9 $\pm$ 4.8
Thickness (μm)	2.1 $\pm$ 0.05	1.8 $\pm$ 0.09	2.1 $\pm$ 0.02	2.6 $\pm$ 0.21
Spheric index	3.6 $\pm$ 0.10	4.3 $\pm$ 0.25	3.8 $\pm$ 0.6	3.0 $\pm$ 0.36
Poikilocytes (%00)	21.8 $\pm$ 2.2	113.8 $\pm$ 14.4	24.8 $\pm$ 2.4	56.2 $\pm$ 5.3
Erythrocytes:				
with perforations (%)	2.8 $\pm$ 0.2	18.2 $\pm$ 2.1	3.1 $\pm$ 0.3	13.9 $\pm$ 1.2
with protrusions and processes (%)	5.2 $\pm$ 1.2	30.5 $\pm$ 4.6	5.0 $\pm$ 1.5	15.4 $\pm$ 2.7
with crypt-like hollows on the surface (%)	9.1 $\pm$ 0.8	72.8 $\pm$ 5.4	8.6 $\pm$ 0.7	26.4 $\pm$ 2.9
	7.9 $\pm$ 1.1	51.4 $\pm$ 8.0	8.4 $\pm$ 1.0	18.0 $\pm$ 1.6

Fig 1 Functional morphology of erythrocytes of peripheral blood in normal subject: (a) ccc = concavo-concave erythrocytes; fp = flat parallel-sided erythrocyte; cvc = concavo-convex erythrocyte ($\times 10\,000$); (b) concavo-convex spherocyte ($\times 10\,000$); (c) spherocyte ($\times 10\,000$); (d) spherocyte with fine granular surface ($\times 15\,000$); (e) spherocyte with rough-granular surface ($\times 15\,000$).

corresponded to the norms. In both occupational groups (table) there was roughly the same fall in erythrocyte number (average $1 \times 10^{12}/l$) as compared with the normal and haemoglobin content (men, up to 71.2%, women up to 76.5% of normal level).

Results and discussion

In the study of the erythrocytes of healthy people with the scanning electron microscope on the basis of a three-dimensional picture it is stated that the main part of the cells are concavo-concave; concavo-convex, sometimes with parallel flat sides; or spherical. Most of the surface of the erythrocyte is smooth. A few spherical red blood cells have decreased size and a rough granular surface (fig 1).

Besides these main types of cells there can normally be found single erythrocytes of atypical shape and irregular outline—for example, with a comb-like projection on the surface, cells with parallel flat sides with cupola-like protrusions, cells with sharply changed forms, perforations, single or multiple protuberances and appendages, and perhaps other forms. Spherical and atypical erythrocytes show the processes of physiological aging and destruction.

The blood of patients with anaemia had the same types of erythrocyte as in the normal but the correlation between them was greatly changed. A decrease in the number of normocytes and development of anisocytosis was noted. The number of erythrocytes with regular concavo-concave shape had greatly decreased. The number of concavo-convex and spherical cells and atypical misshapen cells and dwarf cells had greatly increased (fig 2). Sometimes the surface of the erythrocyte looked granular, rough, or roughly folded with microhollows or microprominences in the cell membrane.

In pathological conditions the process of fragmentation and sequestration of cell parts, which is

Fig 2 Erythrocyte morphology in anaemia: (a) erythrocyte with a peripheral protuberance ($\times 10\,000$); (b) erythrocyte with central and peripheral protuberance. Hollow in central protuberance is 200 nm ($\times 12\,000$); (c) erythrocyte with perforation ($\times 20\,000$); (d) erythrocyte with crypt-like holes on edge ($\times 12\,000$); (e) erythrocyte with protuberance and micropores (70 nm) ($\times k5mIII$); (f) erythrocyte of decreased size with two protuberances ($\times 12\,000$).

one of the main mechanisms of gradual erythrocyte destruction, has been observed. In the forms of anaemia studied here, most erythrocytes had one or more prominences about $1.5\,\mu\text{m}$ in height on the front or lateral surfaces. Study of several different tuberos erythrocytes allowed us to see the gradual evolution of the breaking-off of parts of the cytoplasm from the cell surface. The first projection was a bit elongated, then narrowing occurred, and finally the protuberance was seen to be broken off. Sequestration of the cell in the marginal zone led to the development of crypt-like hollows $0.3\text{--}0.7\,\mu\text{m}$. Sometimes erythrocytes with projections of cytoplasm from a free edge had sequestration of one or

more protuberances, leaving holes of $1.0\text{--}1.5\,\mu\text{m}$. Repeated fragmentation and sequestration of erythrocytes led to reduction in their size, and promoted the development of cells of irregular shapes or schizocytes ($2\text{--}3\,\mu\text{m}$). All these changes in erythrocyte structure are considered as morphological evidence of one possible mechanism of the development of anaemia.

Thus the application of scanning electron microscopy can show changes in the shape and structure of the erythrocyte surface in toxic anaemia caused by lead and chlorobenzene. There were no specific changes for the types of anaemia studied but we noticed a decrease in the proportion of normal erythrocytes and a great increase in the number of cells showing the stages of aging and destruction of red blood corpuscles (spherocytes, atypical misshapen cells, erythrocytes with fragmentation, and schizocytes).

An increase in the number of mature cells in the erythrocyte population is thought to depend both on an increase in the cell degeneration process and also on a decrease in their production.¹² Light-optics investigations of erythrocytes showed that the mechanism of erythrocyte population change was different in some forms of anaemia studied. Groups of patients were distinguished both by cytometric characteristics and also by morphological signs in erythrocytes (table). In lead intoxication there was reticulocytosis, many granular basophils, and macroplanocytosis. The erythrocytes of women with anaemia and working with chlorobenzene are both smaller than normal and more spherical. The reticulocyte count and the number of punctate basophils did not exceed the normal.

Macroplanocytosis, reticulocytosis, a great number of punctate basophils as described in lead anaemia, together with data on stimulation of erythropoiesis^{13 14} are evidence of the activation of erythrocyte output.

Young erythrocytes coming into the circulation, however, are not perhaps structurally and functionally fully developed, both because of a direct effect on the cell coat and consequent pathological disturbance of haemsynthesis by lead. There is evidence that the life duration of such cells is decreased and they are largely destroyed.^{15 16} This investigation on the diagnosis of reticulocyte maturation shows an essential decrease of the erythrocyte life duration in the group of patients with lead intoxication.¹⁷ The results of scanning electron microscopy give morphological confirmation of increased destruction of erythrocytes in lead intoxication and indicate the great importance of fragmentation and sequestration in this process.

Morphological features of activation of erythro-

Fig 3 Light-optics morphology of non-typical erythrocytes: (a) erythrocyte with hole; (b) erythrocyte with crypt-like hollow; (c) erythrocyte with appendages. (Stain, Giemsa-Romanovsky $\times 2000$.)

poiesis (reticulocytosis, macrocytosis) are absent in the anaemia caused by chlorobenzene. Biopsy of bone marrow showed a moderate reduction of erythroid growth in these cases and decrease of the proliferative activation of the red cells. These data together with microspherocytosis found at cytometry allow us to state that in this form of anaemia there is decreasing entrance into the circulation of young cellular forms and relative accumulation of old erythrocytes at the end of their life cycle.

Observation of the shape and surface of erythrocytes with the help of scanning electron microscopy became the basis for additional criteria for assessing erythrocyte morphology during light-optics investigation. Analysis of the corresponding peripheral blood smears showed that some shape and surface changes of erythrocytes may be identified by light-optics microscopy. Besides cells of irregular shape (poikilocytes and schizocytes), it became possible to detect erythrocytes with protuberances, processes, holes, and crypt-like excavations (fig 3). The possibility of making quantitative calculations of the findings easily is an advantage of light-optics investigation. But only by using scanning electron microscopy is it possible to identify peculiarities in the structure of the erythrocyte—the actual shape of the cell, the degree to which it is spherical, rough and folded surfaces, and the number of small hollows and prominences on it.

Counting the number of erythrocytes per 10 000 with defects of shape and surface showed different frequencies of erythrocyte changes in the forms of anaemia studied despite the same degree of the disease (table). The frequency of deviations due to lead intoxication was large. The data confirm differ-

ent mechanisms for the accumulation of deformed erythrocytes from lead and chlorobenzene intoxication.

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