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Studies on erythrocyte membranes of patients with Huntington's disease

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SUMMARY Several red cell membrane properties and activities of membrane-bound enzymes were investigated in blood samples of patients with Huntington's disease. $(Na^{+}+K^{+})$ATPase activity and cell deformability appeared to be normal, in contradiction to preceding reports from other laboratories. With other techniques sensitive to relatively small changes in membrane structure, no abnormalities were found in Huntington's disease red cell membranes. These investigations do not support the concept that a generalised membrane abnormality is present in Huntington's disease.

In recent studies it has been postulated that Huntington's disease (HD) is associated with a diffuse, generalised membrane defect. This concept is based mainly on the apparently abnormal surface properties of erythrocytes and skin fibroblasts in Huntington's disease, including altered electron spin resonance parameters,[1 2] increased $(Na^{+}+K^{+})$ ATPase activity,[3] decreased cell deformability[2] and an increased number of stomatocytes[4] in erythrocyte studies, and anomalous cellular proliferation and fluorescence spectroscopy in studies with fibroblasts.[5 6] The altered election spin resonance parameters and the increased $(Na^{+}+K^{+})$ATPase activity of the red blood cell membrane have been interpreted as an alteration in conformation or organisation of membrane proteins (or both) in Huntington's disease. Demonstration of such generalised membrane defects would be important both for further studies on the biochemical basis of the disease and for the development of diagnostic tests. Therefore some aspects of red blood cell membrane structure and function were investigated in detail. These studies included the measurement of membrane-bound $(Na^{+}+K^{+})$ATPase activity and red cell deformability, both claimed to be abnormal in Huntington's disease. Other experiments concerned membrane properties which might be sensitive to relatively small changes in membrane conformation and organisation. It will be shown that these investigations do not support the concept of a generalised membrane defect in Huntington's disease.

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Materials and methods

Heparinised blood of patients with Huntington's disease and of normal, healthy donors was stored at 4°C and centrifuged 0·5–2 hours after collection. The red blood cells were washed three times in buffered, isotonic NaCl solution (154 mM NaCl, 9·6 mM Na_2HPO_4 and 1·5 mM NaH_2PO_4) and resuspended in the same medium. This red blood cell suspension was used the same day. The diagnosis of Huntington's disease in the group of patients was beyond doubt, based on the clinical history and a detailed pedigree analysis. Five patients received drug therapy. In the experimental results no differences were observed between treated and untreated patients. The control group had the same sex and age distribution.

Red cell deformability measurements were carried out in a Brookfield cone-plate viscosimeter, as described by Weed *et al.*[7] Hypertonic cryohaemolysis was determined as described by Dubbelman *et al*[8] in 0·9 and 1·0 M NaCl. Dielectric breakdown of red blood cells was measured as described by Riemann *et al.*[9] After subjecting the cells to electric shock they were incubated for 30 min at 0 or 37°C, before measuring haemoglobin release.

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Haemoglobin-free red cell membranes were prepared according to the method of Fairbanks *et al.*[10] Spectrin was extracted as described by Bennett and Branton[11] and purified by precipitation at pH 5·1. Sodium dodecyl-sulfate polyacrylamide gel electrophoresis of membrane proteins was performed according to Fairbanks *et al*,[10] with subsequent staining of protein bands with Coomassie Brilliant Blue and of glycoproteins with the periodic acid-Schiff procedure. Isoelectric focusing of spectrin was done on polyacrylamide gels according to Boivin and Galand.[12] (Na^++K^+)ATPase and Mg^{2+}—ATPase were measured as described by Hodson and Pleasure.[13]

Phosphorylation of membrane proteins was studied essentially as described by Hosey and Tao.[14] The assay mixture contained 10 mM $MgCl_2$, 0·2 mM ATP, 20 mM TRIS/HCl pH 8·0 and TRIS/HCl-washed, freeze-thawed ghosts (0·3–0·4 mg protein). The final volume was 0·2 ml. When present, the cAMP concentration was 2 μM. After 30 min the incubation was stopped by adding electrophoresis buffer with 1% SDS and heating the mixture at 70°C for 10 min. Electrophoresis was done with gels, cross-linked by N,N-diallyltartardiamide as described by Anker[15] After fixation and staining, the band 1+2, 3 and 4·5 were cut out and dissolved in 2% periodic acid. Subsequent radioactivity analysis was done by liquid scintillation counting.

Fluorescence of membrane-bound 8-anilino-l-naphthalene sulfonic acid (ANS) and perylene was measured by the following procedure. A mixture of 100 μl packed ghosts, 2 ml 0·25% NaCl and either 20 μl ANS solution (1 mg/ml alcohol) or 20 μl perylene (0·52 mg/ml acetone) was incubated at a constant temperature between 20–60°C. After incubation for 10 min at this temperature the mixture was incubated for another 10 min in melting ice. Subsequently fluorescence was measured at 0°C, utilising an Aminco-Bowman spectrophotofluorometer with constant-temperature cell compartment.

Results

The deformability of red blood cells of HD patients is shown in fig 1. As the viscosity of erythrocyte suspensions with a haematocrit in excess of 60 is completely determined by the visco-elastic properties of the cell membrane,[16] the plots reflect directly the deformability of the cells at different shear rates. These results, as well as the results obtained with blood samples of three other patients with Huntington's disease, were in the normal range (shaded area in fig 1).

Hypertonic cryohaemolysis, measured after preincubation at 25, 30, 40 and 50°C in 0·9 and 1·0% NaCl, is shown in table 1. No significant differences between Huntington's disease and normal blood samples were observed.

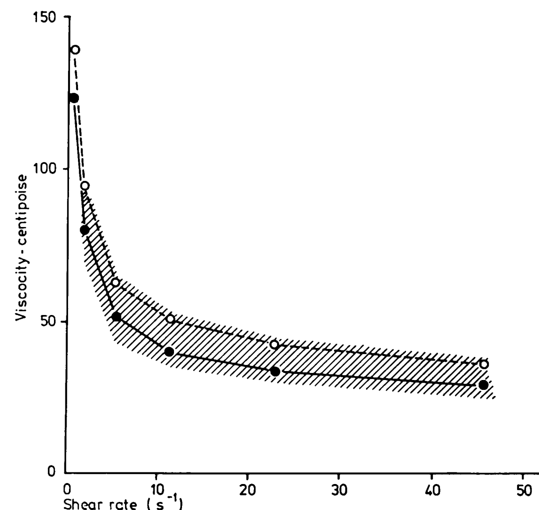

Fig 1 *Viscosity of two samples of HD red blood cells at different shear rates. Haematocrit: 80%. The shaded area represents the normal range, as determined on 36 blood samples of healthy controls.*

Dielectric haemolysis of HD red blood cells is shown in fig 2. With 10 different Huntington's disease blood samples the results were within the normal range. Fluorescence of membrane-bound ANS and perylene, measured at 0°C, is strongly dependent on the preincubation temperature.[8] This is shown in fig 3. Fluorescence measured on six normal and six Huntington's disease blood samples revealed no differences between the two groups, either in the excitation and emission

Table 1 *Percent haemolysis of 0·2% erythrocyte suspensions preincubated at 25, 30, 40 and 50°C in 0·9 and 1·0 M NaCl during 10 min and subsequently for 10 min in melting ice*

Preincubation temperature °C	*0·9 M NaCl*		*1·0 M NaCl*	
	Controls	*Patients*	*Controls*	*Patients*
25°	16·6±5·1 (n=10)	20·2±6·6 (n=6)	36·5±8·5 (n=13)	40·8± 8·3 (n=9)
30°	24·3±7·6 (n=10)	25·7±9·2 (n=6)	47·7±8·1 (n=13)	50·3± 7·2 (n=9)
40°	22·7±8·0 (n=10)	22·4±6·6 (n=6)	34·3±8·6 (n=13)	38·9±11·7 (n=9)
50°	14·3±4·2 (n=10)	17·9±8·4 (n=6)	14·8±3·8 (n=13)	20·5± 7·3 (n=9)

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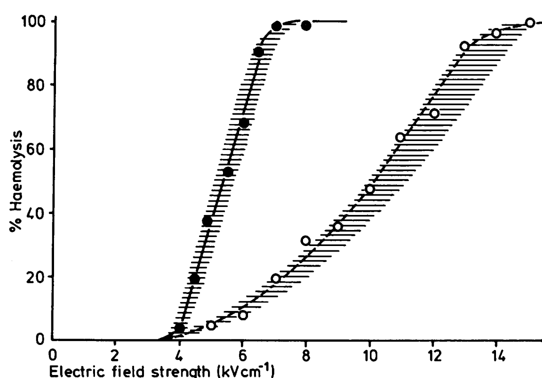

Fig 2 *Hemolysis of HD red blood cells as a function of the electric field strength at a pulse length of 5 μs ●—●: 0°C; ○—○: 37°C. The shaded area represents the normal range.*

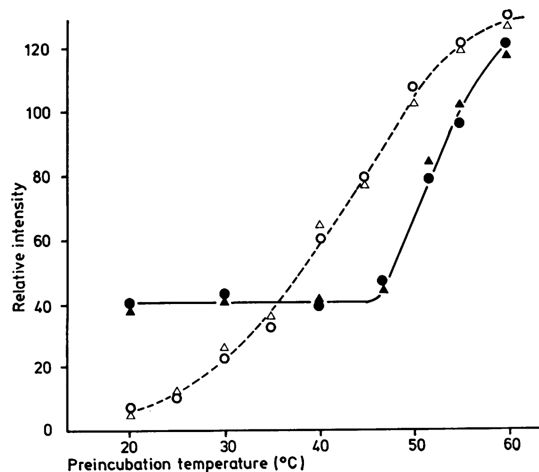

Fig 3 *Relative fluorescence intensity of membrane-bound ANS (closed symbols) and perylene (open symbols) at 0°C, as a function of preincubation temperature. and ○: HD; ▲ and △: control.*

spectra, or in the influence of preincubation temperature on relative fluorescence intensity.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis of membrane proteins and isoelectric focusing of purified spectrin from Huntington's disease red blood cells yielded normal patterns. This was also the case in studies on membrane protein phosphorylation. Finally, the results of $(Na^{+}+K^{+})$- and Mg^{2+}-ATPase assays are summarised in table 2. Again, no significant differences were found between controls and blood samples obtained from patients with Huntington's disease.

Table 2 *$(Na^{+}+K^{+})$- and Mg^{2+}-ATPase activity of control and HD ghost samples, in μmolPi/mg protein h*

	HD	*Control*
Mg^{2+}-ATPase	0·41 ± 0·07 (n = 12)	0·41 ± 0·09 (n = 12)
$(Na^{+}+K^{+})$-ATPase	0·25 ± 0·07 (n = 12)	0·22 ± 0·04 (n = 12)

Discussion

In the present investigations several aspects of red blood cell membrane structure and function have been studied in patients with Huntington's disease. Much attention was paid to appropriate standardisation of all procedures in handling the blood samples and in the various measurements. Cell deformability can be measured with several techniques, including viscosity measurements of concentrated cell suspensions and red cell filtration. Although in previous studies no discrepancies were detected when using viscosity measurements and cell filtration in parallel experiments (see for example reference 17) we prefer the viscosity technique. Using this method, cell deformability is measured at different, well-defined shear rates and thus this technique potentially yields more detailed information.

For cell deformability and $(Na^{+}+K^{+})$ATPase activity, normal values were found. These results contradict reports in recent literature which claim decreased deformability[2] and increased $(Na^{+}+K^{+})$ ATPase activity[3] in HD red blood cells. The reasons for these descrepancies remain as yet obscure.

Fluorescent spectroscopy is a very sensitive technique to detect changes in membrane structure.[18-20] Moreover, in a recent report differences in fluorescence of ANS-labelled normal and Huntington's disease fibroblasts have been described.[6] For these reasons a study of red cell membrane-bound ANS and perylene fluorescence was performed. ANS and perylene fluorescence, measured as described in the methods section, report mainly on the protein part of human red cell membranes, as will be discussed in detail elsewhere. As shown in the results section, no differences were found between normal and HD red cell membranes with this technique.

In further attempts to confirm the suggested alterations in red blood cell membrane organisation in Huntington's disease, some other techniques were used. Rather small alterations might be reflected by changes of the susceptibility of the cells to hypertonic cryohaemolysis.[8 21] Further, possible changes in membrane structure can be

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reflected very sensitively in changes of the critical breakdown voltage, as measured in dielectric haemolysis experiments.[9 22] As shown in the results section, however, normal values were found for all parameters in red blood cells of patients with Huntington's disease. These investigations do not support the notion of a diffuse, generalised membrane defect in Huntington's disease. A similar conclusion was reached by Cassiman *et al.*[22] In a detailed study these authors could detect no differences in the surface properties of normal and HD fibroblasts. In view of further studies on the biochemical aspects of Huntington's disease a critical re-evaluation of arguments in favour of and against the membrane hypothesis seems appropriate.

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