IT has been known for many years that since Duchenne muscular dystrophy is inherited as an X-linked recessive trait only boys will manifest the disease and girls in affected families will have a 50 per cent chance of being genetic carriers of the condition. Although Duchenne dystrophy is a relatively uncommon condition with possibly about 70 patients in Northern Ireland, the number of potential carriers is much greater, since sisters, maternal aunts, and female cousins of the patient all fall into this category. From incidence and prevalence figures (Nevin, 1975) it can be calculated that, as a conservative estimate, there are about 500 potential carriers in Northern Ireland, and the real figure may be much greater.

A considerable amount of effort has been put into the development of methods of detecting carriers. All of the methods used are based on the "Lyon hypothesis" (Lyon, 1961) that carriers suffer from a sub-clinical form of the disease. This hypothesis has been criticised recently, but nevertheless it has formed a useful basis for the investigation of the carrier state. Occasionally, clinical signs and symptoms of the disease have been found in carriers and these findings include pseudo-hyper trophy and weakness (Chung, Morton, Peters, 1960; Emery, 1963). If then, the carrier has a sub-clinical form of the disease, it follows that the methods which have been used for carrier detection have been mainly those which are used in diagnosing the fully expressed disease. Muscle morphology has been used at light and electron microscopic levels, and abnormalities have been reported (Pearson, Fowler and Wright, 1963; Gardner-Medwin, Pennington and Walton, 1971; Craig, Allen and McCormick, in preparation). Electromyography has also been used with some reported success (Gardner-Medwin, 1968). Several more exotic methods such as muscle cell culture and muscle protein turnover rate have been used but these methods have not been widely adopted largely due to the technical problems involved.

It was with the development of diagnostic serum enzymology from 1950 onwards that advances in carrier detection were made. The first positive results came in 1960 when Chung et al using aldolase and transaminase reported increased levels of these enzymes in the serum of 15 per cent of mothers of boys with Duchenne muscular dystrophy. Schapiro et al, also in 1960 reported increased levels of aldolase and creatine phosphokinase (CPK) in 30 per cent of known carriers. Following progressive technical improvements in the method of serum CPK analysis, detection rates steadily increased until detection rates of up to 75 per cent of known carriers were possible (Walton and Gardner-Medwin 1969).
In the present paper we would like to present our results for carrier detection in Northern Ireland during the last few years, dealing exclusively with the results from CPK analysis. Light microscopy, electron microscopy and electromyography are also used, but because of the ease of obtaining the material (clotted blood) it has been possible to investigate more carriers using CPK analysis than by the other techniques.

MATERIALS AND METHODS

In our survey of serum CPK in carriers we have up to the present analysed specimens from 125 potential carriers who were divided into three categories according to the classification of Walton and Gardner-Medwin (1969):

Definite Carriers: Mothers of an affected son who have also an affected brother, maternal uncle, sister's son or other male relative in the female line of inheritance.

Probable Carriers: Mothers of two or more affected sons, who have no other affected relatives.

Possible Carriers: Mothers of isolated cases and sisters and other female relatives of affected males.

Five millilitres of clotted blood samples were taken from the subjects and serum CPK was estimated, usually within two hours, using the Boehringer Test Combination 15790 (Boehringer, Mannheim, G.M.B.H.).

RESULTS

In using CPK analysis to maximum advantage it is important that a well defined normal range is determined and that one is aware of the many possible factors which can interfere with serum CPK levels. Our normal range for females was established using blood samples from 163 blood donors. The mean value was found to be 16.3 mU/ml with a normal range of 1.7–37.5 mU/ml.

| No. of cases | No. of cases with elevated CPK | Per cent of cases with elevated CPK |
|--------------|-------------------------------|-----------------------------------|
| DEFINITE CARRIERS | 12 | 11 | 91.7 |
| PROBABLE CARRIERS | 8 | 6 | 75.0 |
| POSSIBLE CARRIERS | 105 | 31 | 29.5 |

The percentage of individuals with elevated CPK in each of the categories of carrier is presented in Table I. From this it can be seen that elevated CPK was found in all but one of the definite carriers. The one exception in this category was
found to have normal levels of CPK on three separate occasions. In the group of probable carriers 75 per cent of individuals had increased levels of CPK. Elevated levels were found in about 30 per cent of potential carriers, this being a very heterogeneous group consisting of aunts, cousins and sisters of dystrophy patients and mother's of isolated cases.

![FIG. 1. Values for serum CPK in potential carriers with elevated levels. The normal range is represented by the vertical bar.](image)

The scatter of CPK values in potential carriers with elevated levels is illustrated in the figure. There is a wide range with values up to 15 times normal with a marked cluster of results just above the upper limit of normal.

**DISCUSSION**

A consideration of the effectiveness of any method of carrier detection must clearly be limited to the definite carriers since they are the only ones with known genetic status. In the present investigation 11 of the 12 definite carriers had elevated CPK indicating that CPK analysis is a very efficient indicator of the carrier state. However, it is significant that one individual in this group had normal levels on three occasions, raising the possibility that some carriers may be inherently undetectable by the CPK method. This confirms the advisability of using more than one
method of carrier detection as it is suspected that normal CPK is not necessarily accompanied by normal results in other carrier detection tests. Although CPK estimation is in general the most sensitive test we have found two possible carriers with normal CPK levels but marked abnormalities in muscle histology (Craig, Allen and McCormick, 1975).

Since the "possible carrier" group is a heterogeneous one it is impossible to estimate the number of carriers which it contains. It is obviously the group in which carrier detection tests are very important since these subjects, at the time of testing, have one or no dystrophic children and are of unknown genetic status. Our results in the other categories suggest that most of the actual carriers within this group will be detected by CPK analysis, but we again stress the desirability of using as many criteria of carrier status as possible e.g. muscle histopathology, electron microscopy, electromyography.

Although some carriers have grossly elevated levels of CPK there are many in whom the increase is much less marked. For an accurate assessment of those results it is necessary to have a precisely defined upper limit of normal, and subjects who have values in the "borderline" range (just above or just below the limit) should be tested on a number of occasions. Although multiple testing is most important in this "borderline" group it should be done routinely in all subjects if possible since occasional high values are found in normal individuals as a result of factors such as unaccustomed muscular exercise and sub-clinical disease states (Griffiths, 1966; Graig and Smith, 1965). In particular, it should be noted that electromyography and surgical procedures cause increased levels of CPK (Maeyens and Pitner, 1968; Phornphutkul et al, 1974). For this reason it is essential that on hospital admission CPK estimation is performed before electromyography or muscle biopsy.

SUMMARY

Serum CPK estimation is a simple investigative procedure requiring only small samples of blood and the present survey indicates that it produces a detection rate of about 90 per cent for carriers of Duchenne muscular dystrophy. The importance of a well defined normal range, multiple CPK analyses and an awareness of the possibility of false positive results is stressed. In addition it is desirable when possible to use other methods for carrier detection such as muscle histopathology and electromyography.
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