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Lysis of red cells from haematological disorders

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Erythrocytes from patients with acute and chronic leukaemias or myeloproliferative disorders treated with N-acetyl-L-cysteine are more sensitive than normal erythrocytes to lysis in acidified serum and in the 'sucrose' lysis test. Furthermore, erythrocytes from acute leukaemias and myeloproliferative disorders exhibit a lower acetylcholinesterase activity than normal red cells. Erythrocytes from aplastic anaemia and thalassaemic syndromes behave normally. Several workers have shown that normal erythrocytes, following incubation with a variety of substances, behave like erythrocytes of paroxysmal nocturnal haemoglobinuria (PNH). N-acetyl-L-cysteine (N-Ac) is one of these substances (Dé Sandre, Vettore, Corrocher, Cortesi, and Perona, 1968).

Materials and Methods

Erythrocytes were obtained before any treatment from patients suffering from various haematological disorders; erythrocytes from 20 normal subjects were used as controls. One volume of venous blood was mixed with 3 volumes of Alsever solution. After centrifugation the erythrocytes were separated and treated with a solution of 0.613 M N-Ac (BDH) as described by De Sandre *et al* (1968).

The haemolysis test in acidified serum (pH 6.2) was performed as described by Dacie and Lewis (1968). A pool of fresh human sera from healthy group AB and Rh(D)-positive donors was used. The 'sucrose' lysis test was performed as described by Jenkins, Hartmann, and Kerns (1967). The AchE activity of the erythrocytes was measured as described by Michel (1949).

Results

Table I shows that whilst erythrocytes from patients with aplastic anaemia and thalassaemic syndromes treated with N-Ac behave like normal erythrocytes, erythrocytes from patients with acute leukaemia, chronic leukaemias (both myeloid and lymphatic),

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Erythrocytes	No. of Samples	Haemolysis (%)	
		In Acidified Serum	In 'Sucrose'
Normal	20	16.95 ± 4.83 ¹	12.85 ± 3.80
Thalassaemia	10	18.20 ± 3.71 (P > 0.5)	14.60 ± 2.90 (P > 0.5)
Acute leukaemia	13	23.53 ± 7.52 (P < 0.01)	20.23 ± 2.90 (P < 0.01)
Chronic myelocytic leukaemia	10	28.7 ± 6.22 (P < 0.01)	23.90 ± 5.95 (P < 0.01)
Chronic lymphocytic leukaemia	10	32.50 ± 4.9 (P < 0.01)	28.20 ± 8.24 (P < 0.01)
Myeloproliferative disorders	8	31.90 ± 4.51 (P < 0.01)	27.20 ± 5.44 (P < 0.01)
Aplastic anaemia	10	19.50 ± 6.35 (P > 0.2)	16.10 ± 5.93 (P > 0.05)

Table I Haemolysis of red cells treated with N-acetyl-L-cysteine in Ham and sucrose lysis test

¹Means ± SD.

Erythrocytes	No. of Samples	AChE Activity (ΔpH/hr)		AChE Activity (% of initial value)
		Before Treatment	After Treatment	
Normal	20	0.773 ± 0.116 ¹	0.5840 ± 0.078	75.68
Thalassaemia	10	0.782 ± 0.126 (P > 0.5)	0.5810 ± 0.097	74.29
Acute leukaemia	13	0.644 ± 0.141 (P < 0.01)	0.4753 ± 0.098	73.73
Chronic myeloid leukaemia	10	0.7225 ± 0.093 (P > 0.2)	0.545 ± 0.070	75.4
Chronic lymphatic leukaemia	10	0.760 ± 0.126 (P > 0.5)	0.574 ± 0.083	75.5
Myeloproliferative disorders	8	0.5512 ± 0.143 (P < 0.01)	0.416 ± 0.103	75.5
Aplastic anaemia	10	0.789 ± 0.138 (P > 0.5)	0.59 ± 0.133	74.7

Table II Acetylcholinesterase activity of red cells from various haematological disorders before and after treatment with N-acetyl-L-cysteine

¹Means ± SD.

and myeloproliferative disorders exhibited higher sensitivity to lysis in the acidified serum as well as the 'sucrose' lysis test. The difference was statistically significant (P < 0.01 for each of the groups in each test).

Table II shows that the AChE activity of the erythrocytes of aplastic anaemia, chronic leukaemias (both myeloid and lymphatic), and thalassaemic syndromes was similar to that of normal red cells. In contrast, the AChE activity of the erythrocytes of acute leukaemias and myeloproliferative disorders was significantly lower (P < 0.01 and P < 0.001 respectively). The percentage reduction in the AChE activity of the red cells following exposure to N-Ac was the same as that of normal erythrocytes.

Comment

The present experiments seem to indicate that, in contrast to erythrocytes from aplastic anaemia and thalassaemia, those from acute leukaemia, chronic leukaemia, and the myeloproliferative disorders treated with N-Ac behave like the red cells of paroxysmal nocturnal haemoglobinuria in acidified and sucrose lysis tests. It could perhaps be surmised that the erythrocytes of paroxysmal nocturnal haemoglobinuria and of the latter haematological disorders may share common non-specific stromal changes, and our findings may relate to the connexion between these disorders and paroxysmal nocturnal haemoglobinuria (Jenkins and Hartmann, 1969;

Holden and Lichtman, 1969; Kaufmann, Schechter and McFarland, 1969; Hansen and Killmann, 1970; Dameshek, 1969). But this is not supported by our experiments with red cells from aplastic anaemia, which behave like normal erythrocytes although aplastic anaemia and paroxysmal nocturnal haemoglobinuria are also connected (Lewis and Dacie, 1967).

Reduced erythrocyte AChE activity, known to be low in paroxysmal nocturnal haemoglobinuria (Auditore and Hartmann, 1959; Metz, Bradlow, Lewis, and Dacie, 1960) has been found in acute leukaemia and in myeloproliferative disorders. Evans (1969), also, has reported low figures in myeloproliferative disorders. These findings, together with the fact that in some instances the sucrose lysis test has been slightly positive, lend support to Dameshek's (1969) view that these disorders and paroxysmal nocturnal haemoglobinuria are in some way connected.

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Estimation of serum and urinary muramidase with the eel aggregometer

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In view of recent interest in the assay of muramidase in biological fluids, especially in the diagnosis of the acute monocytic leukaemias, it became necessary to organize such a method in our department. The methods available depend on measuring the lysis of a suspension of the bacterium *Micrococcus lysodeikticus* either in an agar plate (Osserman and Lawlor, 1966) or by nephelometry in a spectrophotometer cuvette (Parry, Chandan, and Shahani, 1965; Gorin, Papapavlou, and Wang, 1971). An EEL 169 aggregation meter with an attached recorder appeared ideally suited for the latter technique. Accordingly the latter method was modified as follows to suit our requirements.

The substrate is a 50 mg% suspension of ultra-violet-killed and lyophilized *M. Lysodeikticus* in M/15 phosphate buffer as supplied by Difco. To 0.75 ml of this suspension in a plastic disposable aggregometer cuvette is added 0.25 ml of a 0.3M saline solution. This mixture is then brought to 37°C by incubation for 90 sec on the heated block of the aggregometer. At the end of this period 0.5 ml of serum or standard enzyme solution is added and the timer and stirrer are started, the output of the machine being passed to a Servoscribe recorder set at a sensitivity of 5 mV and with a chart speed of 600 cm/hr. Recording continues for five minutes.

A standard curve is constructed for each batch of tests using crystalline egg-white muramidase as obtained from BDH Pharmaceuticals with an activity of 25000 IU/mg. A stock solution is made up containing 5 mg in 100 ml phosphate buffer which is then further diluted also with buffer to give 11 final standards of 0.5 µg/ml 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0. Unknown samples are then run consecutively using 0.5 ml neat serum or urine instead of the dilutions of standard enzyme.

Substrate suspensions and enzyme standards are made up the night before a run of tests which are at present carried out on one day per week. The standards lose about 40% of their activity after one week at 4°C. After one week at 4°C the substrate suspensions have begun to putrefy and are no longer suitable for use.

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