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Caffeine and Halothane Contracture Testing in Swine Using the Recommendations of the North American Malignant Hyperthermia Group

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Caffeine and halothane contracture testing is widely used to detect malignant hyperthermia (MH) susceptibility. The accuracy and reliability of the 3% halothane test and the incremental caffeine test, as recommended by the North American MH Group, were assessed in 11 swine (five MHS, six control). Nine swine were tested twice, 4–6 weeks apart. Accuracy of the *in vitro* diagnosis was also assessed by *in vivo* anesthetic challenge. Of all muscle bundles from MH-susceptible swine, 65% reacted positively to 3% halothane and 70% to 2 mM caffeine. Only 35% had a positive caffeine-specific concentration, and 25% developed an increase in baseline tension ≥ 7% at 2 mM caffeine. However, when only the most positive response to 3% halothane or to 2 mM caffeine was used (a minimum of three fresh muscle strips is recommended), these two tests were highly sensitive and specific. In control swine one of 30 muscle bundles reacted positively to 3% halothane. A positive caffeine-specific concentration developed in one of 25 control muscle bundles exposed to caffeine. The variability in the results of these tests mandated that at least three muscle bundles be used for each test. Nonviable muscle bundles could not be relied upon to provide accurate results. In this porcine model, MH susceptibility could be detected by performing the Caffeine Halothane Contracture Test (CHCT) according to the guidelines of the North American MH Group. However, only the 3% halothane test and the response to 2 mM caffeine produced adequate diagnostic results in this breed of swine. (Key words: Anesthetics, volatile: halothane. Complications: malignant hyperthermia. Hyperthermia, malignant: contracture testing; porcine. Neuromuscular blocking drugs: succinylcholine.)

THE CAFFEINE CONTRACTURE TEST, used to diagnose malignant hyperthermia (MH), was first described by Kalow *et al.*[1] They showed that muscle fiber bundles from MH-susceptible (MHS) patients had a lower *in vitro* contracture threshold to caffeine than that in nonsusceptible patients. Ellis *et al.*[2] later reported that MHS skeletal muscle developed contractures following exposure to halothane *in vitro,* an effect not seen in control tissue.

Since then, attempts have been made to define and improve the sensitivity and specificity of the *in vitro* contracture test. Responses to other pharmacologic agents have been evaluated, and the methodology has begun to be standardized.[3]

In November 1987 representatives from each MH diagnostic center across North America met to begin to standardize the Caffeine Halothane Contracture Test (CHCT). A consensus was reached on the methodology and diagnostic criteria to be used by all member laboratories of the North American MH Group.[4] These guidelines are subject to ongoing revision by the members of the group.

The group accepted two diagnostic tests for determining MH susceptibility in humans, the halothane test and the incremental caffeine test.[4] A minimum of three fresh muscle strips are used for each test. If even one viable muscle strip develops a positive contracture response in either test, the patient is considered MHS.

A positive halothane contracture test is defined as a contracture > 0.2–0.7 g after exposure to 3% halothane bubbled through the tissue bath for 10 min. The exact value of this abnormal range is determined by each laboratory.

The caffeine test uses incremental increases in caffeine dose from 0.5 to 32 mM. The concentration is changed every 4 min if no contracture develops. A positive caffeine test is defined as: 1) a contracture of ≥0.2 g tension at 2 mM caffeine, 2) a caffeine-specific concentration (CSC) of <4 mM caffeine, or 3) a change in tension at 2 mM caffeine ≥ 7% of peak tension generated at 32 mM caffeine. Each laboratory determines the method of interpretation that it will use for diagnosis.

The CSC is defined as the caffeine concentration required to produce a cumulative contracture of 1 g. The per cent peak tension method, proposed by Gronert,[5] attempts to express data in a manner that compensates for considerable differences in fascicle size. By calculating the increase in tension at 2 mM caffeine as a percentage of peak tension at 32 mM, each muscle bundle acts as its own reference standard.

The major drawback to the caffeine halothane test in humans is the inability to validate its results by subsequent *in vivo* anesthetic challenge. It would be unethical to administer known MH triggering agents to a patient with a

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positive *in vitro* contracture test (IVCT). However, such studies can be performed using the swine model of MH.

Therefore, we evaluated the diagnostic utility of the CHCT, as recommended by the North American MH Group, in swine known to be either MH(+) or MH(−). We also assessed other contracture tests that have been proposed to have potential diagnostic value.

Methods

The experimental protocol was approved by the Hahnemann University Animal Welfare Committee. All swine were obtained from Iowa State University through Biomedical Alternatives International, Inc. (Raleigh, North Carolina). Control swine were 80% Yorkshire, 20% Duroc/Pietrain, and were free of MH. MHS swine were from an inbred colony of Yorkshire X Duroc animals. MH susceptibility was determined by resting CPK activity, H-blood typing, and by 6% halothane challenge at 54 days of age,[6] prior to shipment of the animals to Philadelphia.

For muscle biopsy each animal received preanesthetic medication comprised of im xylazine, oxymorphone, and ketamine 1 h preoperatively.[7] Anesthesia was induced with pentobarbital 24 mg/kg iv. Oral tracheal intubation was then performed and the animal breathed 50% N_2O in O_2 spontaneously *via* a semiclosed circle circuit using a CO_2 absorber.

All 11 swine underwent biopsy of the left gracilis muscle for *in vitro* contracture testing. In addition, nine of the 11 swine had a second muscle biopsy for repeat contracture testing 4–6 weeks after the first biopsy. *In vitro* contracture testing was performed as previously described for human biopsy specimens[8–10] and in keeping with the guidelines of the North American MH Group.[4]

A positive response to 3% halothane was defined as a contracture ≥ 0.7 g within 10 min, based on our experience with human *in vitro* contracture testing.[8] A positive response to 2 mM caffeine was defined as a contracture ≥ 0.2 g.[4] A CSC < 4 mM, as recommended for humans,[4] was initially used to indicate MH susceptibility. A threshold of ≥7% at 2 mM caffeine defined a positive response by the peak tension method.[4]

In vitro contracture testing was performed in an unblinded manner because the test is subject to little observer bias. All *in vitro* testing was completed within 2–5 h after muscle biopsy. Muscle bundles were used for testing only if viable, *i.e.*, a twitch ≥ 0.3 g was present immediately before exposure to pharmacologic agents. When sufficient muscle was available, additional tests, which have been described as useful for MH diagnosis,[8] were also performed (table 1).

In four MHS and three control swine, MH susceptibility was reassessed by *in vivo* anesthetic challenge[11] after the second muscle biopsy was completed. Each swine was exposed to 3% halothane for 5 min, using a Fluotec 3 vaporizer. The halothane concentration was then reduced to 2% for the remainder of the challenge. After 10 min of halothane exposure, succinylcholine 1 mg/kg was injected iv. This dose of succinylcholine was repeated once every 5 min, up to three doses, unless clinical signs of MH developed. MH was diagnosed clinically by the onset of muscle rigidity, heart rate > 150 beats/min, changes in skin color, and rectal temperature increase > 0.5° C.[12]

Data were analyzed by the two-tailed, unpaired *t* test. Calculations of sensitivity and specificity were made using standard equations.[13]

Results

Caffeine Halothane Contracture Test

A total of 108 muscle bundles were available for testing with 3% halothane or incremental doses of caffeine; 10 (9%) had twitch tensions < 0.3 g and were discarded. The results of the 3% halothane test and each of the three

TABLE 1. *In vitro* Contracture Tests Performed on Each Muscle Biopsy Specimen

Test	No. of Bundles Tested per Swine	Pharmacologic Agents
3% halothane*	3	Halothane 3% × 10 min
2 mM caffeine, CSC, percent maximal tension*	3	Incremental caffeine concentrations, 0.5 to 32 mM, every 4 min
HCSC	1–2	Same as caffeine, but in presence of 1% halothane
Halothane/succinylcholine (H/S)	1–2	Halothane 3% × 5 min, then 50 mM succinylcholine, observe 5 min
Succinylcholine alone; succinylcholine/halothane (S/H)	1–2	50 mM succinylcholine, wait 5 min, then 3% halothane × 5 min
1% halothane alone; 1% halothane/caffeine	1–2	1% halothane × 15 min, then incremental caffeine concentrations, 0.25 to 32 mM, every 4 min

* Biopsy specimens from all swine were tested with 3% halothane alone and with caffeine alone. When sufficient muscle bundles were available, other tests listed were also performed.

versions of the caffeine test were significantly different between control and MHS swine (table 2). The MH diagnosis with 3% halothane or 2 mM caffeine showed little variation between first and second biopsy specimens (table 3). Figure 1 illustrates the difference in *in vitro* contracture responses between MHS and MH(−) swine.

In control swine none of the 25 muscle bundles tested with 2 mM caffeine responded positively (table 4). One of 30 control muscle bundles (3%) tested with 3% halothane developed a 0.8 g contracture response (*i.e.*, a false-positive diagnosis). When muscle from this animal (control 3) was tested 6 weeks later, the *in vitro* contracture responses were within the control range (tables 3 and 4).

In specimens from MHS swine, at least one muscle bundle responded positively to 3% halothane and to 2 mM caffeine in each biopsy specimen (table 4). The correct diagnosis was made in all MHS swine. The threshold of ≥0.7 g, used in our laboratory for the 3% halothane test in humans, also allowed accurate diagnosis in these swine.

Not all muscle bundles obtained from MHS swine responded positively (table 4). Overall, only 65% of the specimens exposed to 3% halothane developed contractures ≥ 0.7 g. Likewise, only 70% of MHS muscle bundles responded positively to 2 mM caffeine.

A CSC of <4.0 mM, as recommended for human MH diagnosis,[4] did not separate MHS from control swine (table 3). To avoid a false-negative result in any of the MHS swine,[14] a positive CSC was then calculated to be <7.2 mM (table 3). This resulted in a false-positive diagnosis by CSC in control swine 4. Overall, one of 25 control muscle bundles (4%) had a CSC < 7.2 mM (table 4). Only seven of 20 MHS muscle specimens (35%) had a CSC < 7.2 mM.

The results of the incremental caffeine test were then analyzed using the per cent peak tension method.[4,5] None of the muscle bundles from MH(−) swine increased their tension ≥ 7% at 2 mM caffeine (tables 3 and 4). Only five of 20 MHS muscle bundles (25%) developed ≥7% tension increase at 2 mM caffeine.

TABLE 2. *In Vitro* Contracture Responses for Tests Recommended by the North American MH Group

Test	Control Mean + SEM (n) Range	MHS Mean + SEM (n) Range	*P* Value
3% halothane (g)	0.23 ± 0.03 (30) 0.1–0.8	0.98 ± 0.12 (23) 0.4–2.5	<0.001
2 mM caffeine (g)	0.01 ± 0.01 (25) 0–0.1	0.32 ± 0.07 (20) 0–1.4	<0.001
CSC (mM)	10.14 ± 0.54 (25) 6.6–16.6	8.11 ± 0.73 (20) 1.7–14.7	<0.05
Percent peak tension (%)	0.09 ± 0.07 (25) 0–1.5	4.11 ± 0.84 (20) 0–12.1	<0.001

Responses of all viable muscle bundles are included.

TABLE 3. Most Positive Responses (*i.e.*, largest contracture response, lowest caffeine concentration, or highest peak tension) for 3% Halothane Test and Caffeine Contracture Test

	Swine No.	3% Halothane (g)	2 mM Caffeine (g)	CSC (mM)	% Peak Tension
Control	1A	0.4	0	10.0	0
	B	Nonviable specimen			
	2A	0.2	0.1	7.3	1.5
	B*	0.1	0	7.5	0
	3A	0.8	0	8.0	0
	B*	0.2	0.1	8.9	0.8
	4A	0.2	0.1	6.6	0
	B	0.2	0	10.2	0
	5A	0.2	0	9.5	0
	B	Not done			
	6A	0.2	0	8.0	0
	B*	0.6	0	7.8	0
MHS	7A	1.4	1.4	1.7	12.1
	B*	2.2	0.8	5.1	7.0
	8A	1.6	0.2	6.2	4.9
	B	Not done			
	9A	0.8	0.5	4.0	11.8
	B*	0.8	0.3	5.4	2.8
	10A	Nonviable specimen			
	B*	2.5	0.3	6.9	7.5
	11A	2.0	0.4	5.3	3.4
	B*	1.0	0.2	12.6	1.5

A and B represent first and second biopsies.
* These swine were challenged *in vivo* with halothane/succinylcholine to reassess their MH status.

OTHER TESTS

The results of the other *in vitro* tests that were performed are presented in table 5. The distribution of values for the 1% halothane caffeine test overlapped, making it impossible to separate MHS from control results. This was also true of the results of the succinylcholine halothane test.

Three of seven MHS muscle bundles exposed to 50 mM succinylcholine alone developed 0.1 g contractures compared with none of the nine control specimens. Although this difference was statistically significant (table 5), it was not useful diagnostically. Two of the three contractures seen were from the same MHS swine, biopsied on two separate occasions.

None of the 14 control muscle bundles developed contractures ≥ 0.2 g to 1% halothane. However, four of seven MHS muscle bundles developed contractures ≥ 0.2 g. This difference was statistically significant ($P < 0.001$, table 5).

In muscle bundles exposed to succinylcholine in the presence of halothane, there was considerable overlap in

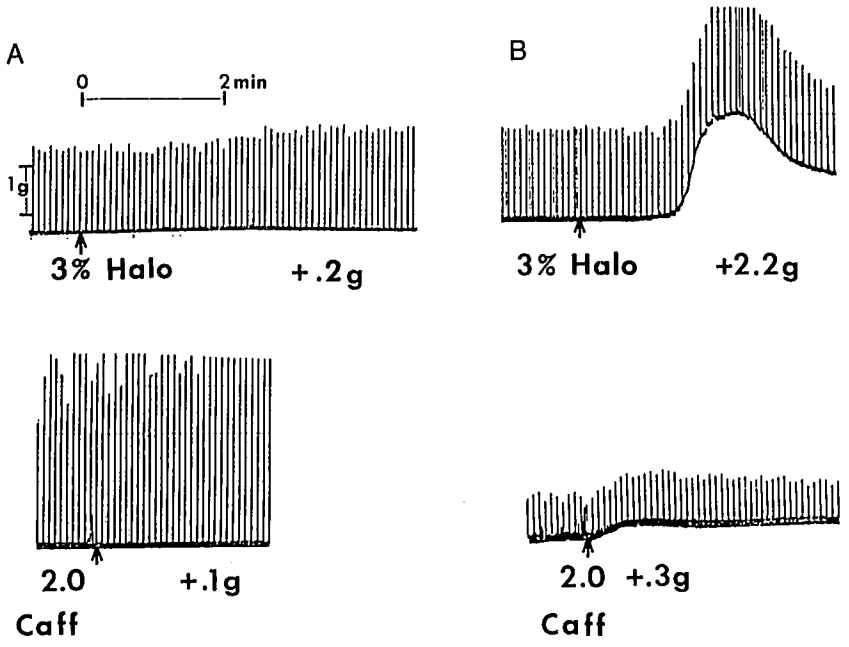

FIG. 1. Typical *in vitro* responses to 3% halothane and to 2 mM caffeine in swine. Muscle from control swine 3 (*A*) exhibited a 0.2 g contracture to 3% halothane and a 0.1 g contracture to 2 mM caffeine. This is a negative response. In MHS swine 7 (*B*), muscle bundles developed a 2.2 g contracture to 3% halothane and a 0.3 g contracture to 2 mM caffeine. This is clearly a positive response.

TABLE 4. Number of Muscle Bundles per Biopsy Exhibiting Positive Responses to 3% Halothane or Incremental Caffeine*

	Swine No.	Halothane 3%	Caffeine 2 mM	CSC	% Peak Tension
Control	1A	0/3	0/1	0/1	0/1
	B	Nonviable specimen			
	2A	0/3	0/3	0/3	0/3
	B	0/3	0/3	0/3	0/3
	3A	1/3	0/2	0/2	0/2
	B	0/3	0/3	0/3	0/3
	4A	0/3	0/2	1/2	0/2
	B	0/3	0/3	0/3	0/3
	5A	0/3	0/2	0/2	0/2
	B	Not done			
	6A	0/3	0/3	0/3	0/3
	B	0/3	0/3	0/3	0/3
		1/30 (3%)	0/25 (0%)	1/25 (4%)	0/25 (0%)
MHS	7A	2/2	2/2	1/2	1/2
	B	3/3	2/3	1/3	1/3
	8A	2/3	1/2	0/2	0/2
	B	Not done			
	9A	1/3	3/3	2/3	2/3
	B	1/3	1/1	1/1	0/1
	10A	Nonviable specimen			
	B	2/3	2/3	1/3	1/3
	11A	3/3	2/3	1/3	0/3
	B	3/3	1/3	0/3	0/3
		15/23 (65%)	14/20 (70%)	7/20 (35%)	5/20 (25%)

* Positive contracture responses consist of ≥0.7 g to 3% halothane, ≥0.2 g to 2 mM caffeine, CSC <7.2 mM, or percent peak tension ≥7% at 2 mM.

results from control and MHS muscle (table 5). Contractures of >0.6 g were observed in four of seven MHS muscle bundles but in none of the nine control muscle bundles.

TEST SENSITIVITY AND SPECIFICITY

For each test having potential diagnostic value, the sensitivity and specificity were calculated (table 6). The most abnormal responses from each of the 18 muscle biopsy specimens (10 control, 8 MHS) were used. All tests were highly specific; however, sensitivity varied from 50% to 100%. The most sensitive tests of MH susceptibility in these swine were the 3% halothane test and the contracture response to 2 mM caffeine.

TABLE 5. *In Vitro* Contracture Responses for Other Tests Proposed for Diagnosis of MH Susceptibility

Test	Control Mean ± SEM(n) Range	MHS Mean ± SEM(n), Range	*P* Value
1% halothane (g)	0.01 ± 0.01 (14) 0–0.1	0.27 ± 0.08 (7) 0.1–0.7	<0.001
Halothane/succinylcholine (g)	0.28 ± 0.05 (9) 0.1–0.6	0.81 ± 0.21 (7) 0.1–1.6	<0.02
50 mM succinylcholine	0 ± 0 (9) 0–0	0.04 ± 0.02 (7) 0–0.1	<0.05
Succinylcholine/halothane (g)	0.91 ± 0.17 (9) 0.4–1.7	1.20 ± 0.17 (7) 0.6–1.8	NS
HCSC (mM)	2.13 ± 0.16 (14) 1.0–3.4	1.77 ± 0.18 (7) 1.0–2.4	NS

Responses of all viable muscle bundles are included.

TABLE 6. Sensitivity and Specificity of Various *In Vitro* Tests in Control and MHS Swine

Test	Threshold	N	Sensitivity (%)	Specificity (%)
Tests recommended by North American MH Group				
3% halothane	≥0.7 g	18	100	90
2 mM caffeine	≥0.2 g	18	100	100
CSC	<7.2 mM	18	88	90
% peak tension at 2 mM caffeine	≥7%	18	50	100
Other proposed tests				
1% halothane	≥0.2 g	15	50	100
Halothane/succinylcholine	≥0.7 g	16	57	100

N = number of viable muscle biopsy specimens.

In Vivo Halothane–Succinylcholine Challenge

In vivo anesthetic challenge with halothane and succinylcholine confirmed the presence or absence of MH susceptibility in four MHS and three control animals. None of the control swine developed any clinical signs of MH after 25–45 min. Heart rate fell from 100–105 beats/min to 80–90 beats/min during halothane exposure. Repeated doses of succinylcholine produced limb and jaw muscle flaccidity. Rectal temperature did not change following either agent.

The MHS swine responded typically to *in vivo* anesthetic challenge. Exposure to halothane alone led to extensor muscle rigidity within 5 min in all MHS animals. Administration of a single dose of succinylcholine was consistently followed by a rapid increase in jaw and limb muscle rigidity, tachycardia (180–240 beats/min), mottled cyanosis, and temperature increases of 0.5–1.5° C over 10–20 min.

Discussion

This study used the recommendations of the North American MH Group[4] to perform the CHCT in known MHS and control swine. Using the response to 3% halothane alone or to 2 mM caffeine alone, we could accurately detect MH susceptibility in these animals.

The 3% halothane test is one of two mandatory tests recommended by the North American standards. A false-positive response (confirmed by *in vivo* challenge) occurred in control swine 3, lowering the test's specificity to 90%. If we had raised the threshold response to >0.8 g, this false-positive result would not occur. However, this would lead to a false-negative diagnosis in MHS swine 9. Clinically, a false-negative diagnosis for MH is less desirable than a false-positive diagnosis.

The caffeine contracture test is also a mandatory test for MH diagnosis. The North American recommendations include three methods for interpreting this test. In this study the most sensitive and specific method was the response to 2 mM caffeine. The CSC was associated with false-positive and false-negative results, lowering both its sensitivity and specificity. The per cent peak tension method of interpretation had a 50% incidence of false-negative results in these swine.

Several factors were found to be important in performing and interpreting the *in vitro* contracture test, as recommended by the North American MH Group:

1. Variability in the *in vitro* response to pharmacologic agents has been reported in human[8,15,16] and porcine muscle,[17] and was also seen in this study. Because of this inherent variability, the halothane test and the caffeine test should each be performed with at least three muscle bundles, as recommended by the North American MH Group.[4]

2. The use of a poorly viable muscle bundle may also affect the *in vitro* contracture response (fig. 2). Viability is usually defined as the presence of a twitch response to electrical stimulation.[4] Because the North American MH Group recommendations do not define a minimally acceptable twitch tension, we arbitrarily chose a twitch tension of ≥0.3 g to indicate viability. However, muscle bundles should ideally exhibit twitch tensions ≥ 1.0 g. In our experience porcine muscle deteriorated more rapidly than human muscle. Nine percent of the original muscle bundles were discarded prior to testing with 3% halothane or incremental doses of caffeine.

3. The results of the 1% halothane caffeine test did not allow the determination of a diagnostic threshold, and we did not find the test useful for MH diagnosis. Two human studies have also questioned the validity of this test because of high false-positive rates.[10,18]

Other tests with potential diagnostic value were also examined. The response to halothane with succinylcholine did not discriminate MHS from control swine. Likewise, a positive response to 50 mM succinylcholine occurred in only two of five MHS swine, making it a poor discriminator of MH susceptibility. Other studies have concluded that the response to succinylcholine alone is not useful diagnostically.[9,18,19,**] The low sensitivity of the 1% halothane test supports the use of higher concentrations of halothane for diagnostic testing. However, unlike the 3% halothane test, no false-positive diagnoses occurred with the 1% halothane test.

The response to succinylcholine in the presence of halothane was a relatively insensitive test. However, contractures > 0.6 g were seen only in MHS swine. We have also seen such large contractures in six patients who tested negative for MH by caffeine halothane contracture testing

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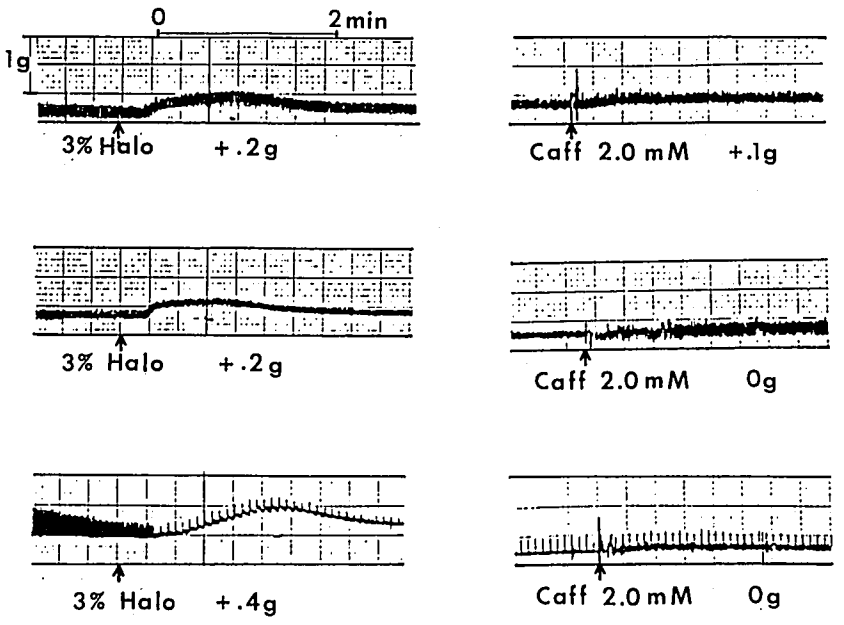

FIG. 2. The muscle bundles tested from the first biopsy from MHS swine 10 are shown. The responses to 3% halothane, on the left, were all <0.7 g. The three muscle bundles on the right were exposed to 2 mM caffeine; no contractures > 0.1 g developed. The twitch tensions of all six bundles were <0.3 g, indicating poor viability. This would have led to a false-negative diagnosis in this animal (see table 3).

(unpublished observations). These patients subsequently have received anesthesia with MH triggering agents without incident. Thus, this test does not appear to be useful for MH diagnosis in humans.

In conclusion, we were able to accurately detect MH susceptibility in swine using the *in vitro* contracture response to 3% halothane alone or to 2 mM caffeine alone. The CSC and percent peak tension methods of assessing the incremental caffeine test were less useful. None of the alternate tests studied, including the 1% halothane caffeine test, were found to be useful for the laboratory diagnosis of MH.

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