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THE EFFECTS OF HYDRAZINE ON SICKLE CELLS

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The response of the red cells from patients with sickle cell disease to hydrazine treatment in vitro is to inhibit the sickled morphology, while the metabolic characteristics and the osmotic fragility of the cells remain unaltered. However, the oxygen affinity of the sickle cell haemoglobin is decreased.

Sickle cell anaemia is characterized by the polymerization of sickle cell haemoglobin on deoxygenation leading to the cellular sickling and membrane damage which culminate in membrane stiffening and the formation of irreversibly sickled cells. Many red cell membrane abnormalities have been reported, including decreased deformability [1,2], elevated calcium content [3], potassium loss and dehydration [4], the decreased bulk lipid fluidity [5], the increased binding of haemoglobin to the membrane [6], and the rearrangment of the membrane lipids [7]. To date, controlled trials of treatment for sickle cell anaemia have shown proposed therapies to be ineffective [8]. Monofunctional [9] and bifunctional [10] imidoesters have been shown to have antisickling properties in vitro by increasing the oxygen affinity of the haemoglobin. Generally, these reagents have only been effective at pH values above physiological levels. We report here on the effects of hydrazine on homozygous sickle cells in vitro at pH 7.4 and have observed evidence consistent with the view that 0.5 mM hydrazine inhibits the sickled morphology of the cells, whilst not affecting their metabolic properties but the oxygen affinity of the erythrocytes is decreased. Although hydrazines have been identified in a small number of biological substances those used in cancer chemotherapy are synthetic derivatives [11].

Fresh heparinized blood samples were obtained from patients with homozygous sickle cell anaemia. Erythrocytes were packed and the buffy coat removed. After washing twice in isotonic Tris/sodium chloride buffer at pH 7.4, the red cells were incubated at 5% haematocrit in hydrazine in isotonic Tris buffer for 30 min at 37°C. Control sickle cells were similarly incubated in the buffer in the absence of the drug. After incubation the treated cells were washed twice to remove excess hydrazine. Samples were taken for adenosine triphosphate and glucose-6-phosphate dehydrogenase assays using the ultraviolet method Test Combination kits from Boehringer. Further samples of the washed packed cells were suspended at a 5% concentration in glutaraldehyde in cacodylate buffer, dehydrated and critical-point dried. Samples for scanning electron microscopy were prepared using carbon and gold in an Ion Tech Saddle field using an ion source sputter coater. Micrographs were taken on a Philips 501 scanning electron microscope. Oxygen dissociation curves were measured on the Aminco Hem-O-Scan at 37°C. Washed erythrocytes were deoxygenated by bubbling nitrogen through for 10 or 50 min.

Osmotic fragility measurements were performed on treated and untreated sickle cells using Drab-

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kin's reagent in varying concentrations of NaCl. There was no change in the susceptibility of the sickle cells to haemolysis on hydrazine treatment nor were the metabolic characteristics altered. ATP levels were unaffected by the treatment up to 10 mM hydrazine, remaining at 15.2 ± 4 mg/100 ml (16 samples), which is lower than the range for normal red cells, 23.7 ± 4 mg/100 ml (nine samples). Concentrations of glucose-6-phosphate dehydrogenase in the sickle cells treated with varying hydrazine concentrations up to 10 mM were not significantly altered from those of the control sickle cells at 161 ± 30 mU/10^9 erythrocytes (12 samples) although they were elevated above the values for normal erythrocytes as expected. Red cell membrane lipid peroxidation was assayed as in Ref. 12 using the thiobarbituric acid method and expressed in terms of the absorbance at 532 nm/mg protein per ml. The untreated sickle cells with $A_{532} = 0.178 \pm 0.028$/mg protein per ml (seven patients) have a greater tendency to peroxidation compared with normal control erythrocytes with $A_{532} = 0.072 \pm 0.013$/mg protein per ml (16 samples). No significant alteration in the degree of lipid peroxidation was found at concentrations of hydrazine treatment up to 3 mM.

Fig. 1a shows a scanning electron micrograph of the oxygenated erythrocytes from a typical patient homozygous for sickle cell disease and demonstrates the presence of irreversibly sickled, partially sickled and reversibly sickled cells. After treatment of these erythrocytes with 0.5 mM hydrazine (Fig. 1b), the irreversibly sickled and partially sickled holly-leaf forms are transformed into

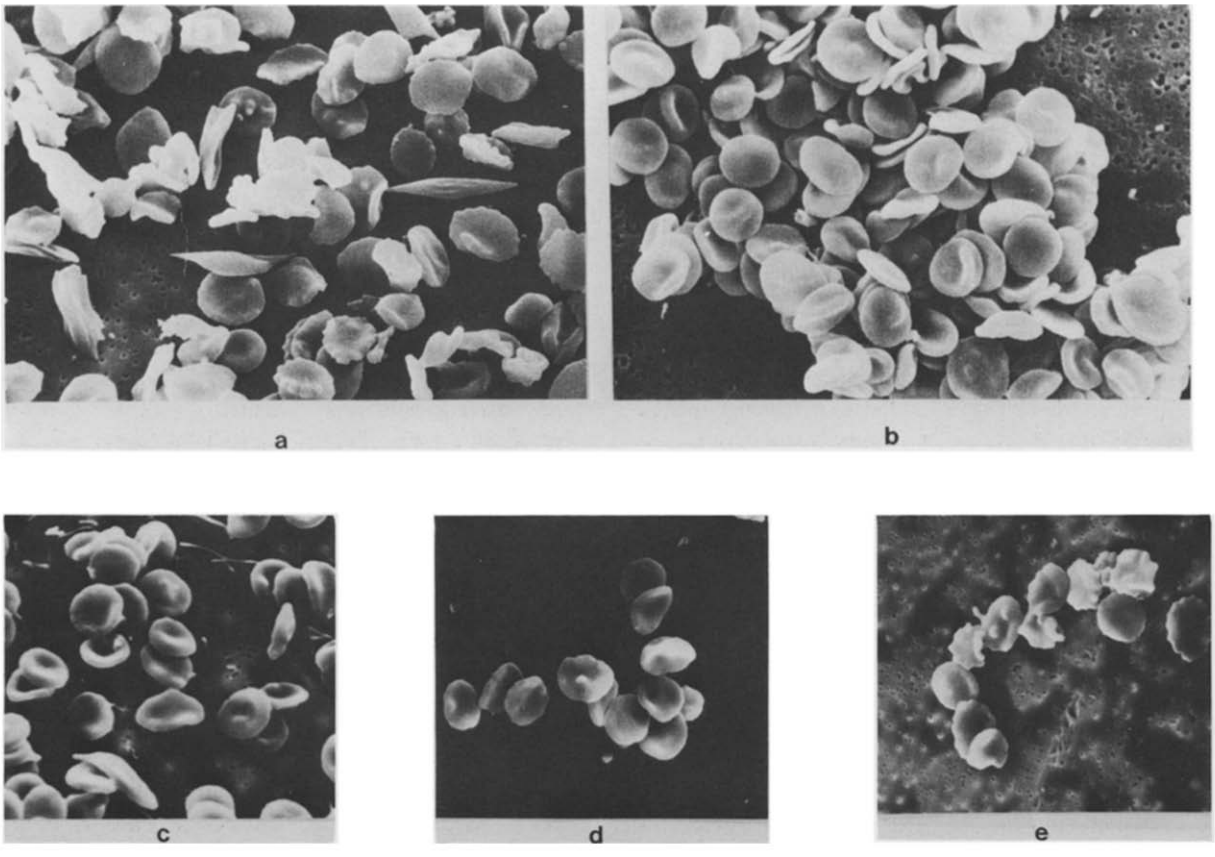

Fig. 1. Scanning electron micrographs of the red cells of typical patients with sickle cell disease: (a) oxygenated sickle erythrocytes (patient S/6, untransfused); (b) 0.5 mM hydrazine-treated oxygenated sickle erythrocytes (patient S6); (c) oxygenated sickle erythrocytes (patient S/16 two months since transfusion); (d) 4 mM hydrazine-treated oxygenated sickle erythrocytes (patient S/16); (e) 10 mM hydrazine-treated oxygenated sickle erythrocytes (patient S/16).

rounded cells which appear morphologically more normal than the drepano-discocytic forms apparent in Fig. 1a. This morphological study was repeated on samples drawn from ten different patients and produced the same effect. A proportion of the treated cells have noticeably abnormal shapes. This is possibly indicative of the attempted transition to the normal configuration of partially and irreversibly sickled cells which had lost membrane during their formation, consequent to microspherulation.

The effects of elevated hydrazine concentrations on sickle cells are demonstrated by a typical experiment in Fig. 1c–e. The red cells become

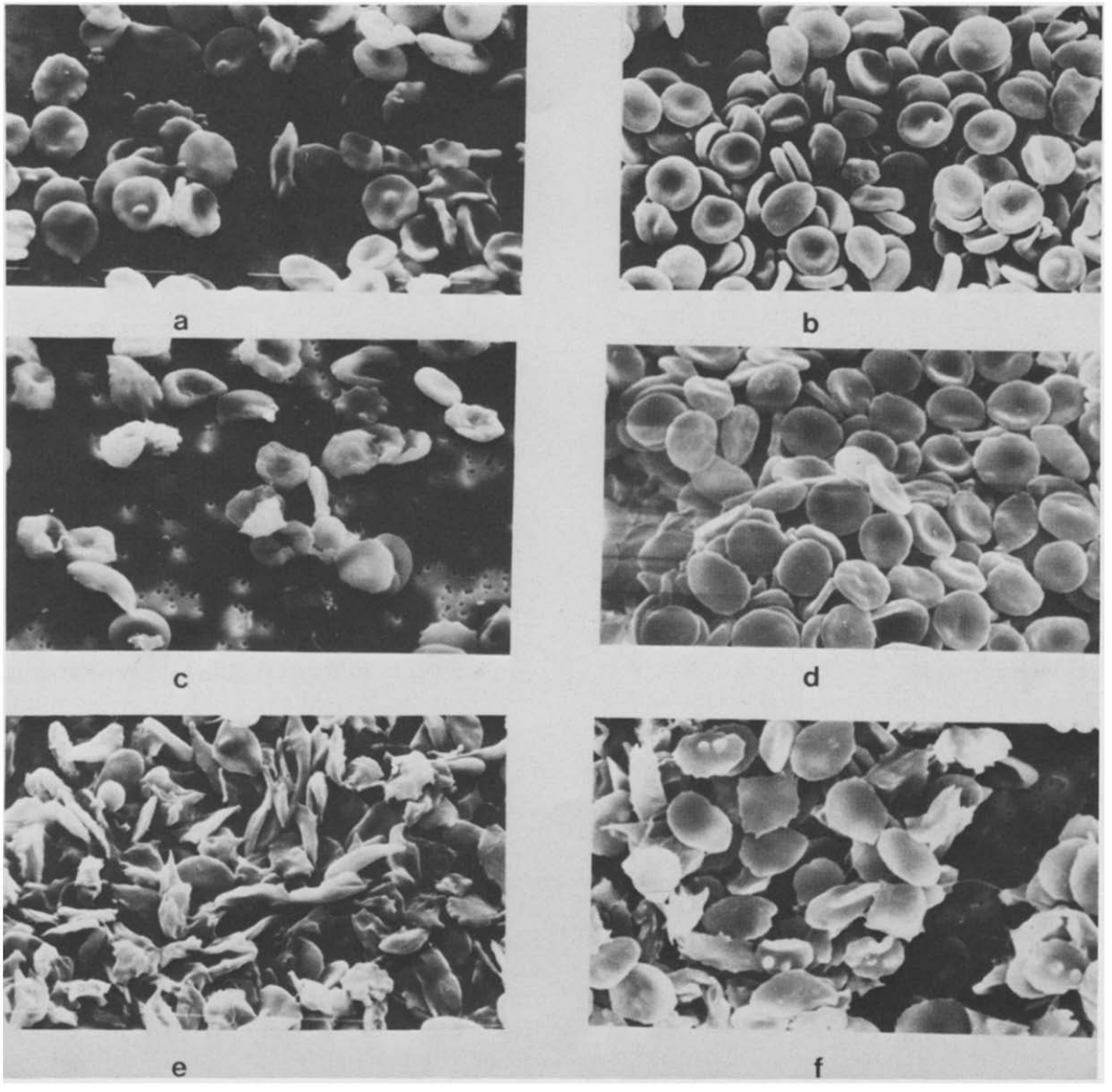

Fig. 2. Scanning electron micrographs showing the effects of deoxygenation on sickle cells treated with 0.5 mM hydrazine: (a) oxygenated sickle erythrocytes (patient S/12, 2 months since transfusion); (b) as above, but treated with 0.5 mM hydrazine; (c) untreated sickle erythrocytes, deoxygenated for 10 minutes (patient S/12); (d) sickle erythrocytes treated with 0.5 mM hydrazine then deoxygenated for 10 min (patient S12); (e) untreated sickle erythrocytes, deoxygenated for 50 min; (f) sickle erythrocytes treated with 0.5 mM hydrazine then deoxygenated for 50 min.

flatter and appear to develop small protrusions on some of their surfaces after incubation with 4 mM hydrazine (Fig. 1d) compared with the untreated cells (Fig. 1c). In Fig. 1e, the 10 mM hydrazine treatment produces a heterogeneous collection of cell shapes with pronounced evaginations developing in some cases. Morphologically, these concentrations of the drug are too high for normalization of the cell shape and the lower concentration of 0.5 mM seems to be the most effective (Fig. 1b).

In Fig. 2. the micrographs of sickle cells deoxygenated for 10 min (c) and 50 min (e) demonstrate the increased formation of sickled cells as expected. Treatment of these cells with 0.5 mM hydrazine prior to deoxygenation appears to protect the cells deoxygenated for 10 min from the sickling process (Fig. 2d). Excessive deoxygenation for 50 min after the treatment with hydrazine causes the cells to assume a variety of abnormal shapes (f) some of which are sickled. Comparison of Fig. 2f with untreated sickle cells which have undergone deoxygenation for 50 min (Fig. 2e) suggests that the hydrazine treatment is still exerting a protective effect as shown by the decreased proportion of sickled cells. In Fig. 3 the oxygen dissociation curves of the treated (0.5 mM) and untreated sickle cells from 3 different patients indicate an average shift in the p_{50} value from 29.0 to 42.0 mmHg on treatment; the oxygen affinity of normal erythrocytes is unaffected at this level of hydrazine concentration.

The central event in the pathogenesis of sickle cell disease is the formation of long rod-like polymers of deoxyhaemoglobin S at low pO_2 values. There occurs a marked increase in the viscosity of the cell contents leading to the abnormal shape and reduced deformability resulting in the local obstruction of the microcirculation and the consequent infarction. It has recently been shown that the major binding site on the membrane for haemoglobin is the anion-transporting protein, band 3 [13], and so the polymerization of membrane-anchored sickle cell haemoglobin can be expected to produce impaired cell shape and deformability. The results presented here indicate that the treatment of sickle cells in vitro with 0.5 mM hydrazine considerably improves the morphology but decreases the oxygen affinity of the cells, without affecting the metabolism or the susceptibility to haemolysis. It is suggested that on treatment of the sickle cells with hydrazine some of the membrane proteins, including band 3, may become chemically modified in such a way as to reduce considerably the binding of the haemoglobin S to the membrane. Furthermore, hydrazine may interact with side-chains on the haemoglobin S molecules and interfere with the stacking process on deoxygenation, thereby preventing the shape distortion charcteristic of this disease, but at the same time decreasing the affinity for oxygen.

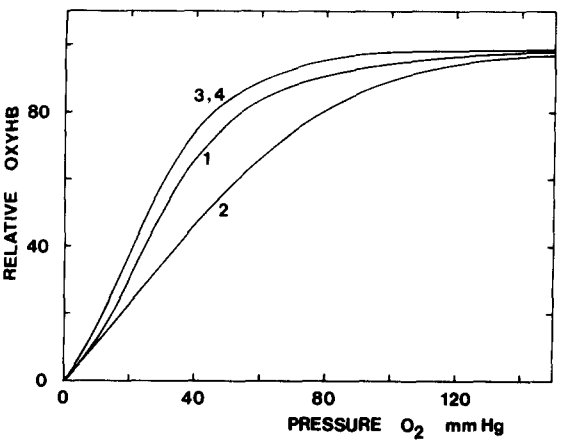

Fig. 3. Oxygen dissociation curves of erythrocytes from patients with sickle cell disease: (1) control sickle erythrocytes; (2) hydrazine-treated sickle erythrocytes; (3) normal erythrocytes, (4) hydrazine-treated normal erythrocytes.

We are currently investigating the mechanisms of this process as well as the effects of hydrazine on the erythrocyte deformability and on the membrane abnormalities known to predominate in sickle cells.

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