Fig. 3 Correlation of erythrocyte spectrin content and sensitivity to hypotonic lysis. Erythrocyte spectrin content was estimated by radioimmunoassay for each patient and is listed in Table 1: ○, ●, dominant inheritance; △, non-dominant or recessive inheritance; ○, △, previous splenectomy. The sensitivity to hypotonic lysis (osmotic fragility) was conducted on fresh blood samples mixed with PBS of concentrations ranging from 0.9% NaCl to distilled water¹. Median lysis was measured by interpolating between points to estimate the concentration of NaCl at which 50% of cells had lysed. The box at the upper left indicates the range of spectrin determinations on erythrocytes from multiple individuals (Table 1) and the range of median lysis as determined on erythrocytes from multiple normal adults and children.

of the mechanical fragility¹⁷. Reductions in other proteins that bind to spectrin would be expected secondary to deficiency in spectrin, and small but variable reductions in band 3 content were noted by radioimmunoassay as well as small reductions in ankyrin, band 4.1 and actin by analysis of SDS-PAGE gels⁸ (for example, Fig. 1). Band 3 deficiency is unlikely to be the primary defect in HS, as this correlated neither with clinical severity nor with osmotic fragility. Small reductions in ankyrin probably result from early degradation of uncomplexed ankyrin, although primary ankyrin defects or deficiencies may exist in certain HS families. A small reduction in band 4.1 is also unlikely to be a primary structural defect in HS, as a 50% deficiency in band 4.1 results, not in HS but in a subclinical form of ovalocytosis^{18,19}.

The spectrin deficiency in human HS probably results from many genetic defects, with each kindred carrying a different mutation. Probable abnormalities are likely to fall into two groups, a reduced spectrin synthesis due to abnormal gene regulation or unstable mRNA, and inherent protein defects in α - or β -spectrin subunits or ankyrin, leading to defective protein-protein associations, intracellular precipitation or increased susceptibility to proteolysis. Bodine and colleagues found spectrin-deficient spherocytic mouse mutants lacking ankyrin (*nb/nb*), with reduced spectrin α -subunit synthesis (*sph/sph*), synthesis of apparently unstable α -subunits (*sph^{na}/sph^{na}*) and reduced spectrin β -subunit synthesis (*ja/ja*)²⁰. Erythrocytes from three human families with dominantly inherited spherocytosis have been shown to possess two populations of spectrin, a normal population and a variant population binding defectively to band 4.1 (refs 21, 22). Members of the latter family have been found recently to have a reduction in spectrin relative to band 3 on SDS-PAGE gels (P. Becker and S. Lux, unpublished). The defect in the spectrin-4.1 interaction may result either in reduced spectrin incorporation into the membrane or increased degradation of membrane spectrin, and as expected, it was the variant population that was quantitatively reduced^{21,22}. Hereditary pyropoikilocytosis is a rare anaemia²³⁻²⁵ in which an inherent spectrin defect probably contributes directly to membrane instability¹⁰ but may also lead to secondary spectrin deficiency, while reduced binding of spectrin to ankyrin is

another mechanism which could lead to reductions in membrane spectrin (for example, ref. 26). Thus, it is likely that a large number of human genetic defects, although variable in degree, will have the common result of an overall deficiency in spectrin. The studies reported here indicate that spectrin deficiency, from whatever cause, will result in a haemolytic, spherocytic anaemia if the erythrocytes contain less than 200,000 copies of spectrin.

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Corrigendum

High rates of *in vitro* synthesis of 1,4- β -D-glucan in cell-free preparations from *Phaseolus aureus*

T. Callaghan & M. Benziman
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This letter described conditions for high rates of *in vitro* synthesis of 1,4- β -glucan in membrane preparations derived from *Phaseolus aureus*. This was achieved with extracts prepared in polyethylene glycol 4000 and assayed in the presence of Mg^{2+} , Ca^{2+} and cellobiose. The 24% KOH-insoluble glucan product, formed from UDP ¹⁴C-glucose, has been characterized as 1,4- β -D-glucan by several criteria, including digestion by purified exocellobiohydrolase and endo-1,4- β -glucanase, methylation analysis and periodate oxidation.

During further studies on the role of cellobiose in this *in vitro* system, more recent analyses of the product (in collaboration with Dr D. P. Delmer) have now revealed largely the formation of 1,3-glucan from UDP-glucose. This finding is puzzling and it is not yet clear why, in our early studies, 1,4-linked glucan was produced, whereas later, under the same conditions, the product was found to contain only 1,3 linkages.

Work is in progress to clarify exactly what conditions are required to obtain reproducible synthesis of cellulose *in vitro* with plant systems.