

We are sure, however, that several cases will continue to show a normal karyotype. The next effort, therefore, should be in the direction of isolating, by careful comparison of the phenotypes, the eventual existence of a new syndrome similar to the Prader-Willi syndrome whose pathogenesis is the result of the genetical imbalance resulting from a 15/15 translocation.

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Echinocytes in families with Duchenne muscular dystrophy

SUMMARY The results of the present investigation have failed to confirm the suggestion that there is a significant increase in the proportion of echinocytes in preparation of fresh erythrocytes in patients with Duchenne muscular dystrophy and heterozygous carriers of this disorder.

It has been reported that the frequency of deformed or more easily deformable erythrocytes is greater in males with X-linked Duchenne muscular dystrophy and heterozygous females than in controls (Matheson and Howland, 1974; Miller *et al.*, 1976; Percy and Miller, 1975). However, a previous study showed that

there was considerable overlap between the results in controls and carriers and between controls and affected boys (Lumb and Emery, 1975). The present more extensive study was undertaken in order to investigate this problem more fully.

Subjects and methods

Blood samples were obtained from 10 families where Duchenne dystrophy had occurred, living within 70 kilometre radius of Edinburgh. The control sample included healthy laboratory and university personnel. Heparinized blood, 20 μ l, drawn not more than 4 hours previously, was added to each of 6 Luckham plastic tubes containing 180 μ l 0.9% NaCl solution previously adjusted to 284 mosm/kg. The contents of the tubes were mixed gently and left for 2 minutes. They were then centrifuged for 3 minutes, the supernatant removed, and a further 180 μ l saline added. After 2 minutes the supernatant was again removed after centrifugation and the cells were fixed by adding 180 μ l of fresh 3% gluteraldehyde in 0.01 M sodium cacodylate adjusted to pH 7.4 and 310 mosm/kg. After 1 hour the tubes were gently agitated to facilitate mixing and the cells remained in fixative for 2 hours. After two washes in saline, 100 erythrocytes from each tube were counted and classified by examining random fields of view. Each cell was classified as either echinocyte (Class I, II, or III) or non-echinocyte (Bessis, 1972). For two families examination under phase contrast was also performed, but since the proportion of echinocytes using this method did not differ significantly from the proportions obtained from the same tubes using standard light microscopy, examination by phase contrast was discontinued.

Serum creatine kinase (CK) determinations were estimated by the Rosalki method using the kit supplied by Calbiochem (Rosalki, 1967).

Results and discussion

The proportions of echinocytes observed together with means and 95th centiles are depicted in the Fig. grouped under the categories of controls, possible carriers, definite carriers, and boys affected with Duchenne muscular dystrophy. There was no significant difference between any of the mean values in these various groups. These results clearly indicate that a simple echinocyte count on a 'fresh' blood sample is of no value in carrier detection which confirms Lumb and Emery's (1975) previous findings. Further, there was no significant correlation between the proportion of echinocytes and the serum level of creatine kinase.

Matheson and Howland's (1975) sample was small

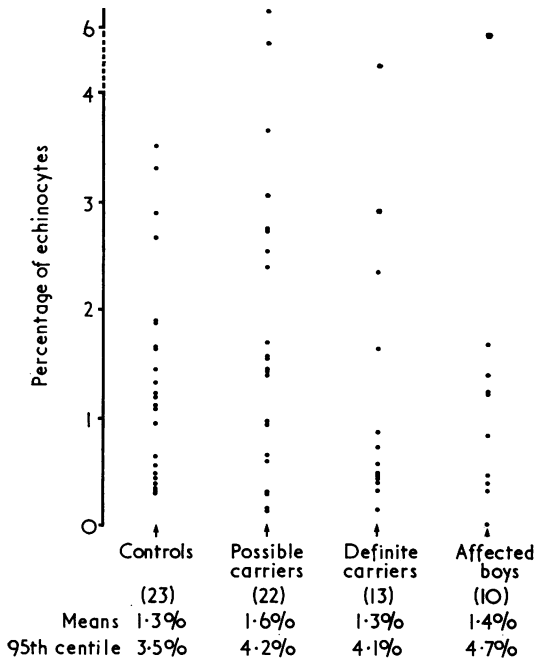


Fig. Frequency of echinocytes in families with Duchenne muscular dystrophy and in controls.

but the frequencies of distorted erythrocytes so high (from 21% to 98% for 4 affected boys and from 34% to 40% for 3 carrier females versus 3% to 7% for 5 controls) that differences between observations with electron microscopy, as opposed to light microscopy, are not likely to account for the discrepancy. Indeed, these authors suggested that echinocytes are visible with light microscopy, using unfixed cells in saline. More significantly our results support those of Miller *et al.* (1976) who, using numbers of subjects comparable to those in the present study, found no evidence for specific echinocytic changes under controlled conditions of sampling and fixing. However, these investigators found that using 'unmanipulated' erythrocytes (that is, cells fixed immediately on venepuncture without saline washes or centrifugation) there was a greater proportion of 'stomatocytes' in boys with Duchenne muscular dystrophy and their mothers compared with controls. In controls these workers found 2 to 5% stomatocytes; in Duchenne muscular dystrophy patients and in their mothers 20 to 80% of the erythrocytes were stomatocytes. In all instances the frequency of stomatocytes was at least four times higher than in controls. Stomatocytic shapes are perhaps the result of intrinsic biochemical membrane differences that respond to fixation in an abnormal manner.

Percy and Miller (1975) have speculated that

possible altered surface contour of patients' erythrocytes may be reflected in abnormal membrane elasticity or deformability. Their preliminary results, using modified cell elastimetry techniques, suggest that significantly more of the erythrocytes of the patients and possible carriers were aspirated into micropipettes at similar negative pressures. The technical difficulties of applying negative pressure to single erythrocytes through micropipettes are considerable, but these kind of studies need to be repeated using a sample of subjects much larger than the 5 patients and 4 carriers of the original study.

Thus, increasing but conflicting data are accumulating on erythrocyte deformation under various conditions of manipulation and fixation in Duchenne muscular dystrophy patients and in carriers.

Further studies to test the utility of scanning electron microscopy and the significance of immediate fixation of erythrocytes, without previous manipulation, are currently in progress in Edinburgh.

We are grateful to all the families and individuals who gave blood samples for this study and particularly to Mr J. M. S. Brodie, Chairman of the Edinburgh Branch of the Muscular Dystrophy Group of Great Britain. Mrs Peggy Lewis assisted in the collection of several samples and Dr Susan Holloway assisted in the statistical analysis of the data. I am particularly grateful to Mrs Letty Bain for technical assistance and to Professor Alan Emery for providing the laboratory facilities for this investigation and for advice. Financial support, making possible this study, was received from the Muscular Dystrophy Association of Canada to the author and the Muscular Dystrophy Group of Great Britain to Professor A. Emery.

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