DIAGNOSIS OF SUSCEPTIBILITY TO MALIGNANT HYPERTERMIA IN MAN

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The reported incidence of clinical malignant hyperthermia (MH) varies, but is altogether fairly low [14, 30, 79, 94, 108]. The need for elective diagnosis of susceptibility to MH, however, is by no means a rare phenomenon. This is first of all because susceptibility to MH is inherited [24], but also because anaesthetists, with the increasing awareness of MH, tend to terminate an anaesthetic if early signs of possible MH occur [31, 49, 127]. This increases the number of patients whose MH status cannot be determined from clinical data alone, but must be established from a diagnostic test.

Many diagnostic procedures have been described over the years, but only few have stood the test of time. Since we do not yet know if clinical MH is caused by one single biochemical defect or by various combinations of several defects, a definitive test for MH susceptibility has not been developed. Moreover, we do not know if the biochemical defects causing MH are the same in man and the pig. Since pigs often are inbred for susceptibility to MH, it seems unwise to extrapolate results obtained in pigs for uncontrolled application in man, therefore I will concentrate on investigations in humans in the following, although we have learnt much about MH from studies of pigs. In relation to diagnostic tests for susceptibility to MH, this creates problems. It is easy to apply a test for MH in pigs and subsequently challenge the animals with halothane and suxamethonium and get a definite answer. In humans, all evidence of accuracy and specificity is indirect, but should nevertheless be obtained. Thus any diagnostic test, before being introduced for general use, should be applied in patients with a previous history of unequivocal, fulminant MH as well as in normal control subjects. If complete separation of these two diagnostic groups is obtained with the test considered, it can be relied on for use in other groups of patients. This simple rule, unfortunately, has been followed for only a few of the described tests.

TESTS ON BLOOD

Serum tests

Creatine kinase. When MH was first recognized as a skeletal muscle disease, determination of creatine kinase activity (CK) was introduced as a diagnostic tool for MH susceptibility [23, 68, 69]. There is no doubt that CK is increased in many individuals susceptible to MH, but in others it is normal [56, 116]. Since increased CK activity is a non-specific sign of muscle injury, it may be increased as a result of factors other than MH. Many studies now show that, although MH susceptible individuals as a group have higher CK values than non-susceptible individuals, determination of CK is unreliable as a predictor of MH susceptibility in the individual patient [3, 11, 28, 96, 113, 116, 134].

Cholinesterase. For some years it was considered a possibility that inheritance of the fluoride resistant gene for plasma cholinesterase and susceptibility to MH were coupled. This was originally based on findings in a few families by Whittaker, Spencer and Searle [146], whose observations were confirmed by Ellis and colleagues [27]. While Whittaker later confirmed her original findings in a larger series of patients [145], Evans and co-workers from the Leeds group were unable to repeat their results, when performing a prospective study on a larger patient population [44]. Likewise, Ørbing, Hanel and Viby-Mogensen [111] did not find any increased frequency of the fluoride resistant gene in MH patients. There may be two explanations for these
discrepancies: The patient populations could be different, since susceptibility to MH was confirmed with in vitro contracture tests in two of the studies [44,111], but not in the third [145]. In the latter study, analysis of cholinesterase was performed at the request of anaesthetists from different parts of Britain, and clinical details were not given. Another explanation could be the difficulty in correctly classifying cholinesterase genotypes containing the fluoride resistant gene [61]. In Denmark, cholinesterase genotype has now been determined in 249 patients suspected of susceptibility to malignant hyperthermia, and only two have been found with the fluoride resistant gene. This gene frequency is not statistically different from that found in the general Danish population [61]. Thus the evidence is not convincing for any correlation between cholinesterase abnormalities and MH.

Erythrocyte tests

Osmotic fragility. Increased osmotic erythrocyte fragility has been observed in some human subjects with MH [75], but decreased fragility has also been found [151], invalidating this test for diagnosis of susceptibility.

Chemiluminescence. The method of chemiluminescence for quantitating auto-oxidation in red blood cells has been described to give different results in MH susceptible and non-susceptible pigs [77]. Recently, Jones and Bready [72] were able to distinguish between diagnostic groups of pigs with known MH genotype using the ratio of chemiluminescence in erythrocytes before and after exposure to halothane. So far, no results with this test have been obtained in humans, although a defective protection against oxidant action has been observed in some survivors of MH [138].

Platelet tests

Platelet aggregation. Since platelets resemble skeletal muscle in that they contain calcium storage vesicles, contractile elements and a calcium dependent ATPase, platelets have been investigated in the search for a non-invasive test for MH susceptibility. One test based on platelet aggregation was suggested by Zsigmond, Penner and Kothary [151], but was not found useful for diagnosis of MH susceptibility by others: Rosenberg and co-workers studied platelet aggregation in five subjects with either a personal or family history of MH. All were found to be MH susceptible based on in vitro contracture tests with halothane and caffeine, and all but one exhibited normal platelet aggregation as compared with 23 normal control subjects [126]. Similar results were obtained by Sullivan, Ardlie and Denborough [136]. Gerrard and colleagues studied platelet aggregation in response to halothane in seven patients suspected of malignant hyperthermia and 35 normal controls [53]. They found that halothane (in concentrations far greater than those attained in blood during clinical anaesthesia) stimulated platelet aggregation in both MH patients and normal controls. Although the results of this study may be questioned because muscle biopsies were not performed in these patients and clinical data on the MH episodes were lacking, platelet aggregation tests do not seem useful for diagnostic purposes.

Platelet nucleotide depletion. This test was developed by Solomons and Masson [133]. The idea of the test involves two assumptions: that platelets are affected in MH, and that halothane decreases nucleotide content more in affected than in normal platelets, as a result of increased metabolism and ATP turnover in the MH subjects. The authors obtained very good diagnostic separation between patients with previous clinical MH and normal control subjects with this method. Later, this test has been evaluated in three independent studies [16,54,84], all of which concluded that this test is not useful for diagnosis of MH susceptibility, since halothane reduces nucleotide content of platelets equally in MH and control subjects. Although clinical and laboratory criteria for MH susceptibility varied in these studies, enough details were presented in all of them to make the conclusions valid. It seems unlikely that possible minor differences in technique should account for this lack of sensitivity of the platelet test, as has been claimed [132]. Should it be the case, however, such a test could not be recommended for large scale use, since the risk of wrong diagnosis because of technical difficulties would be unacceptably high. Hence the platelet test should not be used as a single diagnostic tool. It is not yet settled if platelets are indeed involved in human MH.

White cell tests

Human leucocyte antigen (HLA) type. Lutsky, Witkowski and Henschel [88] investigated the possible association of HLA type and MH
susceptibility in one large family with several deaths attributed to MH. They did not find any association between MH susceptibility and HLA type in this Wisconsin family. On the other hand, Kikuchi and co-workers [78], in a preliminary report described that three out of four MH patients had an identical DR gene, which is not common in Japan. These patients were all MH probands and apparently not related. This observed association still awaits further investigation.

Calcium concentration in lymphocytes. By measuring the fluorescence of the calcium ion indicator quin 2, which was loaded into lymphocytes, Klip and colleagues [80] found that exposure of the lymphocytes to halothane increased the ionized calcium concentration significantly in cells from MHS patients, but not in cells from controls. The values after exposure to halothane were significantly different in the two groups, and there was only little overlap between the groups. This promising, fairly non-invasive test was also evaluated in pigs, where similar results were obtained [81]. In both studies, the concentration of halothane used (38 mmol litre\(^{-1}\)) was much greater than clinical concentrations. It was stated that halothane 1 mmol litre\(^{-1}\) would also elicit an increase in calcium concentration, but results were not given. It will be very interesting to see if similar results can be obtained with this method in other MH centres, since a non-invasive test for MH is obviously much needed.

ELECTROPHYSIOLOGICAL TESTS

Motor unit counting

In 1977 Britt and colleagues described a test based on counting the number of functioning motor units in different muscles [15]. MH patients were found to have a reduced motor unit count compared with control subjects. The sensitivity of this test was found to be high in patients with a previous episode of rigid MH and in their relatives, but low in patients with previous non-rigid MH (but only three were investigated). It was noted, however, that the test was non-specific, and that abnormally low motor unit counts were seen in other myopathies and in denervating conditions as well. Conventional electromyography was also evaluated and found much less sensitive for the diagnosis of MH susceptibility than motor unit counting. Apparently, motor unit counting is not in use any more for diagnosis of MH susceptibility, and has not been further evaluated for this purpose.

Tourniquet test

Stimulation of a motor nerve and recording of the elicited mechanical twitch response has become routine for clinical monitoring of neuromuscular blockade during anaesthesia [142]. The same method has been applied for diagnosis of MH susceptibility by Roberts, Ali and Ryan [123], who recorded twitch height before, during and after ischaemia of the arm. MH patients were found to have an increased twitch height after ischaemia, whereas twitch height was unchanged or lower in normal controls. This “tourniquet test” has subsequently been evaluated by Britt and co-workers [18] in a large series of patients who also had in vitro contracture tests performed. Britt’s group found it impossible to discriminate between susceptible and non-susceptible patients using the tourniquet test. They observed a tendency for young people to have increased and for old people to have decreased post-ischaemia twitch height. Since many of the MH patients in the study of Roberts, Ali and Ryan [123] had been children and the controls mostly adults, Britt and colleagues ascribed the results of the original study to a difference in age between the MH patients and the control group.

Relaxation rates of the elicited twitch response

Recently, Lennmarken, Rutberg and Henriksson [86] reported that relaxation rates of the elicited twitch response in the adductor pollicis muscle were significantly higher in subjects susceptible to MH than in normal controls. Because there was overlap in results between the groups, however, this interesting observation cannot be used for diagnosis of susceptibility without further standardization of the technique.

Recruitment pattern after halothane and suxamethonium

A fourth electrodiagnostic test was developed by Eng, Becker and Muldoon [41]. They investigated the EMG recruitment pattern of the hand muscles during maximal contraction before and after local instillation of halothane and suxamethonium. A significant decrease in the number of negative peaks of motor unit potentials was observed after instillation of suxamethonium in both MH subjects and normal controls.
However, the number of these potentials was significantly less in the MH susceptible individuals than in the controls. In nine subjects in vitro contracture tests were also performed, and in eight the results of in vivo and in vitro tests showed good correlation. Following these promising results, a larger, prospective study was planned, but has not yet been published; neither has this in vivo test been evaluated by other groups.

**BIOCHEMICAL MUSCLE TESTS**

*ATP depletion*

ATP depletion in muscle upon exposure to halothane was first described in 1969 by Harrison and co-workers [64], who could predict susceptibility to MH in pigs with this test. The ratio of ATP content in muscle before and after equilibration with halothane was determined and found decreased in pigs susceptible to MH. The test has been evaluated in humans by Britt and others [10]. They found the ratio to decrease with increasing age and therefore introduced an age correcting factor. Because of overlap between groups, they found it a less accurate method for diagnosis of susceptibility to MH than the caffeine contracture test. Sporn [134], using the halothane contracture test for diagnosis of MH susceptibility, also found the ATP ratios to overlap between MH susceptible and non-susceptible patients. In a study of normal human muscle, Gronert found that the ATP ratio was not decreased after exposure to halothane [57]. In contrast to Britt and others [10], Gronert [57] found no variation in ATP ratio with age. The reason for this discrepancy has not been elucidated, but is not very important, because of the lack of accuracy and specificity of the ATP depletion test.

*Glycolytic metabolites*

Indications of increased glycolytic activity in unstressed MH susceptible humans have been presented [29,70], but were not confirmed in a more recent study, using an electrophoretic micromethod (isotachophoresis) on freeze clamped muscle tissue [141]. In another study, metabolites were determined before and during exercise [129]. MH susceptible subjects were found to have completely normal muscle metabolism. Hence the general metabolic pattern with or without stress cannot be used diagnostically.

**Myophosphorylase ratio**

The activated form of phosphorylase stimulates glycolgenolysis in muscle and, ultimately, lactate formation, which is a prominent feature of clinical MH. Ono and colleagues therefore studied the content of phosphorylase a (the active form) in relation to the total content of this enzyme in pigs and found that this ratio was increased in stress susceptible pigs [107]. An even more increased ratio was found in humans susceptible to MH, in a study by Willner and co-workers [148]. They could predict susceptibility to MH from this test because there was no overlap in phosphorylase ratio between the MH susceptible and the control group. Susceptibility was diagnosed with either a single fibre caffeine test or a muscle bundle caffeine or halothane–caffeine test (vide infra). Ellis and colleagues [33] also found an increased phosphorylase ratio in MH susceptible patients (with overlap between groups), whereas Trayner, Van Dyke and Gronert were unable to confirm this finding [139]. It is not clear whether these observed differences are attributable to variations in the applied methodology (for example anaesthesia) or to the patient populations studied. However, increased sympathetic activity will activate phosphorylase and thereby influence the results. Since the stress factor is uncontrollable, phosphorylase ratio is probably not a very sensitive diagnostic test.

**Adenylate kinase deficiency**

In 1974 Schmitt, Schmidt and Ritter [130] reported a deficiency of adenylate kinase in the mother and a sister of two children, who died from MH. Since adenylate kinase plays an important role in maintaining adequate concentrations of ATP in tissues, the authors suggested that development of MH was the result of decreasing concentrations of ATP, when individuals with hereditary adenylate kinase deficiency were exposed to halothane. This theory was later rejected by Cerri and co-workers [21] and Marjanen and Denborough [91], who found normal adenylate kinase activity and isoenzyme pattern in humans susceptible to MH.

**Adenylate cyclase activity and cyclic AMP**

Adenylate cyclase is located in transverse tubuli and sarclemma of skeletal muscle and indirectly mediates calcium transport in sarcoplasmic reticulum. Willner, Cerri and Wood [147] found
increased activity of this enzyme in patients susceptible to MH as determined by the skinned single fibre caffeine test; also, cyclic AMP was increased in muscle from these patients. In earlier studies in pigs, cyclic AMP concentrations had been found to be increased immediately after slaughter [107], whereas adenylate cyclase concentrations had been similar in MH susceptible and control pigs [106]. Increased concentrations of cyclic AMP has also been reported in blood of MH susceptible patients after physical exercise [135]. None of these parameters, however, seem useful for diagnosis of MH susceptibility.

Adenylate deaminase deficiency

In individuals susceptible to MH, myoadenylate deaminase was either absent or less than one-third of the normal value more frequently than was found in non-susceptible persons [45]. However, the authors stated that the predictive value of this finding was too low for its use as a diagnostic test for MH.

Low weight proteins

Blanck and colleagues in 1984, suggested that two abnormal low weight proteins found in MH susceptible patients could be of assistance in diagnosing MH susceptibility [8]. Since then, these proteins have been found to be present in varying amounts in both normal and MH susceptible muscle [47,143]. It has been suggested that the observed proteins are subunits of contaminating haemoglobin [47], a theory which has later been accepted by Blanck [7]. The conclusion, that electrophoretic separation of muscle proteins, at present, is not suitable for diagnosis of MH susceptibility, has been supported also by studies of Marjanen and Denborough [92] and Whistler, Isaacs and Badenhorst [144].

Calcium uptake by sarcoplasmic reticulum

Sarcoplasmic reticulum has been intensely studied in the search for the basic lesion of MH [37]. In one centre, determination of calcium uptake by the sarcoplasmic reticulum is used for the diagnosis of MH susceptibility [1,2,89,90]. MH susceptible muscle is found to have lower calcium uptake than normal muscle. In another study, 100% of children investigated with this method because of masseter muscle rigidity were found to be MH susceptible [131]. This is a much higher proportion than found in other centres, where approximately 50% of patients with masseter muscle rigidity are found to be susceptible [31,49,113,127]. Also, the incidence of masseter spasm and hence MH susceptibility found in this study was several-fold higher than in other reports [14,30,79,94,108]. The validity of this test has therefore been questioned [32,58,140]. With different methods, calcium uptake has been found to be similar in susceptible and non-susceptible patients in one study [9], and lower in susceptible patients in another study [22]. Only recently has the “Boston test” [1,2,89,90] been evaluated with identical technique in an independent laboratory [98]. In this latter study it was found that neither results of the standard contracture tests (halothane test and caffeine test) nor clinical episodes of MH correlated with the results of the calcium uptake test [98]. Therefore this test can not be recommended as a single diagnostic tool.

Intracellular ionized calcium concentration

In a recent publication, intracellular ionized calcium concentrations were described to be significantly higher in muscle biopsies from subjects susceptible to MH than in control biopsies [87]. The measurements were made with a calcium sensitive microelectrode on biopsies taken between 15 days and 4 months after a clinical episode of MH. Although these observations are very interesting, it cannot be excluded that the results were influenced by the circumstances of excision of the biopsies, for example the degree of stress, since the four MH patients had intercostal biopsies taken during local anaesthesia, whereas the three controls had general anaesthesia for thoracotomy [87]. The results are also in contrast to those obtained in lymphocytes by Klip and co-workers [80,81], who found increased calcium only after exposure of the MH lymphocytes to halothane. However, since muscle is the primary tissue involved in MH, resting intracellular calcium concentrations could well be increased exclusively in MH muscle. Only when more results with the calcium microelectrode have been reported, and the results confirmed in other centres, can the method be further evaluated for diagnostic purposes.

Heat production

Heat production upon exposure to halothane was measured in muscle tissue with a microcalorimetric method [120]. It was found that heat production increased in both normal and
MH muscle, and there was no difference between the groups. At present therefore, this interesting method is not suitable for diagnosing MH susceptibility.

Nuclear magnetic resonance scanning

NMR scanning with phosphorus-31 has been used to demonstrate concentrations of high energy phosphate compounds and pH in vivo during exercise in subjects susceptible to MH [82,93]. The preliminary results seem to indicate that concentrations of metabolites are fairly similar for susceptible and non-susceptible persons, but differences in time course of pH changes could be of informative value. Further studies utilizing this technique will probably clarify this point.

MUSCLE HISTOLOGY

Abnormalities of muscle histology are unspecific. They include internal nuclei, moth-eaten fibres and target or core-targetoid fibres, variations in fibre size, and signs of necrosis and regeneration [62,67,119,121,122,134]. Sometimes signs of denervation are found [67,121]. Changes are rarely seen in children, and the incidence of positive findings increases with increasing age of the patients [34]. Thus approximately 25–50% of MH susceptible individuals can be expected to have one or more abnormalities [63,119,134]. It follows, that histology alone is insufficient for establishing MH susceptibility. However, it is still of major importance to perform muscle histology and histochemistry in order to rule out the presence of other myopathies.

CONTRACTURE TESTS

The most reliable method for diagnosis of susceptibility to MH is at present the in vitro contracture tests with halothane and (separately) caffeine. Similar results with these tests are obtained in many different laboratories all over the world. The basic principle of these tests is as follows: A piece of skeletal muscle, 10–20 mm long and 2–3 mm in diameter is suspended, immediately after excision, in a tissue bath with physiological Krebs solution at 37 °C and bubbled with 5% carbon dioxide in oxygen. Viability is secured by recording the twitches elicited by supramaximal electrical stimulation. Initial length should be adjusted according to the evoked twitches and should be close to optimal length [85]. This is often obtained with a preload of 1–2 g in a specimen with the above dimensions. Tension is measured by a transducer and recorded before and after addition of a test drug. Mostly, a full dose–response curve with the test drug is obtained and the slope of this curve analysed. Because of variations in the ways these tests are performed and interpreted, it has been difficult to compare results of the tests between different MH units.

In Europe, most centres performing these tests have agreed upon a common, standardized procedure describing in great detail how the tests should be performed [42,43]. Each centre is encouraged also to obtain normal control biopsies following the same procedure. A halothane test and a caffeine test (vide infra) are included, and the parameter measured is the threshold drug concentration—that is, the lowest concentration of drug eliciting a sustained increase in basal tension of at least 0.2 g. Three diagnostic groups are recognized: MHS (susceptible) with abnormal results of both halothane and caffeine tests, MHN (non-susceptible) with normal results of both tests, and MHE (equivocal) with differing results (fig. 1). The MHE group has been created in order to minimize the risk of either false positive or false negative diagnosis. For the MHE patient, family studies will usually elucidate the true MH status.

The caffeine contracture test

The use of caffeine for diagnosis of MH susceptibility was introduced by Kalow and...
SUSCEPTIBILITY TO MH

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Concentration of caffeine (mmol litre^{-1})

FIG. 2. Response to cumulative doses of caffeine in diagnostic muscle biopsies from 46 MH susceptible (——) and 54 non-susceptible patients (----). Mean±1 SEM. Statistically significant differences between groups for concentrations 0.5—2 mmol litre^{-1} (P < 0.05) (Redrawn from Ørding, Ranklev and Fletcher [113], with permission.)

colleagues in 1970 [74], and is now generally accepted as a reliable indicator of susceptibility, although problems with this test do exist in some MH units. The caffeine dose–response curve is shifted to the left in MH susceptible individuals compared with non-susceptible individuals (fig. 2). Originally, the caffeine test was performed at room temperature [73,74], but now all MH units perform the test at 37 °C.

Criteria for susceptibility to MH vary between units, although generally it is the lower part of the dose–response curve which is used. As mentioned before, the European MH Group looks for the threshold concentration of caffeine eliciting a sustained increase in tension of at least 0.2 g [42,43]. From analysis of results in normal control biopsies (fig. 1), a normal result of the caffeine test was found to be a caffeine threshold concentration greater than 2 mmol litre^{-1}. In other units, the concentration of caffeine producing a contracture of 1 g is found by interpolation and is called the caffeine specific concentration [10,12,19,98,105]. The normal values for this parameter vary markedly between units (table I). Other “dividing points” are also in use [6,128,139,144]. In one centre, fraction of peak tension is calculated and the entire dose–response curve analysed [57].

Good control data with the caffeine test performed at 37 °C are scarce. Such biopsies should be excised under the same type of anaesthesia as the diagnostic biopsies and should be from the same muscle group. Only one test should be made on each specimen. Some published control data are shown in table I. Differing results are observed with the caffeine dose–response curve at crucial points: in some centres caffeine 2 mmol litre^{-1} never [19] or rarely [109,128] induces contractures in normal muscle, whereas small contractures after caffeine 2 mmol litre^{-1} are regularly seen by others [57,97]. This underlines the necessity of good control data obtained at the individual MH units.

Halothane contracture test

Halothane was introduced as a test drug by Ellis and colleagues in 1971 [36]. Normally, halothane alone produces significant contractures only in MH susceptible muscle, as shown in figure 3. However, in some normal specimens high concentrations of halothane (3–4 %) will elicit slight contractures (fig. 3). Halothane has been found reliable for establishment of MH susceptibility in many MH units and is widely used for this purpose. In a few centres, halothane contractures are not seen regularly and halothane accordingly is not used for testing by itself [12,19].

Another difficulty with the halothane test is that the concentration of halothane in the tissue bath may vary substantially. It is not surprising that halothane, being a volatile anaesthetic with low

2.0
1.5
1.0
0.5
-0.5
0
1.5
3.0
4.0

increase in tension (g)

Concentration of halothane (%)

MH-positive

MH-negative

FIG. 3. Response to halothane in diagnostic muscle biopsies from 60 MH susceptible and 64 non-susceptible patients. Mean±1 SEM. Statistically significant differences between groups at all concentrations (P < 0.001). (Redrawn from Ørding, Ranklev and Fletcher [113], with permission.)
TABLE I. Control data for caffeine test at 37 °C. CSC = Caffeine specific contracture (i.e. the concentration of caffeine eliciting a contracture of 1 g)

<table>
<thead>
<tr>
<th>Reference</th>
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<tr>
<td>[12]</td>
<td>Vastus lateralis muscle. Anaesthesia with Innovar, diazepam, fentanyl and nitrous oxide. n = 20. CSC 5.5 ± 0.63 mmol litre⁻¹ (mean ± SEM).</td>
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<tr>
<td>[13]</td>
<td>Muscle and anaesthesia not specified for control patients. n = 10. CSC 7.53 ± 3.48 mmol litre⁻¹ (mean ± SEM). In all controls CSC &lt; 4.1 mmol litre⁻¹.</td>
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<tr>
<td>[19]</td>
<td>Muscle and anaesthesia not specified. n = 29. CSC &gt; 3.5 mmol litre⁻¹ (range 3.5-10 mmol litre⁻¹).</td>
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<tr>
<td>[57]</td>
<td>Quadriceps muscle. Anaesthesia with potent inhalation agent in 30 patients; use of suxamethonium in 21 patients. n = 33. Caffeine threshold concentration 2-4 mmol litre⁻¹, CSC 2-4 mmol litre⁻¹, fraction of peak tension analysed.</td>
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<tr>
<td>[97]</td>
<td>Rectus abdominis. Anaesthesia: Thiopentone, nitrous oxide. Relaxation: pancuronium, tubocurarine, gallamine or suxamethonium. n not stated. Threshold for increase in tension 2-8 mmol litre⁻¹.</td>
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<tr>
<td>[99]</td>
<td>Rectus abdominis muscle. Anaesthesia not specified. n not specified. Response to caffeine 4 mmol litre⁻¹ in 20 specimens 0.54 ± 0.12 g (mean ± SEM).</td>
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<tr>
<td>[105]</td>
<td>Rectus abdominis muscle. Anaesthesia with thiopentone, fentanyl and diazepam. n = 19. CSC 4.85 ± 2.1 mmol litre⁻¹ (mean ± SEM).</td>
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<tr>
<td>[109]</td>
<td>Quadriceps, mostly vastus medialis muscle. Anaesthesia as used for diagnostic biopsies in individual units, mostly thiopentone-fentanyl-nitrous oxide or regional bupivacaine. n = 73. Threshold concentration for sustained increase in tension of 0.2 g &gt; 2 mmol litre⁻¹ in 68 of 73 patients (fig. 1).</td>
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<tr>
<td>[128]</td>
<td>Quadriceps muscle. Anaesthesia not specified. n = 15 (three of whom required diagnostic muscle biopsy for reasons other than MH). Contracture &lt; 0.2 g at 2 mmol litre⁻¹ in 13 of 15 patients, ≤ 0.4 g in all 15. CSC 7.92 ± 4.48 mmol litre⁻¹ (mean ± SD).</td>
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<tr>
<td>[134]</td>
<td>Vastus lateralis muscle. Extradural analgesia, agent not stated. n = 8. Threshold concentration for contracture &gt; 0.25 g: 3.56 ± 1.16 mmol litre⁻¹ (mean ± SD).</td>
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solubility in water, evaporates from the tissue bath. At a fixed vaporizer setting, therefore, the actual concentration of halothane in solution varies, depending on the shape and size of the bath, the flow of Krebs solution, gas flow and other factors. Hence it is necessary to measure halothane concentrations in the bath and adjust the vaporizer setting to obtain the desired concentration in the bath. The European MH Group in their procedure have specified the desired halothane concentrations, a step towards standardization in performance of the test. Instead of
2% halothane in the gas phase, they aim at halothane 0.44 mmol litre\(^{-1}\) in the Krebs solution.

Good control data with the halothane test have been obtained by the European MH Group [109] (quadriiceps muscle, same anaesthetic technique for diagnostic and control biopsies). They found a small halothane contracture in one of 73 control subjects (fig. 1). The one control with halothane contracture reacted normally to caffeine and was not considered susceptible to MH. Gronert did not observe any contractures following 2% halothane in 33 controls (of whom 30 received halothane or enflurane for anaesthesia, and 21 suxamethonium) [57]. Britt and colleagues [12] saw a contracture of 0.1 g in one of 20 controls (quadriiceps muscle, same anaesthetic technique for patients and controls). Moulds and Denborough [97] observed a small contracture in two of 18 control biopsies upon exposure to halothane (concentration not specified). In contrast, Nelson, Austin and Denborough [99] found that muscle from 18 of 57 normal controls developed contractures greater than 0.1 g with halothane in concentrations between 0.4 and 4% (rectus abdominis muscle, anaesthetic technique not specified). Rosenberg and Reed [128] observed contractures exceeding 0.1 g in three of 15 controls following 1% halothane (anaesthetic technique not specified, three of 15 controls having diagnostic biopsies for reasons other than MH). The observed discrepancies between the investigation units are most likely caused by a variety of factors: differences in halothane concentrations in the tissue baths, the excision of biopsies from different muscle groups and sometimes from patients with other neuromuscular disorders, the administration of a wide variety of drugs for anaesthesia. It is therefore mandatory for each laboratory to obtain its own control data.

A dynamic halothane test was described by Ellis and Harriman in 1973 [35]. In this test, the length of the muscle bundle is cyclically changed. The dynamic halothane test is incorporated in the European MH procedure [42], although not all units in the group are performing this test. Halothane contractures are greater in the dynamic test than in the static test [114], presumably as a result of enhanced calcium release following the stretching. Evaluation of the possible advantage of this test has been undertaken in two units, reaching different conclusions. Ranklev, Fletcher and Blomquist [118] found the dynamic test superior to the static test because 20% of their susceptible patients reacted to halothane only in the dynamic test, and would thus have been classified MH equivocal without the use of a dynamic test. Altogether, the diagnostic result (MHS, MHN, MHE) would have changed in 17% of their patients, if the dynamic halothane test had not been used [118]. In contrast, Ørding and Skovgaard [114] found that, by omitting the dynamic test, only three of 105 patients would have their diagnosis changed: two would be classified as MH equivocal instead of susceptible (in which case family studies would be undertaken), and one MH equivocal patient would be diagnosed non-susceptible. Since the single control biopsy with a halothane contracture reacted only in the dynamic test, Ørding and Skovgaard concluded that the dynamic test was not essential. More control biopsies in the individual units of the European MH Group, and further analysis of cases with previous unequivocal clinical episodes will probably clarify these discrepancies.

**Factors with possible influence on contracture tests**

Age of the patient (within a range of 7–83 yr) does not affect the result of the tests [57, 73, 112].

In one study, size of the individual muscle fascicles was found not to influence the magnitude of caffeine elicited contractures [12]. This is surprising, since the size of the contractures was simply measured in grams, as is the custom. The reason could be that the muscle bundles were fairly uniform in size. To circumvent the expected influence of different size of the muscle bundles and thus make results more comparable, the size of the contractures can be expressed as a percentage of either maximum tetanic tension or the contracture elicited by caffeine 32 mmol litre\(^{-1}\) [55, 57]. The latter parameter is more suitable for routine use, since it is obtained at the end of the test. If the specimen tears apart, as sometimes happens, it does not preclude the possibility of making a diagnosis.

In contrast to findings in animals, there is no correlation between muscle fibre type composition and magnitude of contractures or threshold concentrations in humans [45, 112]. This is presumably so because human muscles are of mixed type with a fairly uniform composition of the muscle groups studied, whereas animals have some muscles composed of a single muscle fibre type.

Composition of the Krebs solution, especially
the calcium and magnesium concentrations, influence the magnitude of contractures and therefore have to be carefully controlled. Thus contracture responses to both halothane and caffeine are significantly decreased when the calcium concentration in the bath is decreased \[25,95,101\]. The contractures elicited by both halothane \[48\] and caffeine \[113\] seem to be weaker with increasing magnesium concentrations.

Temperature of the Krebs solution in the tissue bath influences the magnitude of the elicited contractures. Thus halothane elicited contractures in MH susceptible pig muscle at 37 °C, but not at 25 °C \[100\]. In human muscle, the caffeine contractures were reduced, when temperature was decreased from 37 °C to 25 °C \[99\], or 22 °C \[12\]. This does not seem to create problems now, since all centres are performing the contracture tests at 37 °C.

In pig muscle, it has been shown that deterioration of the muscle bundles used for the contracture tests increased sensitivity to halothane \[50,104\]. Therefore, the time from excision of the biopsy to performance of the contracture tests should be as short as possible, and care should be taken not to damage the fibres during excision or preparation.

The caffeine used for the test should be pure caffeine base, since caffeine citrate or benzoate yield different results as a result of a shift in pH of the Krebs solution \[65\].

In one study, the contractures elicited by caffeine 4 mmol litre\(^{-1}\) were significantly larger when this dose was administered as a single bolus dose as compared with several cumulative doses \[115\]. This phenomenon could be the result of fatigue of the muscle tissue \[17\] or of tachyphylaxis. It illustrates the importance of standardized tests, if results are to be compared between different MH units.

Various drugs have been reported to interfere with the halothane and caffeine contracture tests:

Dantrolene has been shown to change the dose–response curve of caffeine in cat \[26\], rat \[60\], pig \[5,17\] and human muscle, decreasing the caffeine contracture response \[17,102\]. Dantrolene also blocks or attenuates the response to halothane in cat \[26\], pig \[4\], and human \[6,103\] muscle. Conflicting results about the influence of in vivo dantrolene pretreatment on the contracture test results have been reported: one patient had dantrolene 100 mg in divided doses over 12 h before operation and still had abnormal results of the halothane and caffeine tests \[124\]. Another patient had dantrolene 100 mg four times a day for 2 days before operation and subsequently had a normal result of the caffeine test, whereas 1 year later without dantrolene pretreatment she had an abnormal result with the caffeine test \[83\]. In a third report, repeat contracture tests were performed with and without prior administration of dantrolene \[117\]. In four of five patients, the original abnormal results of the caffeine tests were changed to normal values after pretreatment with oral dantrolene for several days. The last patient received dantrolene 1 mg kg\(^{-1}\) i.v. immediately before anaesthesia, and continued to have an abnormal result with the caffeine test \[117\]. In susceptible pigs given i.v. dantrolene 3.5 mg kg\(^{-1}\) 15 min before the second biopsy, results of halothane and caffeine tests were not changed \[104\]. Thus, apparently, both dose of dantrolene and length of preoperative treatment influence the dantrolene effect. Accordingly, it seems wise not to administer dantrolene as prophylaxis against MH during anaesthesia for the diagnostic biopsy.

The calcium channel blocker verapamil changes the dose–response curve of caffeine and blocks halothane contractures in cat muscle \[25\], but was found in a study of MH susceptible pig muscle to potentiate some halothane induced contractures \[51\]. The calcium channel blocker diltiazem blocks or attenuates the halothane induced contractures in MH susceptible pig muscle \[66\], and changes the caffeine dose–response curve of human MH susceptible muscle \[71\] and cat muscle \[20\]. Procaine reduces the size of contractures elicited by caffeine in human muscle \[97\], but was reported not to inhibit halothane induced contractures in pig muscle \[103\]. The β-blocker propranolol has been found to attenuate the response to caffeine in susceptible human muscle without interfering with the response to halothane \[110\]. If possible, therefore, drugs should be discontinued before admission for diagnostic muscle biopsy. If this is not possible, the result of the contracture tests must be evaluated taking into account a possibility of drug interactions.

Other contracture tests

Halothane–caffeine contracture test. Halothane’s potentiating effect on caffeine elicited contractures
Susceptibility to MH was first described by Kalow and colleagues in 1970 [74]. It is used in some American centres for diagnostic purposes [13,105], but is found to be less accurate than the halothane or caffeine tests in other units [19,98,115,128,139]. Nelson, Flewellen and Gloyna [105] use the halothane-caffeine test to diagnose a third MH phenotype, the K-type, which they claim react to trigger agents differently from both susceptible and normal individuals. It is not yet settled if this group represents a true biological phenomenon or not. One of the disturbing points is that this phenotype is found in 10-20% of control biopsies [125]. Another is that the K-type is only found in patients with very mild signs suggestive of possible MH, never in patients with unequivocal clinical episodes.

Halothane-suxamethonium contracture test. Fletcher and Rosenberg have described the in vitro effect of halothane in the presence of suxamethonium in patients negative to the standard tests for MH susceptibility (halothane and caffeine contracture tests) [46]. Muscle from patients with a history of masseter muscle rigidity developed halothane contractures, whereas muscle from patients without masseter muscle rigidity did not develop contracture. A new subgroup of patients was thus identified by clinical history and in vitro tests. They suggested that this subgroup of patients could be identical to the K-type patients described by Nelson, Flewellen and Gloyna [105]. Recently, this finding has been confirmed in a preliminary report from Nelson’s group [76]. At present, however, the clinical implications of this finding are not known.

Caffeine-suxamethonium contracture test. Halsall and Ellis in 1979 described the combined use of caffeine and suxamethonium for diagnosis of MH susceptibility [59]. A graded contracture was seen in response to suxamethonium when this drug was added at the height of the caffeine elicited contracture. The authors speculated that this test could be of value in distinguishing between various possible MH phenotypes. However, Ørding and Skovgaard [115] did not obtain contractures with suxamethonium in this test and did not find it of any value for diagnostic purposes. Other groups apparently have not worked with this test.

Potassium chloride test. Moulds and Denborough in 1974 described the potassium chloride test for diagnosis of MH susceptibility [95]. They wanted to study the effect of complete depolarization of the sarcolemma, and obtained this by applying potassium chloride 80 mmol litre−1 to the surrounding medium. MH susceptible muscle was found to develop a marked contracture in response to potassium, whereas normal muscle did not. In order to increase the general diagnostic sensitivity of in vitro tests, the authors suggested the use of a battery of diagnostic tests instead of relying on one single test, and recommended the potassium test for this purpose. In a recent study of human muscle, agreement between the results of the conventional contracture tests (halothane and caffeine tests) and the potassium test was found in 75–80 % of subjects [115].

Suxamethonium test. This test was first introduced by Moulds and Denborough [95], who found MH susceptible muscle to develop contracture in response to suxamethonium 10 mg. In other laboratories suxamethonium induced contractures were not seen in the muscle preparations [46,59,115]. Later, Galloway and Denborough have shown that the responses to suxamethonium was evoked by the preservatives of the drug and not by suxamethonium itself [52].

Single, skinned muscle fibre tests. The method of skinning single muscle fibres, either chemically or mechanically so that they contain myofibrils and sarcoplasmic reticulum (SR) but no sarcolemma, has been described by Endo, Tanaka and Ogawa [39] and Wood [149]. As the in vitro contracture tests on whole muscle bundles are invasive and can be performed only on fresh tissue, the technique with single, skinned muscle fibres is attractive, since with this method a needle biopsy provides enough tissue. It is also possible to store the muscle tissue for some time before the tests are performed. The reaction of the single muscle fibres to calcium, caffeine and halothane is tested. Endo and colleagues [40] have found MH susceptible muscle more sensitive to calcium-induced calcium release from the SR than normal muscle. Both caffeine [38] and halothane [137] are considered to enhance calcium-induced calcium release, thereby eliciting contractures in MH susceptible muscle. The conventional contracture tests are not yet performed in Japan, so comparison of the skinned...
fibre tests with these tests has not been made in Japan. Wood and co-workers [149, 150] developed a caffeine contracture test on single fibres. This test has been evaluated by Britt and colleagues in a large series of patients [13]. They found it a useful test, although some overlap in results were seen between diagnostic groups. Unfortunately, the diagnosis in this unit is based primarily on the combined halothane–caffeine test, which is found less discriminative in other units. The skinned fibre tests therefore need further evaluation of diagnostic sensitivity and specificity.

CONCLUSION

Of all the tests which have been suggested for diagnosis of susceptibility to MH, only the invasive and cumbersome halothane and caffeine contracture tests have been proved reliable. Even with these tests, control data of good quality are scarce. Research for a reliable non-invasive test is continued. However, it is possible that a definitive test cannot be developed until the exact molecular defects causing MH are found. Until this occurs, a non-invasive test which could be used in the selection of patients for muscle biopsy would be of great help. At present, NMR spectroscopy or the lymphocyte quin 2 test seem promising for this purpose, but both need further evaluation. Until a new definitive test has been developed and properly evaluated, it would be of benefit to standardize as much as possible the contracture tests in use. Both a halothane and a caffeine test should be included, as is the case in the procedure agreed upon by the European MH Group [42, 43].

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