

## Screening for Duchenne muscular dystrophy

Duchenne muscular dystrophy can be diagnosed with confidence before it is clinically apparent, and excluded with certainty where it may have seemed clinically obvious. This has been made possible by the demonstration of a grossly raised level of serum creatine phosphokinase (CPK) (Ebashi *et al.*, 1959; Dreyfus, Schapira, and Demos, 1960). Highest levels (up to 100 times normal) are found early in the disease and tend to decline with age and progressive loss of muscle bulk, though remaining raised. A peak is probably reached at about 1–2 years, but high levels are already present soon after birth. Heyck, Laudahn, and Carsten (1966) recorded a high level of CPK (and other enzymes) in an infant 9 days of age from a family at risk, but no one has yet documented a raised enzyme level in the cord blood of a proven case of Duchenne dystrophy. Diagnosis can be further confirmed, even in the preclinical stage, by electromyography and muscle biopsy. A grossly raised CPK by itself is no index of severity or prognosis, since equally high levels can occur in the milder X-linked Becker type of dystrophy.

In families at risk for Duchenne dystrophy early diagnosis can thus be made or excluded in male infants. Zellweger and Antonik (1975) have recently carried this a stage further by suggesting a screening programme for *all* newborn male infants. This may seem meddlesome in a condition where no effective treatment is available, and earlier diagnosis may simply prolong the parental agony, but Zellweger and Antonik argue that it is justified on two grounds—the prevention of further cases in the family by genetic counselling, and the early institution of ‘supportive therapy’. Such a screening programme would depend on a simple and reliable method for assessing CPK on either cord blood or a capillary sample from the newborn infant.

One of the problems that has bedevilled the whole standardization of CPK in relation to clinical diagnosis has been the large number of different methods for the assay, with widely varying degrees of reliability. This has been further compounded in recent

years by the proliferation of prepacked ‘kits’. Two of the most widely used and reliable methods have been those of Hughes (1962) and Rosalki (1967). Another problem is the different range of normal levels in the newborn, compared with older children.

In a study of 95 cord blood samples, Griffiths (1968), using the method of Ennor and Rosenberg (1954), found levels about three times higher (both the median and 95th centile) than that of older children and thought that in view of the overlap to within the dystrophic range, assay of cord blood was unlikely to be a useful method of detecting preclinical cases of muscular dystrophy. Griffiths found no apparent correlation of the CPK levels with obstetrical complications or fetal hypoxia, whereas Rudolph and Gross (1966) had observed a striking influence of perinatal factors on the CPK level. They undertook serial studies on 31 mothers (venous) and infants (venous and/or capillary) and on an additional 32 isolated infants, using the method of Tanzer and Gilvarg (1959). There was no correlation between the individual maternal and neonatal CPK levels and the infants showed a wider range than the mothers and a consistently higher mean level at each time period tested. There was a peak level in the infants at 24 hours. Capillary samples were on average about 10% lower than the parallel venous samples taken in 10 infants. The level at 24 hours was significantly higher in uncomplicated vertex deliveries than in those born by uncomplicated caesarean section, and a considerable rise was found after breech delivery, secondary uterine inertia with oxytocin stimulation, or emergency caesarean section after a trial of labour. There was no correlation between CPK and one-minute Apgar score, suggesting that the rise probably reflects perinatal muscle trauma rather than any other mechanism.

Bodensteiner and Zellweger (1971), using a modification of the Hughes method, found a consistently raised CPK in normal neonates and infants, with a peak during the first 24 hours, but remaining slightly above adult levels throughout the first year of life. Like Rudolph and Gross (1966), they also

found that differences between cord blood CPK and first-day postpartum levels in the infants were greater for vaginally delivered infants than in those born by caesarean section. In an earlier study on the use of capillary blood for CPK estimation they had also observed consistently lower levels than the venous samples (Bodensteiner and Zellweger, 1970).

Zellweger and Antonik (1975) have introduced a completely new micromethod for their screening programme, applicable to a drop of capillary blood dried on filter paper. It is based on the amount of light produced (expressed as 'relative light units') by the ATP of the reaction process on luciferin-luciferase present. The method was tested on the samples from 1500 newborn infants already obtained for the Guthrie test for phenylketonuria. All but 2 gave normal CPK levels. These two, which were slightly raised at 3 to 4 times the normal level, were normal when rechecked some months later and thus constituted false-positives. It is not possible to ascertain the possibility of false-negative results in this survey as there were no proven cases of Duchenne dystrophy.

They also compared the CPK levels by this dry drop method with the 'routine method' used in their laboratory in a series of 16 cases of Duchenne muscular dystrophy. Though the new method does show an age-related extent of raised levels ranging from  $65 \times$  to  $4 \times$  the upper limit of normal, it is difficult to interpret the table of comparative results, since their unspecified routine method has a mean normal level of about 5 to 6 units (according to age) but an apparent ceiling of 40 units, so that the majority of results are given as  $>40$  and could presumably be anything from  $8 \times$  the normal level upwards. The nature of the units is also not specified. The actual figures for the normal range of their new method are also not included in their paper.

In spite of the relatively high estimated incidence of about 1 in 3000 to 4000 male births (Zellweger and Antonik, 1975) and the potential value of genetic counselling in preventing a recurrence in the family, it is doubtful whether there would be any enthusiasm for large-scale screening of newborns for Duchenne dystrophy at the present time. However, should the circumstances change and the possibility of treatment become available, then this would be a very worthwhile undertaking. Under such circumstances the method would have to be practical, economical, and of proven reliability and consistency in general laboratory use. Zellweger and Antonik claim that with their method at least 5 assays can be obtained from a single drop of blood, 600 assays can be performed in a day by

one technician, and samples can be stored at room temperature up to 6 weeks.

Beckmann and his colleagues in West Germany (Beckmann *et al.*, 1974 a, b) have compared this new method with the standard CPK technique and found it to be reliable. They have favoured the introduction of a screening programme and in their initial survey (R. Beckmann and G. Scheuerbrandt, personal communication, 1976) of 16 520 newborn infants (from 120 hospitals in South West Germany) have detected 5 boys with preclinical muscular dystrophy which would give an incidence of 1 in 1700 male births (assuming 52% of births in Germany are male). This would suggest a higher incidence than any previous estimates, which have ranged from 1 in 3000 male births to 1 in 8000. In addition, Beckmann subsequently also identified 5 female carriers in the families of these 5 males. If a screening programme is introduced, it may well be worth screening all infants, both male and female, since potential female carriers of Duchenne dystrophy may well have higher CPK levels early on which may gradually decline with age (as happens in affected boys) and possibly drop to within normal levels by the time they reach adult life, thus accounting for some of the 30% or so of known carrier females who have normal CPK levels.

In a poll of parents, Beckmann and Scheuerbrandt (personal communication, 1976) found that 87% of those with an affected son said they would wish to know that their newborn son had Duchenne muscular dystrophy, as did 86% of parents of healthy schoolchildren.

In order to minimize the risk of false-positives and particularly false-negatives, the range of normal infants would have to be carefully assessed and the influence of perinatal factors on the levels taken into account.

In this issue Gilboa and Swanson (1976) once again highlight the wide range of CPK in normal newborns. In some the level was up to 10 times the normal. Like previous authors they have also noted the lower level in cord blood than in capillary blood and a peak level in the infant during the first 24 hours after delivery. The mean capillary level was 14.6% lower than that of simultaneous venous samples but did not reach statistical significance. In contrast to the experience of previous authors they did not find the same consistent correlations between birth trauma, mode of delivery, and CPK levels. They wisely suggest that any screening for muscular dystrophy should be postponed beyond the immediate newborn period.

The stage does not yet seem set for a nationwide programme of screening for preclinical Duchenne

muscular dystrophy, but when the time is ripe for it the techniques will hopefully be sufficiently standardized for immediate application.

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