INVESTIGATION OF MALIGNANT HYPERThERMIA IN DENMARK AND SWEDEN

H. ØRDING, E. RANKLEV AND R. FLETCHER

SUMMARY

Units for the investigation of susceptibility to malignant hyperthermia (MH) were set up in Denmark in 1977 and in Sweden in 1981. Two hundred and ten patients from 76 families have been investigated. The diagnosis of MH susceptibility (MHS) was made by \textit{in vitro} exposure of muscle from vastus medialis to halothane and to caffeine. MHS criteria for the patients in this paper were established from examination of 31 control biopsies, obtained from the same muscle and with the same anaesthesia as the MH patients. The criteria have since been changed to those presented elsewhere in this issue. In our laboratories the halothane test (exposure to 0.5–2% halothane) was the more sensitive: 88% of MHS patients reacted to it. The caffeine test was positive in 68% of MHS patients, 0.5–2.0 mmol litre\(^{-1}\) solutions being the most discriminating. Forty-two percent of MHS patients reacted to only one test. Fulminant MH was the most common reason for investigation; all these families contained MHS members. Masseter spasm occurred as sole sign in 21 families, of which 11 were MHS. Only 10% of MHS patients had other signs or symptoms of neuromuscular disease such as muscle cramps or muscular dystrophy. Three families had experienced sudden infant death syndrome (SIDS), and two teenage brothers in a MHS family died suddenly, but death was unrelated to anaesthesia.

Malignant hyperthermia (MH) is a rare, serious pharmacogenetic condition which manifests itself during anaesthesia (Denborough et al., 1962). Susceptibility to MH (MHS) can be determined by \textit{in vitro} exposure of muscle to halothane (Ellis et al., 1978) or caffeine (Kalow, Britt and Richter, 1977). Units were set up in 1977 and 1981 in Denmark and Sweden, respectively, for the purpose of investigating patients and families suspected of being MHS. This paper reports the results of the first 210 investigations. In 1983 the \textit{in vitro} test procedure was altered, following the formation of the European MH Group (The European Malignant Hyperpyrexia Group, 1984). It is therefore appropriate to review the 210 investigations performed before this change.

PATIENTS AND METHODS

Families were investigated if a member (the proband) exhibited symptoms or signs associated with MHS. The proband was investigated first. If the proband had not reached puberty the parents were investigated in Sweden, whereas in Denmark the investigation was postponed until the proband reached about 10 years of age. Relatives of positive probands were then offered investigation.

One hundred and twenty-five patients in Denmark and 85 patients in Sweden were investigated. Clinical examination was performed with special regard to neuromuscular signs known to be associated with MH (King, Denborough and Zapf, 1972; Pollock and Britt, 1983). Blood was drawn for determination of Hb concentration, and the serum concentrations of Na\(^+\), K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\) and inorganic phosphate. Creatine kinase (CK) concentration was measured before and after muscle biopsy. An electrocardiogram (ECG) was taken routinely in Denmark, and if indicated by history or findings in Sweden.

Biopsy procedure

Susceptibility to MH was diagnosed by \textit{in vitro} testing of muscle obtained from a motor point biopsy of the vastus medialis muscle. The biopsies were taken under thiopentone, fentanyl and nitrous oxide in oxygen anaesthesia in all the Danish patients and in 23 of those in Sweden. The rest of the Swedish patients received femoral nerve blockade (\(n = 40\)), extradural anaesthesia (\(n = 16\)) with 2% mepivacaine, or spinal anaesthesia (\(n = 6\)) with 2% tetracaine. During anaesthesia the ECG and rectal temperature were monitored continuously and arterial pressure was measured intermittently. Patients found to be normal on MH testing were observed following the procedure for 6–24 h, and
those found to be MHS for at least 24 h. The biopsy specimens for the in vitro test were transported and stored in Krebs solution at room temperature.

In vitro test procedure

For the in vitro test, pieces of muscle 10–20 mm long and about 3 mm in diameter were dissected and suspended in a tissue bath perfused with Krebs solution at 37°C and bubbled with preheated 5% carbon dioxide in oxygen (carbogen). Viability of the muscle tissue was demonstrated by supramaximal electrical stimulation at 0.2 Hz. The specimens were exposed to either halothane or caffeine in increasing concentrations and the muscle tension measured by a displacement transducer, and a record obtained.

Two different halothane tests were performed, one static and one dynamic. In the static test, the specimens were stretched, initially, to a tension of approximately 2 g and left for about 10 min. After this halothane was added to the carbogen in concentrations of 0.5, 1, 2, and 4% for 3–5 min each. In the dynamic test the muscle was first allowed to rest at zero or low tension for 3 min, and was then stretched at a rate of 4 mm min⁻¹ for 1.5 min to a tension of approximately 2 g, left for 1 min and then shortened at the same rate to the initial length. The whole cycle was thus 7 min (Ellis et al., 1975). After three such cycles 0.5, 1, 2, and 4% halothane was added for one cycle each.

In both countries, a static caffeine test was performed by perfusing the muscle at a resting tension of approximately 2 g for 3–5 min with Krebs solution containing caffeine in increasing concentrations: in Denmark 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 8.0, 16.0 mmol litre⁻¹ and in Sweden 1.0, 2.0, 4.0, 8.0, and 16.0 mmol litre⁻¹. In Sweden, a dynamic caffeine test was performed in the same way as the dynamic halothane test, with one complete cycle at each caffeine concentration. In some patients not all tests were possible because of a lack of suitable muscle.

Criteria for a positive in vitro test were established from control biopsies taken from healthy subjects during minor operations on the leg. The control patients (Denmark 19, Sweden 12) were anaesthetized in the same way as the MH patients. None of the controls had experienced any anaesthetic complications, nor was there any family history of neuromuscular disease. The control biopsies were all from the vastus medialis muscle.

For statistical comparison between groups a Mann–Whitney test was used. Values of $P<0.05$ were considered significant.

RESULTS

In Denmark 125 patients aged between 8 and 75 yr, from 44 families, were investigated with biopsy; in Sweden 85 patients aged between 13 and 64 yr, from 32 families were studied. The material also included 18 non-biopsied probands from the investigated families. No patient developed signs of MH in the perioperative period.

In vitro test

Figures 1–3 show the results from the control biopsies. From these we established the following criteria for MHS: Any sustained contracture (1) with 0.5–2% halothane in the static test; (2) above 0.2 g at 0.5–1% and above 0.3 g at 2% halothane in the dynamic test; (3) with 0.5 or 1 mmol litre⁻¹ of caffeine in Denmark, and above 0.05 g at 1 mmol litre⁻¹ or above 0.15 g at 2 mmol litre⁻¹ caffeine in Sweden. The patients were considered to be MHS if any test fulfilled the above criteria. Patients not fulfilling these criteria were deemed to be MH negative. However, in order to accommodate to the criteria applied presently by the European Malignant Hyperpyrexia Group, our criteria have since been changed to theirs, presented elsewhere in this issue, mainly to include a group of patients with equivocal in vitro test results.

![Fig. 1. Cumulative contracture response of control muscle to dynamic halothane test.](https://academic.oup.com/bja/article-abstract/56/11/1183/316624)
The reasons for investigation, and the results of the in vitro test of the patients are shown in tables I and II and figures 4–6. The in vitro test of one Danish and one Swedish patient were inconclusive and are not included in the figures. Not all the probands in table I were biopsied; some died during MH crises and some were too young. The results for the latter have been deduced from the parents' in vitro test. The group “others” in table I includes two Swedish teenage brothers who died suddenly and unexpectedly. The cause of death could not be established at postmortem (Ranklev, Fletcher and Krantz, 1984). The mother (included in this material) had a normal in vitro test, whereas the father (tested later and not included here) was MH-positive.

One Danish patient died during treatment for acute self poisoning with anticholinergic and neuroleptic drugs. He was rigid and received suxamethonium to facilitate intubation without any relaxation being obtained. His temperature at this time was 40°C. His symptoms could have been attributable to the malignant neuroleptic syndrome, to an overdose of anticholinergics or to MH. His mother had committed suicide 2 years earlier. The father and two siblings had normal in vitro contracture tests, and the patient was therefore considered to be MH-negative.

### Table I. Type of reaction in probands from the investigated families.

Number of probands biopsied in parentheses. *Two or more of the following: tachycardia, arrhythmia, hypertension, rigidity, acidosis, increase in CK, myoglobinuria, fever. †Central core disease, unexplained deaths unrelated to anaesthesia.

<table>
<thead>
<tr>
<th>Type of reaction</th>
<th>Positive</th>
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<th>Negative</th>
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<td></td>
<td>Den</td>
<td>Swe</td>
<td>Den</td>
<td>Swe</td>
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<td></td>
<td>Den</td>
<td>Swe</td>
<td>Den</td>
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<tr>
<td>Fulminant MH reaction</td>
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<td>11(5)</td>
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<td>0</td>
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<td>0</td>
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<td>4(4)</td>
<td>4(3)</td>
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<td>0</td>
<td>3(3)</td>
<td>2(1)</td>
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<td>0</td>
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<td></td>
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<tr>
<td>Others †</td>
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<td>3(1)</td>
<td>1(0)</td>
<td>0</td>
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<td></td>
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<tr>
<td>Total</td>
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<td>14(13)</td>
<td>10(6)</td>
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### Table II. Results of in vitro test of relatives to MHS-probands

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<td>Den</td>
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<tr>
<td>Member of family with fulminant MH</td>
<td>40</td>
<td>16</td>
<td>32</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Member of family with possible MH</td>
<td>4</td>
<td>12</td>
<td>16</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Others</td>
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<td>0</td>
<td>3</td>
<td>1</td>
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<td>51</td>
<td>39</td>
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Fig. 4. Mean (± SEM) contracture responses of diagnostic biopsy specimens in dynamic halothane test. ***Statistically significant difference between positive and negative groups (P < 0.001).

Fig. 5. Mean (± SEM) contracture responses of diagnostic biopsy specimens in static halothane test. ***Statistically significant difference between positive and negative groups (P < 0.001).

Fig. 6. Mean (± SEM) contracture responses of diagnostic biopsy specimens in caffeine test. —— Denmark, MH positive; —— Denmark, MH negative; —— Sweden, MH positive, static test; —— Sweden, MH negative, static test. P < 0.001 between positive and negative Danish groups for caffeine concentrations 0.5–2 mmol litre\(^{-1}\). Significant difference between positive and negative Swedish groups for caffeine concentrations 2 and 4 mmol litre\(^{-1}\) in static test. For the sake of clarity, the results of the dynamic test (Sweden) have been omitted.

Table III shows the relationship between the halothane and caffeine test results. Thirty-five MHS patients reacted to one of the \textit{in vitro} tests and 48 reacted to both tests. Spontaneous contractures were noted in three Danish and 13 Swedish patients. With one exception, this finding was observed in muscle which reacted positively to one or both \textit{in vitro} tests. The exception was the mother of a girl who died from MH. She has one brother and one son who are MH-positive. Thus, on the basis of the family history and the reactive muscles, we considered her positive.

Table IV. Type of operation during which MH occurred or was suspected in the proband. Pooled results from both countries. Number of acu operations in parentheses

<table>
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<th>Type of operation</th>
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<td>Abdominal surgery</td>
<td>18(12)</td>
<td>7(7)</td>
</tr>
<tr>
<td>Otorhinolaryngological surgery</td>
<td>12(0)</td>
<td>12(1)</td>
</tr>
<tr>
<td>Surgery for trauma</td>
<td>10(5)</td>
<td>1(1)</td>
</tr>
<tr>
<td>Eye surgery</td>
<td>2(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Others</td>
<td>6(1)</td>
<td>3(0)</td>
</tr>
<tr>
<td>Total</td>
<td>48(18)</td>
<td>23(9)</td>
</tr>
</tbody>
</table>
The types of operation during which MH occurred or was suspected are shown in table IV. Age distribution and sex of the MHS probands are shown in figure 7. Ten patients died from MH, eight of these during acute surgery. Thus, the overall mortality rate in this material was 21%; the mortality rate in fulminant MH was 38%.

**History and clinical examination**

There were no symptoms or signs of neuromuscular disease in any of the MHN patients or in 89 MHS patients. There were positive findings in 12 MHS patients. These included one with dystrophia myotonica, one who was mentally retarded and deaf, and one with atrophy of the neck muscles after arthrodesis of the cervical column (all three referred because of spasm of the masseter muscles after suxamethonium). There was also one patient with suprapatellar atrophy of the quadriceps. Five patients suffered from severe muscle cramps, and one of these experienced recurrent patellar luxation. One patient was referred because of central core disease (CCD) and one was a known carrier of Duchenne muscular dystrophy (she had two sons with this disease, one of whom survived a fulminant episode of MH). One proband, an 18-month old boy, had a severe unspecified muscular dystrophy (Fletcher et al., 1982). The sudden infant death syndrome (SIDS) was encountered in one Danish and two Swedish families.

Before surgery all patients had a normal temperature. Hb concentration, and the serum concentrations of Na\(^+\) and K\(^+\) were within normal limits. A few patients had Ca\(^2+\), Mg\(^2+\) or inorganic phosphate values just outside the normal limits, but there were no significant differences in values between MHS and MHN patients. Preoperative CK values are shown in figure 8. There is considerable spread of values, and significant difference between MHS and MHN patients.

**DISCUSSION**

In vitro muscle tests are used in many centres for the investigation of susceptibility to MH. However, no universal agreement exists as to which pharmacological agent best separates MHS from MHN patients or how the test should be performed. This problem is augmented by the scarcity of acceptable control data. For this reason our controls were selected from healthy individuals with no family history of neuromuscular disease. Biopsy site and type of anaesthetic were identical to those used for diagnostic biopsies. The results from the controls are fairly uniform at low concentrations of halothane and caffeine, whereas considerable variation is seen with 4% halothane and more than 2 mmol litre\(^{-1}\) of caffeine (figs 1–3). Our diagnostic criteria for MHS are therefore based on the responses to 0.5–2% halothane and caffeine 0.5–2 mmol litre\(^{-1}\). The nine patients investigated who had previously experienced an unequivocal MH reaction all had positive in vitro tests, suggesting that the above criteria are reliable in identifying MHS individuals.

Mainly because of the choice of caffeine concent-
rations and differences in the composition of the Krebs solution, we have not applied the European criteria (The European Malignant Hyperpyrexia Group, 1984) to our patients, who were all biopsied before the European "protocol" was formulated. We have been able, in our laboratories, to discern increases in tension smaller than those at present used in the European procedure. In addition, we were previously prepared to make the diagnosis MHS on a single positive test result, whereas the new procedure demands, at present, positive results from both the halothane and caffeine tests. However, the results obtained from the control biopsies were sufficiently uniform to allow diagnosis to be made with a reasonable degree of confidence.

In our hands the halothane test is the most reliable diagnostic test for MHS, since 88% of the positive patients had an abnormal response to halothane, but only 68% to caffeine. Rosenberg and Reed (1983) reached the same conclusion. However, 11% of the positive patients in our material reacted to the caffeine test only, and therefore this test has its place also.

It is apparent from figures 4 and 5 that fairly uniform results are obtained in Denmark and Sweden for the halothane test, whereas there was a marked difference in sensitivity to caffeine (fig. 6). This could be the result of different magnesium concentrations in the Krebs solution (Denmark 1.2 mmol litre\(^{-1}\), Sweden 1.8 mmol litre\(^{-1}\)) because the calcium-releasing action of caffeine from the sarcoplasmic reticulum is antagonized by an increase in magnesium concentration (Weber, 1968). The difference between the halothane and caffeine results supports the suggestion that halothane has other modes of action as, for example, on the sarclemma (Gallant, Godt and Gronert, 1979).

In 16 patients spontaneous contractures were noted immediately after transfer of the specimen to the muscle bath. This finding, which has also been observed by others (Ellis, F. R., personal communication; Moulds, R. F. W., personal communication) may be regarded as an extreme expression of muscle disease.

Fifty-two percent of patients investigated because of suxamethonium-induced masseter spasm were found to be MHS. This is in agreement with the 57% reported by Rosenberg and Reed (1983) for this group. However, for the group investigated because of atypical reactions suggestive of MH, our results differ from theirs: they found 8% to be MHS whereas we found 56% to be MHS. This difference is probably the result of variation in the clinical history. First, seven of 12 patients were investigated because of unexpected fever, and in this group we found a low prevalence of MHS (one out of six patients). Second, most of our patients in this group had a combination of symptoms suggestive of MH. This would be expected to result in a higher proportion of MHS when compared with a group such as Rosenberg and Reed's with only single symptoms such as unexplained tachycardia or rigidity. Clinically, masseter spasm alone or combined with other symptoms suggestive of MH must be regarded as such and the patient treated as if MH were developing.

Most MH reactions in our material occurred during three types of surgery: abdominal (38%), otolaryngological (25%), and surgery for trauma (21%) (table IV). Twenty-one percent of MH cases reviewed by Ellis and Halsall (1980) were also seen during surgery for trauma. Recently, Pollock and Britt (1983) reviewed a large series of MH reactions, and from their data it can be calculated that approximately 25% of the cases had occurred during ototrinolaryngological surgery, —a value identical with our figure. It is interesting that in our material all surgery in this group was elective, because overall, we found a high proportion of reactions (38%) during emergency surgery. This might reflect the impact of stress on the probability of a susceptible individual developing MH. Certainly mortality was much higher during acute surgery: eight of 18 reactions vs. two of 30 reactions during elective surgery (\(P < 0.01\)).

Remarkably few patients had any neuromuscular symptoms or signs known to be associated with MHS (King, Denborough and Zapf, 1972; Pollock and Britt, 1983). Only 5% of the MHS patients suffered from muscle cramps, pain or stiffness which have been stated to be common amongst MHS individuals (Pollock and Britt, 1983). One MHS patient had dystrophia myotonica (DM). In vitro tests with normal results have been performed in patients with DM at other MH units (Moulds and Denborough, 1974; Brownell et al., 1983). Thus DM per se presumably does not affect the sensitivity to caffeine or halothane, and the diagnosis of MHS in this patient is considered appropriate. Three other neuromuscular diseases were encountered in our MHS patients: Duchenne muscular dystrophy, which has been increasingly reported in association with MHS (Brownell et al., 1983; Rosenberg and Heiman-Patterson, 1983), central core disease
which has a documented, high incidence of MHS (King, Denborough and Zapf, 1972; Frank et al., 1979) and an unspecified muscular dystrophy (Fletcher et al., 1982).

Creatine kinase (CK) was of little value in diagnosing MHS (fig. 7) even in families with fulminant MH. Sixty-two percent of MHS patients had increased CK concentration—a proportion similar to that reported by Ellis, Cain and Harriman (1978). However, in approximately 25% of the MH-negative patients without any muscular symptoms the concentration of CK was increased.

Our results of the in vitro contracture test for relatives are compatible with the theory that MHS is caused by a single autosomal dominant gene (King, Denborough and Zapf, 1972). The high proportion of MH negatives in the Danish families with possible MH is the result of reclassification of some families with possible MH after completion of the control biopsies.

ACKNOWLEDGEMENTS

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REFERENCES


INVESTIGACION DE LA HIPERTERMIA MALIGNA EN DINAMARCA Y SUECIA

SUMARIO

Se crearon unidades para la investigación de la susceptibilidad a la hipertermia maligna (MH) en Dinamarca en 1977 y en Suecia en 1981. Se llevaron a cabo investigaciones sobre 210 pacientes provenientes de 76 familias. El diagnóstico de la susceptibilidad a la MH (MHS) se realizó mediante la exposición in vitro de músculo vasto interno al halotano y a la cafeína. Se establecieron los criterios de MHS para los pacientes a partir del examen de 31 biopsias de control obtenidas en el mismo músculo y con la misma anestesia que con los pacientes MH. Desde entonces, cambiaron los criterios a los que se presentan en otro lugar en la presente publicación. En nuestros laboratorios, el ensayo al halotano (exposición al halotano al 0,5–2%) era más sensitivo: el 88% de los pacientes MHS reaccionaron al mismo. El ensayo con cafeína fue positivo en un 68% de los pacientes MHS y fueron las soluciones de 0,5–2,0 mmol litro⁻¹ las más discriminatorias. Un 42% de los pacientes con MHS reaccionaron a un ensayo solamente. La MH fulminante constituía la razón más común de la investigación; todas dichas familias tenían miembros con MHS. El espasmo del masetero ocurrió como señal única en 21 familias de las cuales 11 tenían MHS. Un 10% solamente de los pacientes con MHS presentaban otras señales o síntomas de enfermedad neuromuscular tales como calambres o distrofia muscular. En tres familias, se produjo el síndrome de muerte infantil repentina (SIDS) y dos jovencitos hermanos de una familia con MHS murieron repentinamente, pero la muerte no tenía relación con la anestesia.