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ACETYLCHOLINESTERASE DURING AGING OF HUMAN ERYTHROCYTES

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Acetylcholinesterase activity differs in the membranes of young, mature, and old human erythrocytes: It is highest in the mature and lowest in the old cells. The enzymes in young and mature erythrocytes is in the form of three, but in the old cells in the form of two molecular components. The results suggest that changes in the structural organization of acetylcholinesterase in the erythrocyte membrane have a direct bearing on the aging of red blood cells.
KEY WORDS: acetylcholinesterase; aging of erythrocytes.

Considerable progress has recently been made in the study of the structural chemical organization of the acetylcholinesterase (ACE) of erythrocytes, but the functional role of this enzyme, localized on the outer surface of the cell membrane, still remains uncertain [5, 6, 9, 12]. At the same time, it is known that during aging of the erythrocyte population the activity of this enzyme changes appreciably and becomes minimal in the osmotically most fragile old cells [8, 10].

The object of this investigation was to study whether a connection exists between the weakening of the osmotic resistance of the cell membrane (leading to aging and to subsequent death of the erythrocytes) and a disturbance of the molecular organization of ACE.

EXPERIMENTAL METHOD

To separate erythrocytes on the basis of their maturity, stepwise hemolysis [10] with NaCl solutions of different concentrations (0.40, 0.38, and 0.36%) was used. Membranes of hemolyzed erythrocytes were washed with solutions of low ionic strength to remove hemoglobin [7]. Acetylcholinesterase was solubilized by incubation of the cell "ghosts" in 0.5% (final concentration) solution of Triton X-100 for 3 h at 37°C, after which it was fractionated by electrophoresis in a disk of polyacrylamide gel (PAG) [2, 12]. The PAG was prepared with the polymer and copolymer in a ratio of 41 : 1 and polymerized at 60°C for 40 min. After completion of electrophoresis in gel, the activity of the enzyme was revealed [3]. The approximate molecular weight of the enzyme component was determined by electrophoresis of marker proteins of known molecular weight: yeast catalase

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TABLE 1. Membrane ACE Activity of Erythrocytes Separated by Stepwise Hemolysis into Age Groups (M ± m)

Erythrocytes	Hemolysis in NaCl solution	ACE activity, units/ml	Number of cells in population, %
	Hemolyzed in NaCl solution:		
Old	0,40%	2,71±0,24	5,2±0,8
Mature	0,38%	5,65±0,45	45,0±8,5
	0,36%	5,43±0,20	33,2±5,7
Immature	Not hemolyzed in 0.36% NaCl solution	2,96±0,60	16,3±2,1

Legend. Erythrocytes from 12 persons were studied.

TABLE 2. Approximate Molecular Weight of Components of ACE (mean results of four experiments)

Molecular forms of enzyme	Age groups of erythrocytes of healthy persons		
	young	mature	old
1	540 000—480 000	540 000—480 000	540 000—480 000
2	420 000—380 000	285 000—270 000	420 000—380 000
3	88 000—66 000	88 000—66 000	—

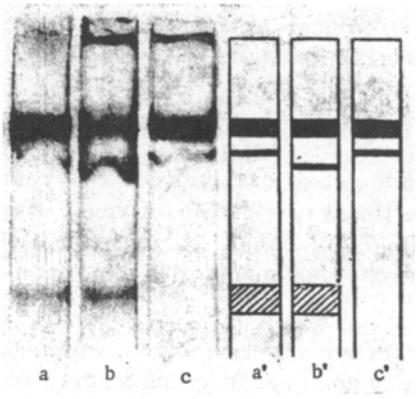

Fig. 1

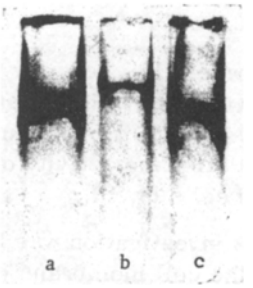

Fig. 2

Fig. 1. Electrophoresis (with scheme) of ACE from human erythrocytes in PAG: a, a') young, b, b') mature, c, c') old cells.

Fig. 2. Electrophoresis of ACE from erythrocytes hemolyzed in 0.40% NaCl solution in PAG: a, c) healthy person, b) patient with Marchiafava- Micheli disease.

and hexokinase, human transferrin and albumin. Activity of the enzyme in the membranes was determined by potentiometric titration [1].

EXPERIMENTAL RESULTS

The investigations confirmed the fact described in the literature that minimal ACE activity is present in old erythrocytes [8]. At the same time, they showed that mature erythrocytes possess maximal enzyme activity (hemolysis in 0.38-0.36% NaCl solution), which accounts for more than two-thirds of the total erythrocyte population of human peripheral blood (Table 1). In immature erythrocytes, maximally resistant to hypotonic shock, much lower ACE activity was discovered than in mature erythrocytes, a result which does not agree on the whole with data in the literature obtained by the use of a basically different method of fractionation of the cell population into age groups [4].

The study of ACE from the erythrocytes in PAG showed that this enzyme in the immature and mature human erythrocytes consists of three molecular forms with different molecular weights (Table 2). In old red blood cells, the lifespan of which in healthy persons is 100-120 days [10], the structure of the ACE was abnormal, for it consisted of only two fractions (Fig. 1).

To discover whether the increase in osmotic fragility of the erythrocytes (aging of the cell and its subsequent death) is connected with disappearance of one of the components of ACE from the cell membrane, erythrocytes from patients with Marchiafava–Micheli disease, in whom the lifespan of the osmotically most fragile erythrocytes does not exceed 8-12 days, were investigated [11].

The results showed that the osmotically most fragile erythrocytes of both healthy subjects and patients had an identical spectrum of molecular components of ACE (Fig. 2). This suggests that the aging of red blood cells, manifested primarily as weakening of the osmotic resistance of their membrane, has a direct bearing on the appearance of the structurally abnormal ACE.

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