SCANNING ELECTRON MICROSCOPY STUDIES OF ERYTHROCYTES IN HUNTINGTON'S DISEASE

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SUMMARY: Scanning electron microscopic study of unmanipulated erythrocytes from Huntington's Disease (HD) patients disclosed an increased number of stomatocytes compared to controls. This morphological alteration may reflect a membrane abnormality and supports our previous electron spin resonance results (Butterfield et al, Nature (1977) 267, 453-455) which suggests that HD may have diffuse membrane involvement.

Huntington's Disease (HD), a degenerative disorder of the central nervous system inherited as an autosomal dominant trait, is characterized clinically by progressive involuntary movements and dementia. Pathologically neuronal degeneration is observed in the basal ganglia and cerebral cortex. The etiology and pathogenesis of this disorder are unknown. Although HD has been considered a disorder of the basal ganglia, recent electron spin resonance (ESR) studies in our laboratory (1) have suggested more widespread membrane involvement. Alterations in the physical state of membrane proteins in HD erythrocytes were observed by a protein-specific spin probe (1).

This report presents the results of scanning electron microscopy (SEM) studies of erythrocyte morphology in HD.

METHOD

Erythrocytes from seven patients with HD from six different families were studied. Patients in early, mid and late stages of the disease on regular at-home diets or normal hospital diets were used. Three were on no medication, one was on a phenothiazine, one was on a butyrophenone, another was taking a phenothiazine and a butyrophenone, and another was on diazepam. Controls consisted of six unrelated, healthy, sex and approximately-aged matched drug-free individuals with no family history of neurological disorders. Separate experiments employing two subjects without neurological disease on phenothiazines and appropriate controls were also performed.

HD and control blood was drawn by venipuncture through a short catheter infusion set and were processed after the method of Miller et al (2). Three

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drops of blood were dripped directly from the infusion set into 1% glutaraldehyde in phosphate buffer at pH 7.4 or 5.0 in a polyethylene tube. The unmanipulated erythrocytes were gently inverted and allowed to settle for 2 hours. One drop of cells was then pipetted to collagen-coated coverslips in a moist petri dish. Cells were then dehydrated through ascending ethanol solutions, processed through critical point drying in liquid carbon dioxide and coated with gold-palladium. Samples were studied in a Cambridge Steroscan MK II-A scanning electron microscope at 20 KV. Between 200 and 1200 cells were counted for each subject. Erythrocyte shapes were compared to previous morphological descriptions (3,4).

RESULTS

The great majority of control erythrocytes were biconcave in shape (Fig. 1A). Comparison of HD erythrocytes to those of controls (N) revealed an increase in stomatocytes (cup shaped cells) (Fig. 1B) at pH 7.4 (mean % ± standard error : N = 7.7 ± 1.6; HD = 22.9 ± 3.8, $P < 0.02$) (Table 1). At a fixative pH of 5.0 an increased percentage of stomatocytes was observed in both control and HD erythrocytes (Figs. 1C & 1D). However, the difference between the mean values of HD and control increased slightly over that at pH 7.4 (mean % ± standard error : N = 11.5 ± 2.0; HD = 29.6 ± 4.9, $P < 0.02$) (Table 1), suggesting a pH effect may be important in this phenomenon. The number of stomatocytes varied considerably in HD patients, but was always greater than the control for each experiment. In only one HD patient did the number of stomatocytes at pH 7.4 exceed those at pH 5.0 and a similar trend was present in the control for that experiment.

Counts of stomatocytes from two non-HD patients on phenothiazines at pH 7.4 and 5.0 (mean % 14 and 17.5, respectively) were greater than those of corresponding controls (mean % 4 and 7, respectively), but fewer than those of HD subjects (Table 1).

DISCUSSION

The present SEM study of unmanipulated erythrocytes from patients with HD showed a marked increase in stomatocytes compared to controls. This alteration in morphology of intact erythrocytes may be the result of an abnormal response to fixation of a defective membrane in HD.

Stomatocytes make up a small percentage of erythrocytes in normal sub-

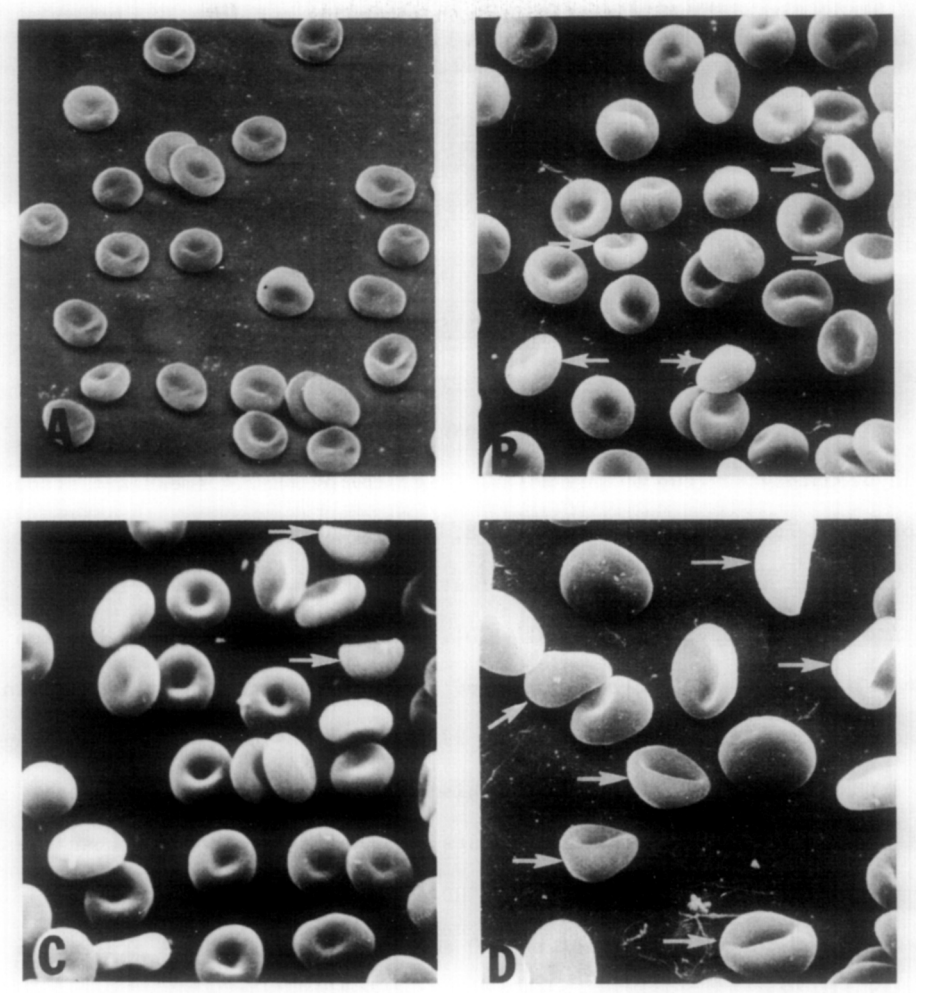

FIGURE 1. Scanning electron micrographs of erythrocytes in HD and controls. 1A. Control at fixative pH 7.4, X 1,850. IB. HD at fixative pH 7.4 showing stomatocytes (arrows), X 1,750. IC. Control at fixative pH 5.0 showing rare stomatocytes (arrows), X 2.275. ID. HD at fixative pH 5.0 showing numerous stomatocytes (arrows), X 3,000.

jects. Their numbers can be increased by decreasing the pH of the fixative in which the blood is processed (4). The cause of the slight increase in the difference of the mean value of HD and control stomatocytes at lower fixative pH is not known but is currently under investigation.

Deuticke (5) has shown that a number of pharmacologic agents, including phenothiazines, can produce stomatocytic changes in erythrocytes. Our study

TABLE I. Percent Stomatocytes in Huntington's Disease and Control Erythrocytes

	pH 7.4		pH 5.0	
	Range[a]	Mean % ± SE	Range[a]	Mean % ± SE
NORMAL (n=6)	2 - 12	7.7 ± 1.6	8 - 21	11.5 ± 2.0
HUNTINGTON'S DISEASE (n=7)	14-38	22.9 ± 3.8 ($P < 0.02$)	11-48	29.6 ± 4.9 ($P < 0.02$)

a = % of total number of cells

of non-HD patients on phenothiazines revealed fewer stomatocytes than in HD patients, but greater numbers than in normal controls. This finding coupled with the increase in stomatocytes in HD patients on no medication suggests that our findings are not due to medication alone.

Considerable variation in erythrocyte morphology as observed by SEM has been reported in other disease processes (3). With reference to other neurologic disorders, a large percentage of echinocytes were originally reported in patients with Duchenne muscular dystrophy (DMD) and other forms of muscular dystrophy (6). Subsequently Matheson et al (7) were not able to identify any erythrocyte abnormalities in DMD. Miller et al (2) reported large numbers of stomatocytes in DMD and myotonic dystrophy, disorders in which our ESR studies have found alterations in the physical state of erythrocyte membranes (8-11).

SEM investigation of HD erythrocytes may be a valuable adjunct to other biophysical and biochemical means of studying HD. However, it should

be emphasized that SEM evaluation of erythrocyte morphology is presently not a diagnostic test for HD. Our current results are consistent with our previous ESR studies (1) suggesting an alteration in the physical state of erythrocyte membrane proteins in HD. These studies together lend support to the hypothesis that HD, a disorder whose major clinical and pathological abnormalities are in the basal ganglia and cerebral cortex, may be associated with a more widespread abnormality of membranes.

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