# Studies of the carrier state in the Duchenne type of muscular dystrophy

## 2. Quantitative electromyography as a method of carrier detection

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The best method for detecting female carriers of the X-linked gene for muscular dystrophy of the Duchenne type is estimation of the serum creatine kinase (CK). Pearce, Pennington, and Walton (1964a) reported their early experience of the method in this unit. They found the serum CK to be raised in five of their seven definite carriers and in five of their eight probable carriers. Since then more subjects have been tested and the detection rate at present stands at 14 of 20 definite carriers (70%) and six of 11 probable carriers (55%). These detection rates for carriers are in agreement with those from many other laboratories in which about 70-75% of known carriers are found to have increased serum CK activity.

In the first of the present pair of papers Hudgson, Gardner-Medwin, Pennington, and Walton (1967) discussed whether estimation of the serum CK after exercise might improve the discrimination between carrier and control subjects, but they found this method to be of little value.

Quantitative electromyography was advocated by van den Bosch (1963) as a method of improving carrier detection. In general, however, those who have tried to use this technique have not found it reliable. Some possible reasons for this will be mentioned in the discussion. The present paper is an account of one successful and two unsuccessful electromyographic techniques which have been tried in this laboratory. Preliminary reports were given by Gardner-Medwin (1967) and Walton, Gardner-Medwin, and Hudgson (1967).

### MATERIALS AND METHODS

The subjects are listed in Tables I and II. The normal controls were drawn from hospital patients and technical, secretarial and medical staff. The nature and purpose of the investigation was explained to all before they were asked to volunteer. None of them had any personal or family history of neuromuscular disease. The 'carriers' were all female relatives, in the maternal line, of proven cases of the severe (Duchenne) or, in two specified cases, the benign late onset (Becker) type of X-linked muscular dystrophy. *Definite carriers* are women with an affected son and also an affected brother, maternal uncle or sister's son; or women with affected sons by different fathers. *Probable carriers* are women with more than one affected son but no other affected relatives. *Possible carriers* are the mothers of isolated cases and the sisters, maternal aunts or other female relatives in the maternal line of affected boys (Pearce *et al.*, 1964a). In this study the term 'presumed' carrier refers to those probable and possible carriers in whom the serum CK was raised (above 60 international units). The method of CK estimation was that given by Pearce, Pennington, and Walton (1964b) and the units were converted to international units (i.u.) by multiplication by a factor of 16·7. The cases of muscular dystrophy were patients with the Duchenne, limb-girdle, or facio-scapulo-humeral types of the disease. Two cases of polymyositis were included in the interference pattern studies (Table I).

The biceps brachii was used for all studies because it is accessible, and is fairly constantly involved early in the course of Duchenne muscular dystrophy and therefore might be expected to be involved in carriers. No measurements of intramuscular temperature were made, but the room used was warm, the subjects were given time to adjust to this temperature, and since the biceps is a large proximal muscle it seems unlikely that much temperature variation occurred.

The techniques used were: (1) integration electromyography of the interference pattern at various tensions;

### TABLE I

THE SUBJECTS OF THE INTERFERENCE PATTERN STUDIES

| Subjects | Integration E.M.G. | Frequency Analysis |
|---|---|---|
| Normal controls | 14 | 8 |
| Muscular dystrophy | 9 | 9 |
| Polymyositis | 2 | 2 |
| Definite carriers | 8 | 7 |
| Presumed carriers | 7 | 2 |
| Possible carriers | 16 | 9 |
| Total | 56 | 37 |

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## TABLE II

### RESULTS OF THE ACTION POTENTIAL MEASUREMENTS IN 69 SUBJECTS[1]

| Subjects | No. | Age (Mean and Range) | Numbers of Potentials Measured (Mean and Range) | Mean Duration (Deviation % from Buchthal's Norms) | Mean Amplitude ($\mu V$) | Mean % Polyphasic Potentials | Mean Number of Phases per Potential |
|---|---|---|---|---|---|---|---|
| Normal controls | 20 | 31·7 (16-58) | 37·5 (21-70) | +6·3 (± 18·6) | 219 (± 89) | 10·3 (± 10·8) | 3·00 (± 0·43) |
| Muscular dystrophy patients | 8 | 20·5 (13-34) | 29·8 (20-37) | − 7·4 (± 25·4) $P = <0.001$ | 184 (± 44) $P = <0.005$ | 30·0 (± 18·4) $P = <0.001$ | 3·85 (± 2·89) $P = <0.001$ |
| Definite carriers | 14 | 42·8 (26-64) | 34·6 (21-56) | − 7·7 (± 24·8) $P = <0.001$ | 209 (± 90) $P = <0.2$ | 18·8 (± 15·7) $P = <0.001$ | 3·38 (±1·82) $P = <0.001$ |
| Definite and presumed carriers | 26 | 40·1 (20-64) | 38·8 (21-65) | − 7·4 (± 27·0) $P = <0.001$ | 196 (± 103) $P = <0.005$ | 20·3 (± 18·6) $P = <0.001$ | 3·44 (± 1·08) $P = <0.001$ |
| Other probable and possible carriers | 15 | 32·2 (19-47) | 37·0 21-69) | +8·2 (± 32·0) | 223 (± 103) | 9·1 (± 13·8) | 2·94 (± 1·54) |

[1]The means are given ± 2 standard deviations. The significance of the difference between each mean and the corresponding normal mean (using Student's *t* test) is also indicated where appropriate.

(2) automatic frequency analysis at a set tension; (3) measurement of individual muscle action potentials.

INTEGRATION E.M.G. The subjects sat with the elbow supported and flexed to 90° and pulled against a spring balance by means of a sling around the wrist and a single pulley. The forearm was kept fully supinated and recordings were made only during steady pulls. The skin over the biceps was cleaned with ether and a bipolar surface electrode was placed over the middle of the muscle belly. This consisted of two circular metal discs 1 cm in diameter and fixed 5 cm apart, each smeared with electrode jelly. Potentials were led off *via* a Medelec MS3 preamplifier and amplifier to a Medelec integrator. The integrating capacitor (1·0 $\mu F$) automatically discharged when the potential reached a set level of approximately 10 V. Thus the number of discharges in a unit of time gave an arbitrary unit of 'counts per sweep' as a measure of the mean voltage of the interference pattern. A sweep duration of 1,000 msec and a gain of 1,000 $\mu V$/cm were used in all recordings. The interference pattern and the integrator ramps, together with the frequency analyser histogram pattern to act as a time calibration, were displayed on three channels of the C.R.O. and were photographed and subsequently projected at a magnification of about 10 ×. The frequency analyser histogram was found to have a constant duration of 920 msec. The number of integrator ramps occurring in this arbitrary period of time was measured in at least 15 successive sweeps at each of at least five muscle tensions from 0 to 20 lb. (0 to 9·1 kg) and also as many times as possible at maximum effort. The counts were averaged for each tension and successive recordings at the same tensions gave closely similar results. Each 10 counts per sweep represent a mean voltage of approximately 92 V/sec at the integrating capacitor.

FREQUENCY ANALYSIS Recordings during isometric contraction were made using the same method but with Medelec 5 cm concentric needle electrodes and a Medelec FA3 frequency analyser. (For needle specifications see under Action Potential Measurements.) Preliminary studies indicated that recordings during sustained maximal contraction were too painful and difficult and

that at above about 10 lb. the tension used did not significantly affect the frequency distribution obtained. An arbitrary tension of 15 lb. (6·8 kg) was therefore used in all carriers and controls and lesser tensions in some of the weaker patients with muscular dystrophy. The frequency analysis patterns obtained at this constant tension from at least 20 different sites in the muscle were displayed on a C.R.O. at a sweep duration of 1,000 msec and gain of 500 $\mu V$/cm, photographed and subsequently projected. The heights of all the histogram columns were measured. Because the absolute height of the whole pattern as well as the relative heights of the 12 columns varied from place to place it was felt necessary in the quantitative studies to subtract from the measured height of each column the height of the equivalent column in the 'resting' histogram (see Fig. 1) and to express the results as proportions of the total activity in each pattern.

ACTION POTENTIAL MEASUREMENTS Three Medelec 5 cm coaxial needle electrodes were chosen because they gave identical values for the mean duration in the same muscle and these were used in all experiments. Their specifications were as follows: external diameter, 0·465 mm; core diameter, 0·12 mm; recording area, 3 × $10^4$ $\mu^2$; impedances measured at 160 c/s, 0·534 M$\Omega$ ($L13°$), 0·934 M$\Omega$ ($L17°$), and 1·0 M$\Omega$ ($L10°$).

The potentials were amplified as described above, the time constants of the amplifier being set to H.F. 30 $\mu$sec and L.F. 100 msec; they were displayed simultaneously with a time calibration derived from a frequency-controlled oscillator at a sweep of 100 msec and a gain of 100 $\mu V$/cm. Photographs were taken on 35 mm negative film with a Cossor Camera. An amplitude calibration signal was recorded on each film.

The biceps was probed with a single needle electrode according to a set pattern of nine different muscle insertions. The needle tip was moved at least 0·5 cm between recording sites. No attempt was made to obtain the maximal amplitude of the potentials and as many as possible were recorded at minimal effort, usually 30-40 and never less than 20. Mixed batches of normal and potentially abnormal films were measured 'blind' after being allotted random numbers by an assistant. Every potential over 50 $\mu V$ in amplitude which had at least one
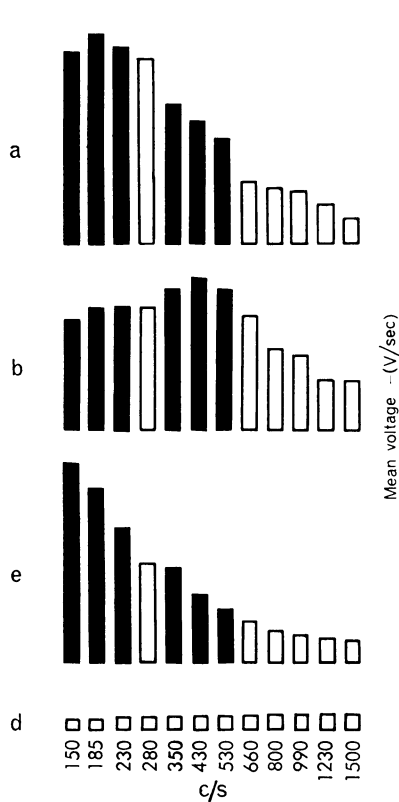
FIG. 1. Frequency analysis histograms. a. *Intermediate pattern seen in normal subjects and carriers.* b. *Typically myopathic pattern.* c. *Normal pattern.* d. *Baseline, with optimum resonating frequency of each column (c/s). The black columns are those used in calculating the ratio referred to in the text.*

FIG. 2. Integration E.M.G. *Mean amplitude of interference pattern (V/sec) at various tensions for different groups of subjects. 1 lb. = 0·4 kg.*

FIG. 1                     FIG. 2

definite spike and which could be clearly delineated and identified at least three times was accepted. They were marked, projected at a magnification of about 10×, and measured to the nearest 10 $\mu$V and 0·5 msec. Phases were defined as oscillations which crossed the baseline, except that initial and terminal oscillations of less than 10 $\mu$V were excluded. Polyphasic potentials were taken as those with more than four phases. The term 'double potential' refers to those in which two apparently different spike components with a constant time interval between them formed part of a single motor unit action potential. The components were often of similar form and amplitude but many were not so.

RESULTS

INTEGRATION E.M.G. This was performed in 56 subjects as indicated in Table I.

The results in terms of the mean voltage of the interference pattern at various tensions are expressed in Figs. 2 and 3. Although the average voltage-tension gradients for the groups of carriers fell between those for the normal and dystrophic subjects (Fig. 2) the normal range (indicated by the
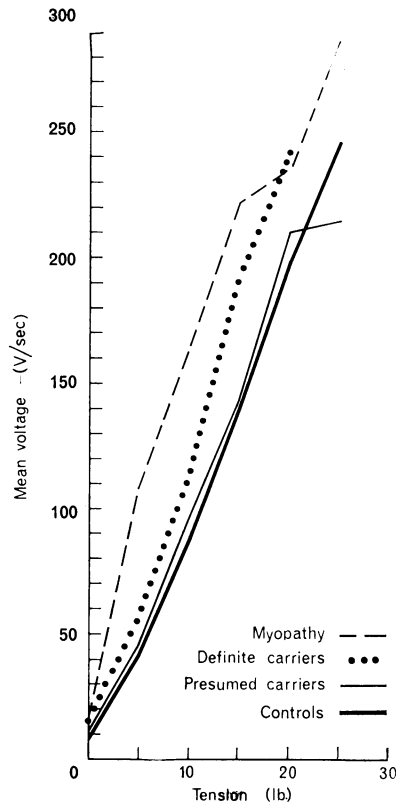
shaded area in Fig. 3) was wide. The voltage-tension gradients of several of the definite carriers lay near the upper limit of normal but only one lay beyond two standard deviations from the normal mean. The gradients for the presumed carriers and for all the possible carriers were perfectly normal. Thus integration electromyography appears to be of little practical value for carrier detection.

FREQUENCY ANALYSIS The 37 subjects tested are shown in Table I. Inspection of the histograms from normal subjects showed that the peak frequencies were normally between 150 and 230 c/s, but that there was much variation between one site in the muscle and another. In the myopathic patients the peak frequency was shifted, usually to between 230 and 530 c/s and only occasionally as far as 800 c/s. In mild cases the peak was correspondingly less shifted and many individual histogram patterns were apparently normal. Inspection of the carriers' patterns revealed no definite abnormality.

An attempt was made to quantify the results, although the frequency analyser is essentially a
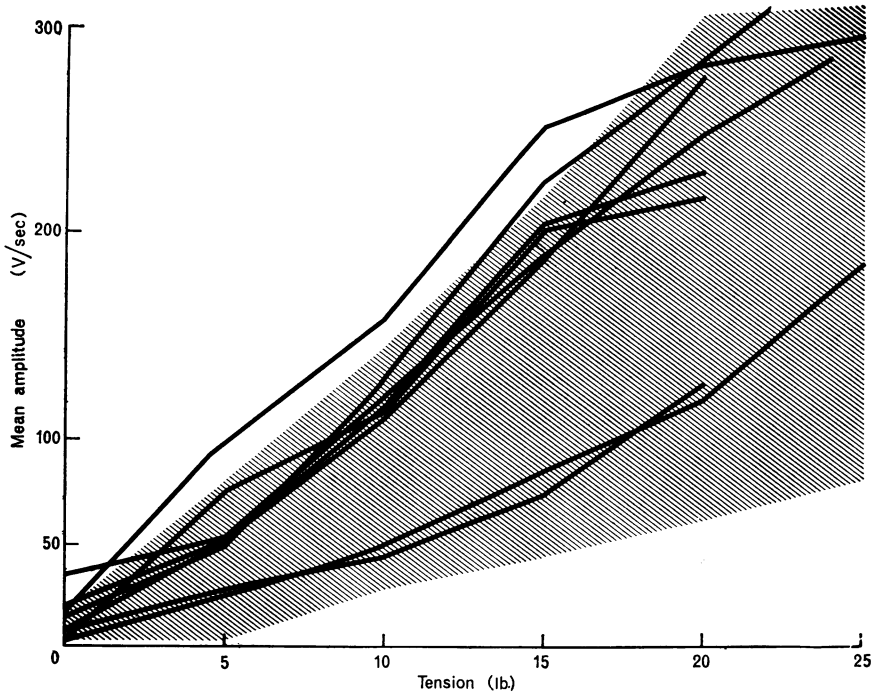
FIG. 3. Integration E.M.G. *Mean amplitude of interference pattern at various tensions. The normal range (mean ± 2SD) is hatched and the value for each of the eight definite carriers is shown.*
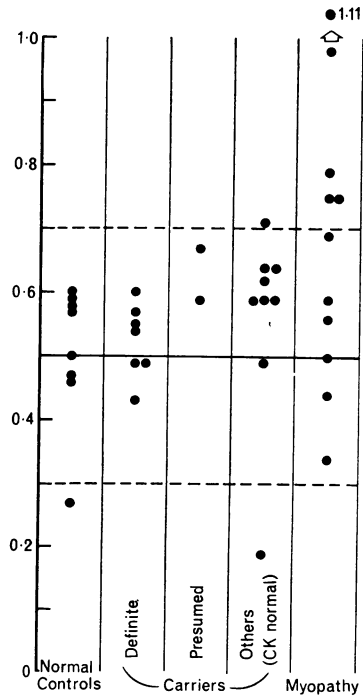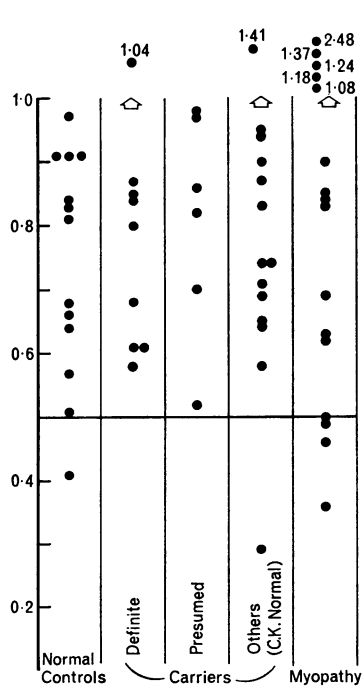


FIGS. 4 and 5. Frequency analysis. *The ratio of activity in the range 350-530 c/s to that in the range 150-230 c/s. The normal mean and the range ± 2SD are indicated. In Fig. 4 the values are calculated from all the histograms obtained and in Fig. 5 from the single most abnormal histogram.*

FIG. 4                    FIG. 5

device for providing an immediate visual impression of the frequencies present in the interference pattern and cannot be expected to measure them accurately. The measured amount of activity in the frequency bands 350-530 c/s was divided by that in the range 150-230 c/s, the resultant ratio being above unity in 'myopathic' patterns and below unity in normal ones (Fig. 1). The activity in each of the corresponding columns of the histograms obtained from each subject was summed and the resulting mean ratios of 350-530 c/s to 150-230 c/s activity are shown in Fig. 4. The ratios in Fig. 5 are those taken from the single most 'myopathic' histogram in each subject. Only one possible carrier falls beyond the normal range in each figure and these were two different young unmarried women in whom the serum CK activity, the muscle biopsy findings, and the other E.M.G. measurements were all normal. Thus there is no evidence from these results that this method of automatic frequency analysis detected any electro-myographic abnormality in the carrier subjects.

ACTION POTENTIAL MEASUREMENTS   The subjects and results are shown in Table II. The definite carriers are shown separately and also combined with the presumed carriers. The two carriers of the gene for the Becker type of muscular dystrophy are also included but they have been indicated separately in Figs. 7-9 which illustrate the individual findings.

The known increase of the action potential (A.P.) duration with age poses a problem. Very large numbers of control subjects would be needed to determine the normal value at every age. The absolute values for mean duration obtained by different observers using different equipment are emphatically not comparable but it seems reasonable to assume that the rate of change with age should be constant. The values for mean duration obtained in the 20 normal subjects (Fig. 6) have therefore been compared with the normal mean values published by Buchthal (1957). The mean duration for each subject was compared with Buchthal's norm for her age and was expressed as a percentage deviation from that norm. The mean deviation for all the normal subjects was $+ 6.3\%$ and in Fig. 6 a line parallel to Buchthal's gradient represents this deviation, and is taken as the mean for the normal subjects in this study. The range of two standard deviations from this mean was $- 12.3\%$ to $+ 24.9\%$ and $- 12.3\%$ was therefore taken as the lower limit of the normal range. Figure 7 shows the results for the definite and presumed carriers compared with this mean and lower limit of normal. Only four carriers gave results which lay above the normal mean; of these one had previously suffered from poliomyelitis (without clinical involvement of the tested biceps) and her results were excluded from Table II. Several carriers gave results lying below the normal range. The mean A.P. durations of the dystrophy cases, definite carriers, and the definite and presumed carriers as groups differed significantly from those of the normal subjects (by Students $t$ test $P = < 0.001$).

Mean amplitude measurements and phase counts require no age correction. The mean amplitudes of the A.P.s for both muscular dystrophy patients and the known carriers were significantly lower than normal (Table II) but the normal variation was wide and no individual case fell outside the normal range.
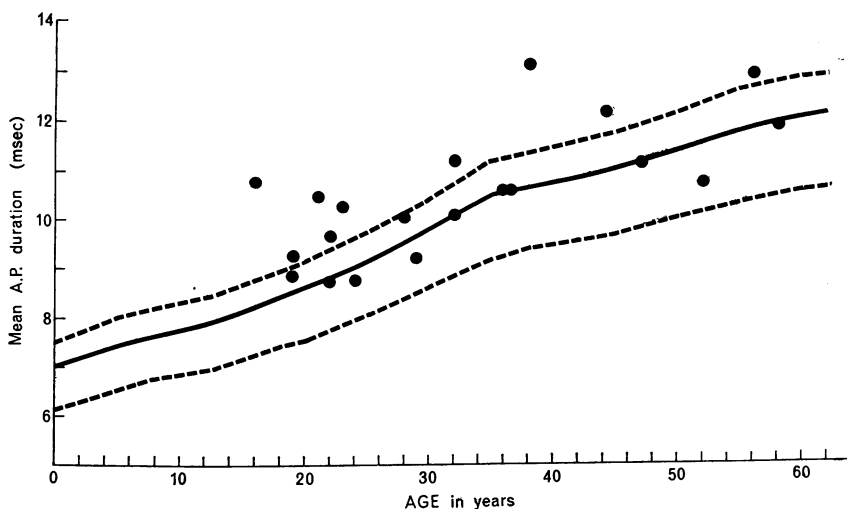
In the normal subjects the mean proportion of



FIG. 6. *Mean action potential duration—normal controls. The continuous line indicates the mean values obtained by Buchthal (1957). The upper interrupted line is 6·3% above this and is the normal mean value for this study. The lower interrupted line is 2 SD below the upper and represents the lower limit of normal.*
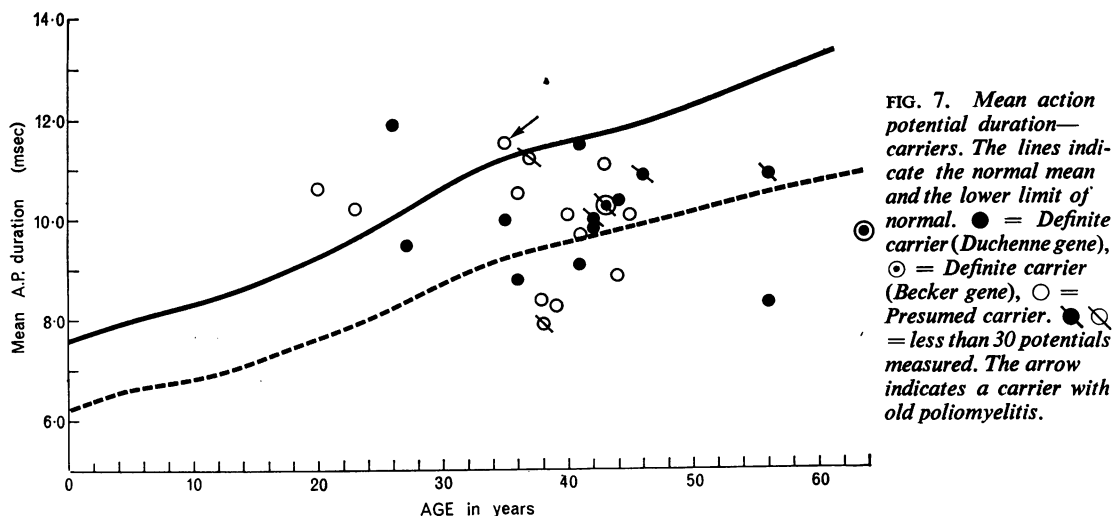
FIG. 7. *Mean action potential duration—carriers. The lines indicate the normal mean and the lower limit of normal.* ● = *Definite carrier (Duchenne gene),* ⊙ = *Definite carrier (Becker gene),* ○ = *Presumed carrier.* ◕ ◔ = *less than 30 potentials measured. The arrow indicates a carrier with old poliomyelitis.*

polyphasic potentials was 10·3% and the upper limit of normal was 21·1%. The known carriers had about twice as many and the cases of muscular dystrophy three times as many polyphasic units. Corresponding to this was an increase in the mean number of phases per potential from the normal (3·00) to 3·38 for definite carriers and 3·85 for cases. All of these results are significant ($P = < 0.001$). Ten carriers (one a possible carrier) had more than 21% of polyphasic potentials and 10, some of them different individuals, had abnormal numbers of phases per potential (Figs. 8 and 9).

For all subjects the ratio of the mean number of phases to the mean A.P. duration was calculated, and is given the symbol Ø (van den Bosch, 1963). Since this figure must vary with age, the normal range was established in the same way as the range of the normal mean duration—that is, by the use of Buchthal's values to derive the rate of decline with age and subsequent calculation of each normal subject's percentage deviation from the normal mean. The normal range (2 SD from the mean for any given age) was ± 19·0%. The dystrophy patients had much higher Ø values (+ 42·0 ± 19·1%) and the results for the carriers are illustrated in Fig. 10. Nine definite, one possible, and eight presumed carriers had Ø values above the normal range.

Taken as a group the possible and probable carriers with normal serum CK levels did not differ from the normal subjects by any of the criteria of measurement. However, in one individual the deviations of the mean A.P. duration and Ø were abnormal (− 15·6% and + 24·8 respectively) and another had 24·3% of polyphasic potentials although her other results were normal.

At the time of the E.M.G. recording a subjective assessment of the E.M.G. appearances was made and any definite abnormalities were noted. The record was described as normal, abnormal, or doubtful, and these categories are indicated in Tables III and IV. Some of the records from normal subjects would have been described as doubtful. Definite abnormalities included fibrillation potentials found in more than two sites in the muscle (two carriers), brief high frequency discharges (two carriers), frequent double potentials (six carriers), and increased numbers of polyphasic or short-duration potentials.

Tables III and IV summarize the detection rates among the definite and presumed carriers. One definite carrier escaped detection altogether. Four were identified by serum CK estimation alone, five by both serum CK and E.M.G., and four (including both of the carriers of the Becker gene) by E.M.G. alone. Two-thirds of the presumed carriers showed some significant electromyographic abnormality. Table IV also shows that, of eight probable carriers tested, four had entirely normal results, while four had increased serum CK activity and of these two had abnormal E.M.G.s. Of 19 possible carriers eight had raised serum CK activity (and six of these had abnormal E.M.G.s) and 11 had normal serum CK activity, two of them (shown in Table IV) having abnormal E.M.G.s.

### DISCUSSION

Van den Bosch (1963) was the first to suggest electromyography as a method of carrier detection. He studied muscle action potentials in the quadriceps. Arguing that the two main features of myo-
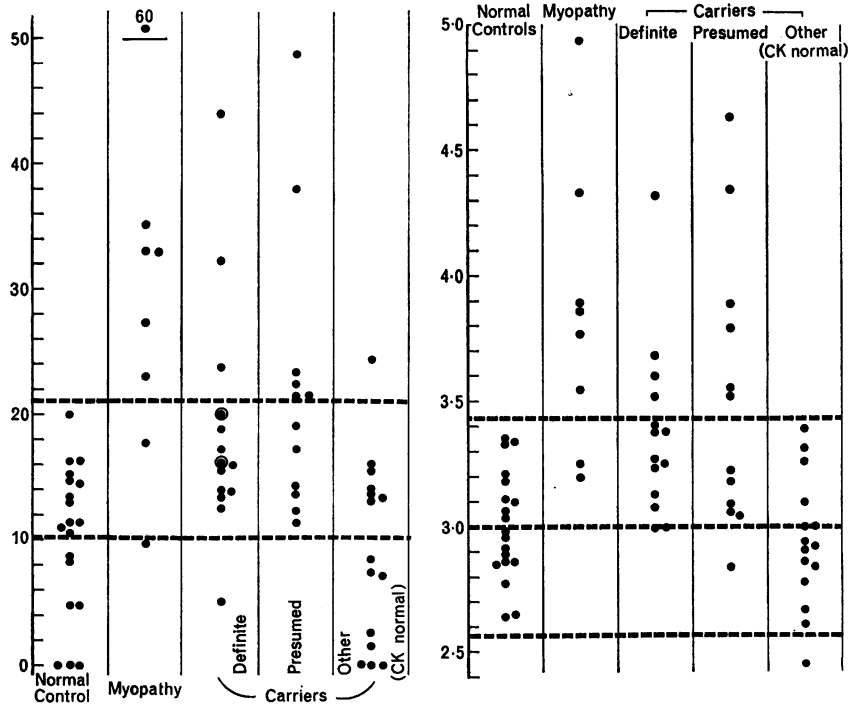
FIGS. 8 (*left*) and 9 (*right*). *Proportion of polyphasic potentials* (%) (*Fig. 8*) *and mean number of phases per potential* (*Fig. 9*). *The normal means* ± *2SD are indicated.*
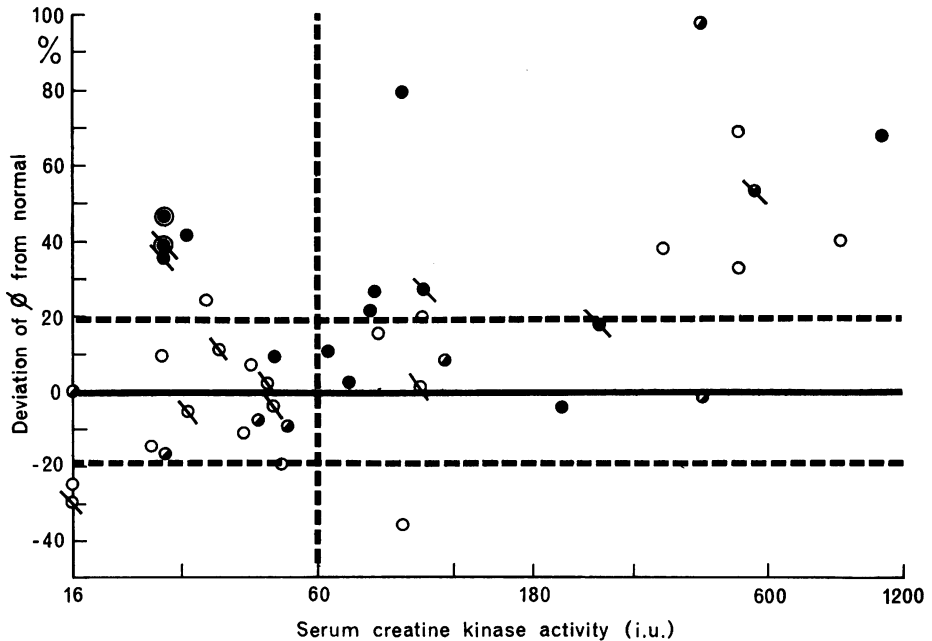
FIG. 10. $\ \overline{\otimes} \left( = \dfrac{mean\ P}{mean\ D} \right)$, *expressed as the deviation % from the normal for the subject's age, plotted against the serum C.K. (i.u.). For* $\overline{\otimes}$ *the mean* ± *2SD is indicated and for CK the upper limit of normal (60 i.u.).* ● = *Definite carrier (Duchenne gene).* ⊙ = *Definite carrier (Becker gene).* ◑ = *Probable carrier.* ○ = *Possible carrier.* ● $\overline{\otimes}$ = *less than 30 potentials measured. Note Log scale.*

pathic action potentials are their short duration and their increased number of phases, he attempted to measure the mean number of phases (or cycles) per second in 11 control subjects and four assumed and 11 possible carriers. He used only potentials of over three phases and divided the number of their phases by their duration to derive a value Ø or phases/second. This value varied from 191-284 in the controls and from 352-392 in the assumed carriers; thus there was no overlap between the two groups. His work has since been criticized mainly on the grounds that his instrumentation was not ideal, that overlapping of several potentials might have occurred, and that bias might have influenced the choice of potentials (Davey and Woolf, 1965; Willison, 1965). Davey and Woolf (1964) were unable to confirm these results

## TABLE III
### SUMMARY OF DETECTION RATES IN THE DEFINITE CARRIERS

| Definite Carriers | Serum Creatine kinase (i.u.) | Subjective E.M.G. Assessment | Abnormal Value Obtained For | | | |
|---|---|---|---|---|---|---|
| | | | Mean A.P. Duration | Mean % Polyphasic Potentials | Mean Number of Phases per Potential | Ø |
| Ma.W | 47 | — | — | — | — | — |
| M.M. | 62 | — | — | — | — | — |
| R.L. | 68 | — | — | — | — | — |
| B.S. | 246 | ? | — | — | — | — |
| My.W. | 202 | ? | — | — | — | — |
| N.M. | 90 | ? | + | + | + | + |
| J.T. | 78 | + | + | — | — | + |
| M.J. | 1,048 | + | — | + | + | + |
| O.Pi. | 77 | ? | — | — | — | + |
| E.P. | 100 | + | — | — | — | + |
| J.R.[1] | 27 | ? | + | — | — | + |
| K.L. | 30 | — | + | — | — | + |
| W.W. | 27 | ? | — | + | + | + |
| G.S.[1] | 27 | ? | — | — | + | + |
| Detection ate | 64% | (21%) | 29% | 21% | 29% | 64% |
| | | Quantitative E.M.G. criteria combined = 64% | | | | |
| | | Overall = 93% | | | | |

[1]Indicates the two carriers of the Becker gene.

## TABLE IV
### SUMMARY OF THE DETECTION RATES IN THE PRESUMED CARRIERS, IN THE FOUR OTHER PROBABLE CARRIERS AND IN TWO OF THE POSSIBLE CARRIERS

| Presumed Carriers | Serum Creatine kinase (i.u.) | Subjective E.M.G. Assessment | Abnormal Value Obtained For | | | |
|---|---|---|---|---|---|---|
| | | | Mean A.P. Duration | Mean % Polyphasic Potentials | Mean Number of Phases per Potential | Ø |
| O.P.[1] | 416 | + | + | + | + | + |
| K.S.[1] | 549 | + | + | + | — | + |
| M.J.[1] | 111 | — | — | — | — | — |
| A.T.[1] | 420 | — | — | — | — | — |
| C.J. | 496 | + | + | + | + | + |
| D.P. | 80 | — | — | — | — | — |
| D.C. | 98 | — | — | — | — | — |
| F.E. | 212 | ? | — | + | + | + |
| M.K. | 849 | + | + | — | — | + |
| P.H. | 100 | — · | — | — | + | + |
| E.S. | 503 | + | — | + | + | + |
| L.T. | 341 | ? · | — | + | + | + |
| Detection rate | 100% (by definition) | (42%) | 33% | 50% | 50% | (66%) |
| | | Quantitative E.M.G. criteria combined = 66% | | | | |
| *Other probable and possible carriers with normal serum CK activity* | | | | | | |
| C.K.[1] | 17 | — | — | — | — | — |
| E.A.[1] | 27 | — | — | — | — | — |
| Ma.J.[1] | 50 | — | — | — | — | — |
| E.F.[1] | 43 | — | — | — | — | — |
| R.M. | 33 | — | + | — | — | + |
| M.D. | 40 | — | — | + | — | — |

[1]Indicates women with more than one affected son.

using the biceps muscle. In a second paper (Davey and Woolf, 1965) they reported results in nine carriers and 33 controls showing that the carriers had small but not statistically significant increases in the values of Ø and that they tended to have more polyphasic potentials. In none of these papers did the authors take account of the effect of the subjects' ages on the duration or Ø value of the potentials.

Barwick (1963) made a 'blind' subjective assessment of the E.M.G. and frequency analysis patterns of control and carrier subjects which revealed a definite difference between the two groups; but the method was not sufficiently reliable to be used for identifying individual carriers. Caruso and Buchthal (1965) studied a number of relatives of cases of muscular dystrophy using measurements of action potentials and of the absolute refractory period of muscle. Among these were one definite carrier and one or possibly two probable carriers of the Duchenne gene (Caruso and Buchthal, 1966, personal communication). Two of these three individuals had normal values for action potential measurements (the third had no E.M.G.) but all three had significantly abnormal refractory periods. Thirteen possible carriers were also studied; two showed slightly increased polyphasia, one had an abnormally short A.P. duration, and five had significantly abnormal refractory periods.

Hausmanova-Petrusewicz, Prot, Niebroj-Dobosz, Emeryk, Wasowicz, Slucka, Hetnarska, Bandarzewska, and Pucek (1965, 1966) studied 66 families of boys with the Duchenne type of muscular dystrophy using a number of tests including quantitative electromyography. There are no details about normal control subjects except that they were of the same age. However, the known carriers (definite and probable) as a group had abnormally low mean potential durations and increased numbers of polyphasic potentials. Three of 12 known carriers had mean A.P. durations below the normal range (not specified) and eight out of 12 had more than 5% of polyphasic potentials. A number of the possible carriers tested also showed significant E.M.G. abnormalities.

Emery, Teasdall, and Coomes (1966) studied 22 definite or probable carriers and 12 controls. They found no difference in the mean A.P. duration or amplitude between the two groups but age was apparently not taken into account. The mean number of polyphasic potentials was 3·43% in the controls and 5·16% in the carriers but the difference was not significant.

Jacobs (1967) in a pilot study in Newcastle studied six definite, two probable, and nine possible carriers and compared them with 12 age-matched controls. Three of the carriers (two definite and one probable) had significantly short mean A.P. durations and two (one definite, one probable) had an increased proportion of polyphasic potentials.

Smith, Amick, and Johnson (1966) in a semi-quantitative study found that many carriers had patchy E.M.G. abnormalities which correlated well with serum enzyme and biopsy results. They emphasized the frequency with which fibrillation potentials were found in both carriers and cases of muscular dystrophy.

In summary, most of the authors who have tried quantitative electromyography as a method of carrier detection have found it to be of limited value. Significant differences between groups of carriers and controls have been found in several studies but individual carriers could rarely be identified. Exceptions were the early study of van den Bosch (1963), the work of Caruso and Buchthal on muscle refractory period, and the measurements of A.P. duration and polyphasia by Caruso and Buchthal (1965), Hausmanova-Petrusewicz et al. (1966), and Jacobs (1967).

The methods of analysis of the E.M.G. interference pattern used in the present study have both proved too insensitive to be of value in carrier detection. Integration electromyography as a method of studying muscle disease was discussed by Lenman (1959a, b). He showed that in primary muscle diseases the voltage-tension curve was steeper than normal. However, so many other factors may influence the slope of this curve—particularly muscle training, fatigue, imperfectly isometric contraction, and the use of trick movements or the simultaneous contraction of antagonist muscles—that the normal range is inevitably wide even if efforts are made to eliminate these factors. Furthermore, the electrophysiological changes which occur in myopathic muscle tend to influence the mean voltage of the E.M.G. in opposing ways; the early recruitment of many mechanically inefficient units increases it, while the reduction in their amplitude and duration tends to decrease it. It is, therefore, hardly surprising that integration electromyography is an insensitive index of mild myopathic changes.

Frequency analysis has been equally unsuccessful in the present study despite the promising results obtained by Barwick (1963). This too is an insensitive method, chiefly because it reflects the frequency components of the interference pattern only indirectly by their effect upon the tuned circuits of the analyser. Devices which make direct measurements of certain features of the interference pattern, such as the spike counting equipment developed by Willison (1963, 1964, 1965, 1966) and Fitch and Willison (1965) seem intrinsically more likely to be of value for this purpose.

The apparent success of the action potential measurements as aids to carrier detection requires special justification. The results obtained by this method may be influenced by many variables (Buchthal, Guld, and Rosenfalck, 1954; Buchthal, Pinelli, and Rosenfalck, 1954) and as far as possible the techniques used here have been standardized to avoid these. The most serious influence on all such investigations is observer bias (Barwick and Gardner-Medwin, 1967). This is extremely difficult to eliminate; even the 'blind' assessment of mixed groups of normal and abnormal films labelled only with random numbers (the method used in this study) allows the observer to be biased by any 'obviously abnormal' potentials into erring on the short side in his measurements of A.P. duration. Bias may also have an important influence at the earlier stage of the recording of the potentials. There is a great temptation to linger and take extra photographs where units look abnormal or unusual. In such areas the needle may be moved too little between recording sites so that the same unit may be recorded twice. The early recruitment of many units in 'myopathic' areas of muscle tends to lead to the simultaneous recording of several units, while in more normal parts of the muscle only one or two units may be recruited and photographed. All these sources of bias, and perhaps others which have escaped notice, have undoubtedly influenced the present results in some degree despite strenuous efforts to avoid them. It is difficult to imagine however that they can account for more than a small part of the differences between the carrier and control subjects.

Measurements of mean action potential amplitude are never accurate because the amplitude recorded is very dependent upon the distance of the motor unit from the needle tip and upon other factors. This explains the wide normal range and the fact that no 'abnormal' values were obtained.

The duration of an action potential is the most difficult of its dimensions to measure. It requires experience to obtain consistent results and, since these can never be checked against the true duration of a potential because this can never be known, the experience amounts in effect to entrenched personal opinion about the correct method of measurement. It is chiefly for this reason that one observer's results cannot be compared with another's unless they have worked together long enough to share their opinions. Equally, it is upon this measurement that unconscious bias has the greatest effect. The only remedy for these difficulties is blind assessment of the films. The durations of individual action potentials vary more widely in carriers than in normal subjects. This means that rather more than the usual 20 potentials ought to be counted in order to eliminate random variation. This was not recognized at first and in six known carriers in the present series less than 30 potentials were recorded. These are indicated in Figs. 7 and 10; in only one case were less than 25 potentials counted.

The values obtained for mean A.P. duration in myopathic subjects may be increased if double potentials are included, as they must be since they seem to represent the activity of a single motor unit. This has had an important effect on some of the results reported here, especially in the cases of the Duchenne type of muscular dystrophy. One boy had a mean duration 8 % above normal despite severe and obvious myopathic E.M.G. changes; among his 30 measured potentials were eight double potentials without which the mean duration would have been 16 % below normal. Double potentials affected the mean duration to a lesser extent in several carriers, but in all such cases the phase counts were abnormal. It is particularly important to avoid confusing superimposed potentials with true double potentials; in this instance, each doublet had usually to be identified at least five times before it was accepted.

The polyphasic potential is an arbitrary concept which is useful in routine electromyography. For accurate assessment of myopathic change the mean number of phases per potential is probably a more reliable index. Certainly in the normal subjects reported here its normal range was more discrete (coefficient of variation 7·2 % as opposed to 52·5 % for the proportion of polyphasic potentials). However, by each criterion 10 carriers lay outside the normal range, eight were abnormal on both counts. The concept of van den Bosch (1963) of an increase in the number of phases/sec in myopathic action potentials provides a useful device for increasing the sensitivity of these tests. Several carriers had values for mean A.P. duration (mean D) or mean number of phases (mean P) just within the limit of the normal range. Calculation of the ratio mean P/mean D for the normal subjects gave a normal range which was exceeded by all but one of the carriers with any other single E.M.G. abnormality and by two other definite carriers whose E.M.G.s were otherwise not quite significantly abnormal. This index may be càlled Ø, although it has been calculated from all the potentials measured, while in van den Bosch's study only potentials with more than three phases were used. The present results support the claim of van den Bosch that Ø is a valuable index of electromyographic abnormalities in the carrier state.

This investigation has been limited to a study of one muscle, the biceps brachii. It is possible that examination of several muscles would improve the

detection rate. Subjective assessment of the E.M.G. in four selected muscles (brachioradialis, biceps, quadriceps, and tibialis anterior) in a few definite and possible carriers suggests that, although 'myopathic' change is often patchy within a muscle, there is not much to choose between brachioradialis, biceps and quadriceps and that tibialis anterior may be less abnormal. Ideally, such a subjective search should be a preliminary to quantitative studies in the most abnormal muscle. This would however make it very difficult to obtain comparable normal control records.

A high proportion of definite carriers of the Duchenne gene seem to be detectable by controlled quantitative electromyography; although the numbers reported here are small it is likely that this method could increase the present detection rate of about 70% (by serum creatine kinase) by at least 10 or 15%. This would have great practical importance in genetic counselling. However the method is time consuming and the importance of obtaining control records and of using 'blind' assessment may prevent it from being used routinely except in special centres. If automatic methods of E.M.G. analysis could be refined sufficiently to detect abnormalities of the degree indicated here they would make a very useful contribution to carrier detection programmes.

## SUMMARY

In a controlled trial integration electromyography and automatic frequency analysis have proved insufficiently sensitive to detect myopathic changes in carriers of the gene for the Duchenne type of muscular dystrophy. The mean action potential duration, the number of phases per potential, or the proportion of polyphasic potentials were abnormal in many carriers, and 17 of 26 known carriers have been identified by a combination of these methods. Several of these had normal serum creatine kinase levels. The importance of adequate control measurements and of 'blind' assessment of the records is stressed.

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