

# Halothane-induced ATP Depletion in Platelets from Patients Susceptible to Malignant Hyperthermia and from Controls

Urs Giger, Dr. Med. Vet.,\* and Richard F. Kaplan, M.D.†

Since the cellular defect of malignant hyperthermia (MH) may occur in tissues other than muscle and since platelets share certain contractile characteristics with muscle cells, testing platelets has been suggested as a way to diagnose susceptibility to MH. In analogy to the *in vitro* depletion of muscle adenosine triphosphate (ATP), the authors compared platelet basal nucleotide levels and halothane-induced depletion of ATP from 10 MH-susceptible patients and from 12 unrelated nonsusceptible controls. A rapid and simple isocratic high-pressure liquid chromatography technique was used to analyze acid-extracted platelet nucleotides. Halothane added to platelet-rich plasma at 37° C significantly decreased ATP in platelets in a dose-dependent as well as a time-dependent manner. In contrast, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) were not changed significantly. Other volatile anesthetic agents also depleted ATP in platelets. Although ATP in platelets exposed to halothane was depleted significantly, there was no difference between platelets from MH-susceptible patients and nonsusceptible controls. Therefore, halothane-induced ATP depletion in platelets is not a reliable test for diagnosing MH susceptibility. (Key words: Anesthetics, volatile: halothane. Blood: platelets. Complications: malignant hyperthermia. Hyperthermia: malignant. Measurement techniques, chromatography: high-pressure liquid chromatography. Metabolism, adenosine diphosphate; adenosine monophosphate; adenosine triphosphate. Neuromuscular relaxants: dantrolene. Temperature, body: metabolism.)

MALIGNANT HYPERTHERMIA (MH) is a rare pharmacogenetic myopathy triggered by various inhalational anesthetics, skeletal muscle relaxants, and, possibly, stress.<sup>1</sup> At present, laboratory identification of susceptibility to MH is based on *in vitro* tests of muscle biopsy specimens. The muscle contracture response tests to halothane, caffeine, or potassium chloride are sensitive, widely used, and the most reliable.<sup>2</sup> Since muscle biopsy requires hospitalization, surgery, and regional or general anesthesia, a simpler, less invasive test to detect susceptibility to MH is highly desirable. Elevations in resting creatine phosphokinase (CPK) is observed only in 50-70% of persons susceptible to MH<sup>1</sup> and, therefore, has limited diagnostic value. Other less invasive

tests (erythrocyte osmotic fragility,<sup>3</sup> platelet aggregation<sup>4</sup>) have not been consistently reliable.

Since platelets and muscle cells have a similar calcium ATP-dependent contractile apparatus,<sup>5</sup> platelets have been suggested as a screening test system for MH. In a letter to the editor, Solomons *et al.*<sup>6</sup> reported a highly significant decrease in ATP in halothane-treated platelets from MH-susceptible patients compared with nonsusceptible controls. This finding appears analogous to the ATP-depletion test in muscle biopsy specimens.<sup>7,8</sup>

The purpose of our study was to compare the basal nucleotide (ATP, ADP, and AMP) levels in platelets from MH-susceptible patients and non-susceptible controls, and to investigate the *in vitro* effect of halothane and other volatile anesthetic drugs on platelet nucleotide contents. In addition, we describe a simple, rapid isocratic high-pressure liquid chromatography (HPLC) technique for the analyses of ATP, ADP, and AMP contents in platelets.

## Materials and Methods

### PATIENTS

Ten patients susceptible to MH from five families were compared with 12 unrelated non-susceptible persons (controls). The clinical and laboratory data of the MH-susceptible patients are shown in table 1. MH-susceptible patients had a classic personal history, a strong family history, or both for MH. "Classic signs" (see table 1) include tachycardia, acidosis, increased body temperature, myoglobinuria, and markedly increased CPK induced by general anesthesia. In addition to these problems, patients B2 and D1 developed masseter spasm after exposure to succinylcholine. The patients' susceptibilities to MH were confirmed further by an *in vitro* test of muscle samples, except for one five-year-old patient (D1) who experienced classic symptoms of MH during halothane anesthesia. The muscle contracture response test to halothane and caffeine from patient D2 was performed at the University of Pennsylvania<sup>4</sup> and from family C at the University of Texas.<sup>9</sup> All other muscle biopsy tests were performed at the Massachusetts General Hospital. This latter test, described by Allen *et al.*<sup>10</sup> involves ionized calcium uptake performed on

\* Postdoctoral Research Associate, Department of Pharmacology. Recipient of a Swiss National Research Foundation Young Investigator Award.

† Assistant Professor of Anesthesiology.

Received from the Departments of Pharmacology and Anesthesiology, University of Florida College of Medicine, Gainesville, Florida. Accepted for publication September 17, 1982. Presented in part at the 1982 Annual National Meeting of the American Federation for Clinical Research, Washington, D. C.

Address reprint requests to Dr. Kaplan: Department of Anesthesiology, University of Florida College of Medicine, Box J-254, J. Hillis Miller Health Center, Gainesville, Florida 32610.

‡ Isocratic: The use of the one solvent of constant composition for elution.

TABLE 1. Clinical Features of Ten Patients Susceptible to MH

Family	Patient	Age (Yr)	Sex	Relation	Personal History	Muscle Biopsy Test
A	1	13	F	—	"Classic signs" during halothane anesthesia	†
B	1	36	M	Father	Nonspecific myalgias	†
	2	12	M	Son	"Classic signs" during halothane anesthesia	†
	3	8	M	Son	Myalgias	†
	4	10	F	Daughter	Myalgias, resting CPK elevated	†
C	1	20	F	Mother	Myalgias and strong family history	†
	2	7	M	Son	None but strong family history	†
D	1	5	M	Son	"Classic signs" during halothane anesthesia	ND*
	2	30	M	Father	Muscle stiffness, resting CPK elevated	†
E	1	20	F	—	None but strong family history	†

\* ND = not determined.

† Test indicated MH-susceptibility.

thin muscle sections. In addition, an adjunctive "tourniquet test" was positive in four patients (B2, B3, B4, C2). A positive test was defined as a post-ischemic rise in twitch height of more than 80%.§

The 12 control subjects (six men and six women, aged 22–40 years) were free of any muscular or general medical illnesses and had no personal or family history of MH or problems during previous general anesthesia. The protocol of this study was approved by the Committee on Human Studies at the University of Florida and informed consent was obtained.

#### EXPERIMENTAL PROCEDURE

Patients and control subjects had abstained from medications known to affect platelet function during the week before the platelet studies, had abstained from caffeine-containing drinks for 24 h before the studies, and had fasted for 4 h before venipuncture. One MH-susceptible patient and one control subject were always tested simultaneously. Up to 25 ml of blood were drawn into heparinized tubes (vacuum containers). Platelet-rich plasma (PRP) was prepared immediately by centrifugation at  $170 \times g$  for 12 min at room temperature (22–25° C). After removing the PRP, platelet-poor plasma was obtained by sedimentation of the remaining blood at  $1,000 \times g$  for 20 min. The platelet counts (determined by a coulter counter) in the PRP from MH-susceptible patients and control persons were  $512,000 \pm 44,000$  and  $443,000 \pm 30,000$  platelets/ $\mu\text{l}$  (mean  $\pm$  SEM), respectively. The cell count of the PRP was adjusted to 250,000 platelets/ $\mu\text{l}$  by dilution with plate-

let-poor plasma. Contamination by red and white blood cells was negligible.

PRP (0.8 ml containing  $2 \times 10^8$  platelets) and 0.2 ml of physiologic saline solution (total volume 1 ml) were placed in 1.5-ml capped polypropylene microcentrifuge tubes and incubated at 37° C for 30 min in a shaking water bath (100 rpm). Various amounts of halothane (1–20  $\mu\text{l}$ ) or other volatile anesthetic agents were added with a Hamilton® microliter syringe at different times during incubation, as detailed in the result section. In some tubes, caffeine, potassium chloride, or dantrolene sodium (Dantrium® intravenous) dissolved in physiologic saline was present during the 30-min incubation (see table 4). Immediately after the incubation, 0.1 ml 55 mM EDTA-0.9% saline solution (pH 7.4) was added and platelets were sedimented by centrifugation at  $9,000 \times g$  for 1 min. The supernatant was removed carefully and the pellet was immediately frozen and stored at –20° C. These *in vitro* studies were run in duplicate and completed within 3 h after venipuncture.

Nucleotides were extracted by: 1) addition of 0.4 ml of 0.7 N perchloric acid (4° C) to the frozen platelet pellets, 2) immediately mixing for 1 min, and 3) sonification for 3 min in an ice bath. In order to sediment the cell debris, the acid extract was centrifuged at  $9,000 \times g$  for 1 min at 4° C. The supernatant then was aspirated carefully and alkalized (pH 9–11) with 10 N potassium hydroxide. The precipitating salts were removed by centrifugation as above, and 0.1-ml samples of supernatant were used for nucleotide analysis by HPLC. This optimized procedure allowed complete extraction of nucleotides from platelets (*i.e.*, no nucleotides were found upon further extractions). Furthermore, the technique used resulted in complete recovery of nucleotide standards (Sigma Chemical Company, St. Louis, Missouri) from the platelet solution.

The nucleotides were analyzed by using an isocratic

§ Jones PIE, Britt BA, Steward DJ, Volgyesi GA, Scott E: Tourniquet test as a predictor of malignant hyperthermia susceptibility. (abstracted). *Anesth Analg* (Cleve) 60:256, 1981.

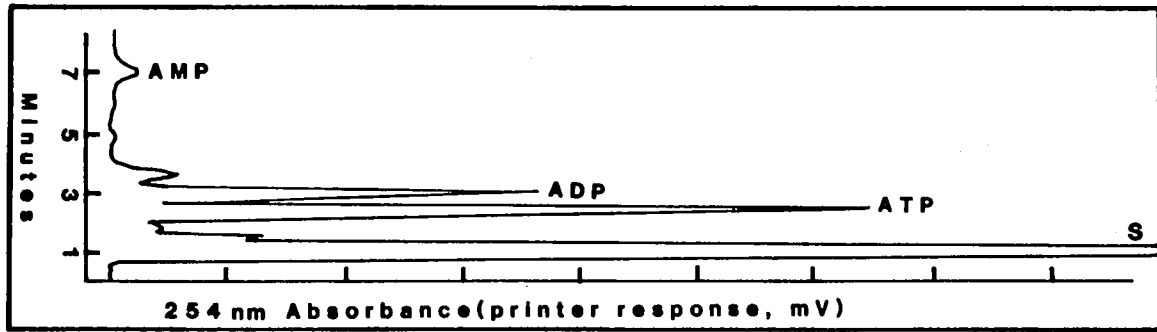


FIG. 1. Chromatogram of acid-extracted adenosine nucleotides from human platelets ( $0.4 \times 10^8$  platelets). S, solvent peak; ATP, 2.84 nmol; ADP, 1.64 nmol; AMP, 0.26 nmol.

HPLC system (HPLC Beckman Model 110A, Palo Alto, California) equipped with a UV-spectrophotometer detector (254 nm), a 0.1-ml injector loop and a C18-Radial-Pak Liquid Chromatography Cartridge column (10  $\mu$ m, Waters Association, Inc., Milford, Massachusetts). According to a preliminary report by Solomons and McDermott,<sup>11</sup> the column was eluted by 0.1 M  $\text{KH}_2\text{PO}_4$ , pH 4.6, and the flow rate was adjusted to 3 ml/min at 800 psi. The separated nucleotides were identified (with external standards) on the basis of retention times (ATP 2.6, ADP 3.1, and AMP 7.0 min). The quantification analysis using the area under the curve was linear between 0.2–20 nmol of nucleotide. This is suitable for analysis in biologic samples. A typical elution profile of a platelet extract is shown in figure 1. All subsequent data are expressed in nmol nucleotide per  $10^8$  platelets and were analyzed for significance by using the Student's *t* test.

### Results

Basal concentrations of ATP, ADP, and AMP in human platelets from PRP kept at room temperature (22–25° C) are shown in table 2. The basal nucleotide levels (before incubation) of control subjects and MH-susceptible patients did not differ significantly. Furthermore, incubation of PRP at 37° C in a shaking water bath for 30 min (after incubation) did not significantly affect the nucleotide contents in the platelets of either group (table 2).

The *in vitro* effects of halothane on nucleotide levels in human platelets are demonstrated in figures 2 and 3. The presence of halothane during 30 min of incubation significantly decreased ATP in platelets of control subjects and MH-susceptible patients. The attenuation of ATP in platelets clearly was dose-related. In contrast, ADP and AMP were not decreased significantly (except for the addition of 20  $\mu$ l halothane when platelet ADP decreased significantly). Furthermore, the effect of halothane (10  $\mu$ l) on ATP in platelets was time-dependent (fig. 3). Depletion of ATP in platelets from MH-susceptible patients and non-susceptible controls did not differ significantly at any dose of halothane or at any time during incubation.

Thymol-free halothane depleted ATP in platelets the same as did the commercial halothane (table 3). Moreover, all of the volatile anesthetic agents studied depleted ATP. When equal volumes of liquid anesthetic agent were used for incubation, enflurane, methoxyflurane, isoflurane, and ether appeared to be less effective than halothane in depleting ATP in platelets (table 3).

Caffeine and potassium chloride have been shown<sup>12,13</sup> by the *in vitro* muscle contracture test to produce a greater response in muscle samples from MH-susceptible patients than from control subjects and to potentiate the contracture response produced by halothane. Therefore, these two compounds were tested in the platelet assay at concentrations equal to that used in contracture tests<sup>12</sup> (table 4). However, neither caffeine nor potassium chloride had any effect on ATP in plate-

TABLE 2. Nucleotide Levels in Platelets from MH-susceptible and Non-susceptible Patients\*

Patients (n)	Incubation	ATP	ADP	AMP
Control Subjects (12)	Before	7.32 $\pm$ 0.19	4.20 $\pm$ 0.09	0.61 $\pm$ 0.04
	After	6.95 $\pm$ 0.23	4.14 $\pm$ 0.13	0.64 $\pm$ 0.03
MH-Susceptible Patients (10)	Before	7.38 $\pm$ 0.24	4.24 $\pm$ 0.20	0.68 $\pm$ 0.10
	After	7.28 $\pm$ 0.24	4.26 $\pm$ 0.19	0.59 $\pm$ 0.04

\* Adenosine nucleotide levels in platelets were measured before and after a 30-minute incubation in a shaking water bath at 37°C.

Data are expressed as nmol/ $10^8$  platelets, mean  $\pm$  SEM.

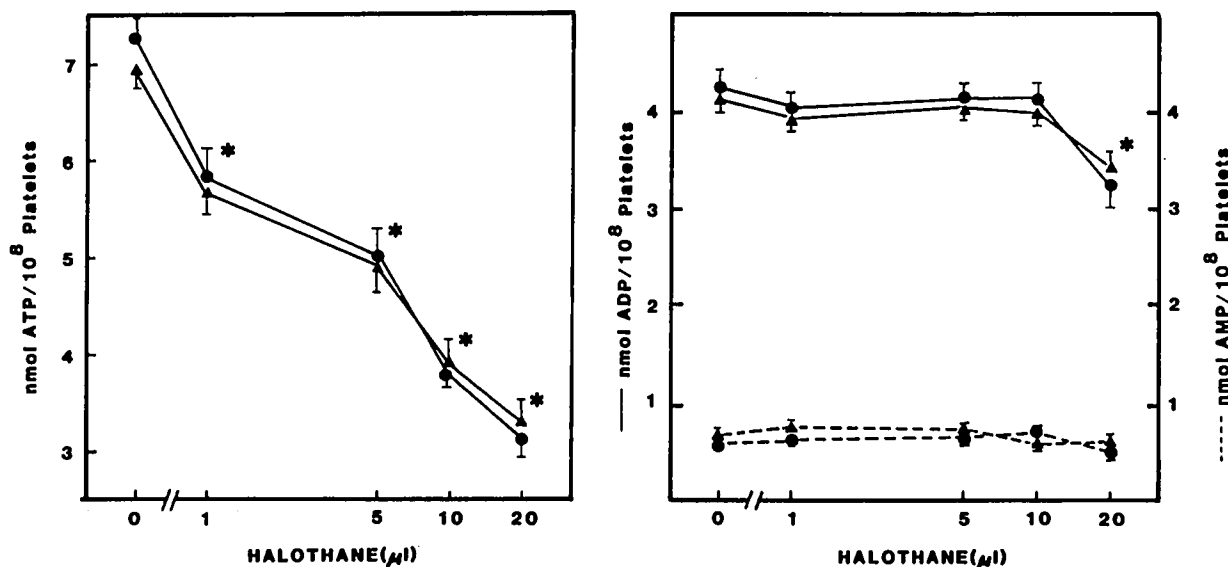


FIG. 2. Dose-response curves of the *in vitro* effect of halothane on nucleotide levels in platelets from MH-susceptible (circle) patients and non-susceptible (triangle) subjects. Platelets ( $2 \times 10^8$  in 1 ml) were incubated 30 min at  $37^\circ\text{C}$  and were either exposed or not exposed to various amounts of halothane. Points represent means  $\pm$  SEM of nucleotide levels from 12 non-susceptible and 10 susceptible patients. \* $P < 0.001$  compared with unexposed platelets. *Left panel*: ATP. *Right panel*: ADP (solid line) and AMP (broken line).

lets or potentiated the depletion of ATP by halothane. Dantrolene sodium attenuates the *in vitro* contracture induced by caffeine in muscle tissue from MH-susceptible patients.<sup>13,14</sup> Concentrations of dantrolene equal to three times that used in muscle contracture tests did not prevent the depletion of ATP by halothane in platelets of either group (table 4).

## Discussion

The rapid and simple isocratic HPLC technique for the analyses of ATP, ADP, and AMP in platelets' acid extract, as described here, appears to be a reliable and valuable tool to determine adenosine nucleotide levels in research and clinical laboratories. Goetz *et al.* have

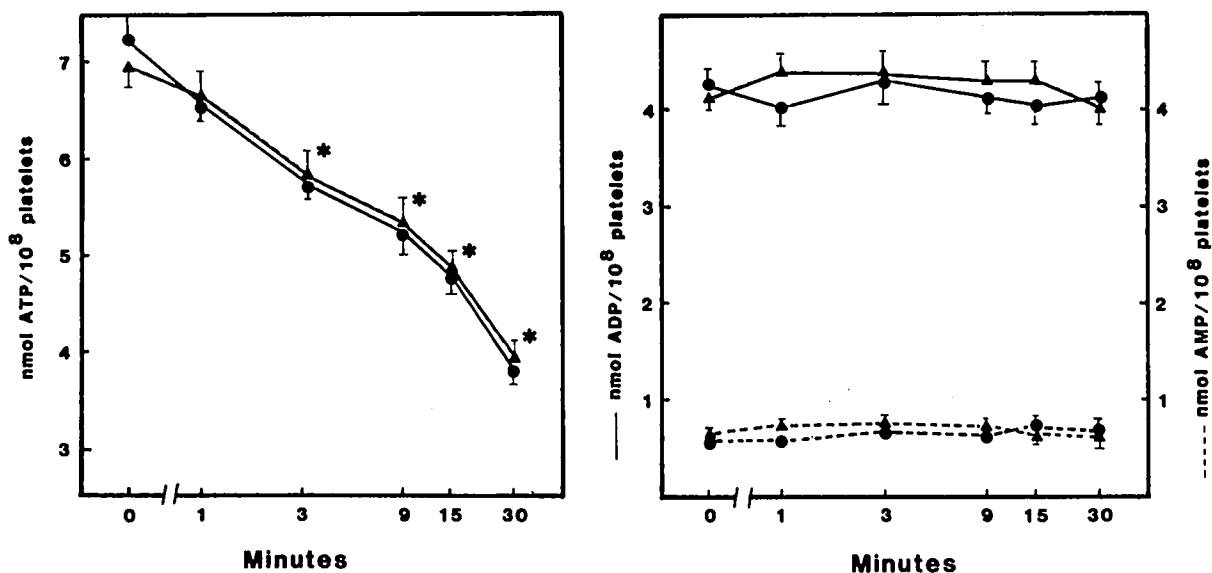


FIG. 3. Time course of the *in vitro* effect of halothane on nucleotide levels in platelets from MH-susceptible (circle) patients and non-susceptible (triangle) subjects. Platelets ( $2 \times 10^8$  in 1 ml PRP) were incubated for 30 min at  $37^\circ\text{C}$  and exposed to  $10 \mu\text{l}$  halothane (final concentration 94 mM) after 0, 15, 21, 27, and 29 min of incubation. Points represent means  $\pm$  SEM of nucleotide levels from 8 to 12 subjects. \* $P < 0.001$  compared with untreated platelets. *Left panel*: ATP. *Right panel*: ADP (solid line) and AMP (broken line).

TABLE 3. Effect of Various Volatile Anesthetics on ATP in Platelets\*

Volatile Anesthetics (10 µl, Incubated 30 min, 37°C)	Non-susceptible Patients	MH-susceptible Patients
None	6.95 ± 0.24 (12)	7.28 ± 0.24 (10)
Halothane (94 mM)	3.90 ± 0.21 (12)	3.82 ± 0.17 (10)
Halothane (without thymol) (94 mM)	3.92 ± 0.12 (9)	3.72 ± 0.20 (6)
Enflurane (82 mM)	5.04 ± 0.27 (6)	4.84 ± 0.44 (5)
Methoxyflurane (86 mM)	4.53 ± 0.25 (6)	4.63 ± 0.26 (5)
Isoflurane (81 mM)	6.04 ± 0.41 (6)	5.03 ± 0.31 (5)
Ether (96 mM)	5.72 ± 0.43 (6)	4.91 ± 0.50 (5)
Chloroform (148 mM)	3.23 ± 0.58 (6)	3.28 ± 0.08 (5)

\* ATP levels from platelets exposed to all volatile anesthetics were significantly different ( $P < 0.001$ ) from untreated platelets. Values are reported as nmol ATP/10<sup>8</sup> platelets, mean ± SEM. (n) = number of patients studied.

shown that 84% of the total amount of nucleotides in human platelets consists of derivatives of adenosine.<sup>15</sup> The sum of adenosine compounds in our platelet studies was 12.2 nmol per 10<sup>8</sup> cells. This closely agrees with published values.<sup>16</sup> A variety of techniques has been used to determine the different adenosine nucleotides.<sup>15,17-20</sup> The most widely used method is the firefly luciferase assay.<sup>17</sup> These techniques yield results that are consistent with the values we obtained for ATP, ADP, and AMP, as well as for the ATP/ADP ratio of 1.7. Previously published HPLC techniques<sup>19,20</sup> used resin anion exchange columns. The advantage of our isocratic HPLC system, which uses a simple C18-column, over the existing chromatographic methods to analyze adenosine nucleotides in platelet extracts are that nucleotides are eluted in less than 10 min and neither a delicate buffer gradient nor a heating system is required.

Halothane is known to affect cellular functions in a variety of tissues. In our study, halothane and other volatile anesthetic agents, when added to PRP, reduced ATP levels in platelets in a dose- and time-dependent manner. Recently, platelets incubated with halothane were shown to lose their ability to aggregate upon addition of ADP, epinephrine, and collagen.<sup>21,22</sup> This in-

hibition of aggregation may have been affected by halothane-induced activation of platelet adenylate cyclase, which increases when platelets are exposed to halothane.<sup>23</sup> This may result in a higher cAMP level, which inhibits platelet aggregation.<sup>24</sup> Enhanced conversion of ATP to cyclic AMP would explain the ATP depletion we observed without affecting ADP and AMP. However, the degradation of other adenosine nucleotide pools beside ATP might have been increased by halothane, but the hydrolysis of ATP might have restored these other pools. More studies are needed to fully understand the mechanism of the halothane-induced inhibition of platelet aggregation and the depletion of ATP in platelets.

Since the cellular defect of MH may be present in tissues other than muscle and since platelets share certain contractile characteristics with muscle cells, platelets have been suggested as a suitable way to diagnose susceptibility to MH.<sup>4</sup> In analogy to the *in vitro* depletion of muscle ATP,<sup>7</sup> we compared platelet basal nucleotide levels and halothane-induced depletion of ATP in platelets from 10 MH-susceptible patients and from 12 unrelated nonsusceptible persons. There were no differences in the two groups. Our data, therefore, do not support those<sup>6</sup> suggesting that MH-susceptibility could be identified from the depletion of platelet ATP by halothane. The methodology and results were not described in detail in that report, so the reasons for the discrepancy are elusive.

The degree of ATP depletion in human muscle has been used as an adjunctive test to detect MH susceptibility.<sup>7</sup> ATP in muscle tissue decreases with incubation at 37° C and this depletion can be potentiated by halothane. The depletion, with or without halothane, was significantly greater in muscle tissue from MH-susceptible patients than from control subjects. Our data plus a report that platelets from MH-susceptible patients have a normal second-wave aggregation<sup>4</sup> may indicate that the metabolic defect of MH manifests in muscle cells but not in platelet metabolism.

In summary, this study describes a simple and rapid isocratic HPLC technique for the analysis of ATP, ADP,

TABLE 4. Effect of Caffeine, Potassium Chloride, or Dantrolene Sodium on Platelet ATP Level in the Presence or Absence of Halothane

Additions	Non-susceptible Patients		MH-susceptible Patients	
	—	Halothane (10 µl)	—	Halothane (10 µl)
None	6.95 ± 0.23 (12)	3.90 ± 0.21 (12)*	7.28 ± 0.24 (10)	3.82 ± 0.17 (10)*
Caffeine (16 mM)	7.31 ± 0.40 (6)	3.53 ± 0.26 (6)*	7.23 ± 0.45 (5)	4.02 ± 3.4 (6)*
Potassium chloride (80 mM)	7.33 ± 0.35 (5)	3.71 ± 0.33 (6)*	6.83 ± 0.66 (4)	3.90 ± 0.36 (4)*
Dantrolene sodium (0.2 mM)	7.12 ± 0.21 (6)	3.64 ± 0.19 (6)*	7.11 ± 0.16 (6)	3.76 ± 0.12 (6)*

Platelets were incubated for 30 min at 37°C with caffeine, potassium chloride, or dantrolene sodium in the absence or presence of halothane. Data represent nmol ATP/10<sup>8</sup> platelets, mean ± SEM with the

numbers of patients in parentheses. \*  $P < 0.001$  significantly different compared with the corresponding platelet preparation in the absence of halothane.

and AMP from acid-extracted human platelets. Our results demonstrate that 1) halothane and other volatile anesthetics decrease ATP levels in platelets *in vitro*, and 2) the halothane-induced ATP depletion in platelets was not significantly different in MH-susceptible patients compared with control subjects. Thus, halothane-induced ATP depletion in platelets is not a reliable test for diagnosing MH susceptibility.

The authors thank Dr. A. H. Neims and Dr. A. S. Keitt, of the University of Florida and the Shands Teaching Hospital and Clinics, Inc., Gainesville, Florida for their support of this study. They also thank Dr. U. A. Meyer and Dr. Ch. Sengupta, Division of Clinical Pharmacology, University of Zurich, Switzerland for initiation of this study. Thymol-free halothane was a gift from Ayerst Laboratories, Inc., New York.

### References

1. Gronert GA: Malignant hyperthermia. *ANESTHESIOLOGY* 53:395-423, 1980
2. Kalow W, Britt BA, Peters P: Rapid simplified techniques for measuring caffeine contracture for patients with malignant hyperthermia, Second International Symposium on Malignant Hyperthermia. Edited by Aldrete JA, Britt BA. New York, Grune and Stratton, 1978, pp 339-350
3. Zsigmond EK, Penner J, Kothary SP: Normal erythrocyte fragility and abnormal platelet aggregation in MH families: A pilot study, Second International Symposium on Malignant Hyperthermia. Edited by Aldrete JA, Britt BA. New York, Grune and Stratton, 1978, pp 213-220
4. Rosenberg H, Fisher CA, Reed SB, Addonizio VP: Platelet aggregation in patients susceptible to malignant hyperthermia. *ANESTHESIOLOGY* 55:621-624, 1981
5. Luscher EF: Microfilaments in blood platelets, *Molecular Basis of Motility*. Edited by Heidmeyer LMG Jr, Ruegg JC, Wieland TH. New York, Springer-Verlag, 1976, pp 175-185
6. Solomons CC, McDermott N, Mahowald M: Screening for malignant hyperthermia with a platelet bioassay (letter). *N Engl J Med* 303:642, 1980
7. Britt BA, Endrenyi L, Kalow W, Peters P: The adenosine triphosphate (ATP) depletion test: Comparison with the caffeine contracture test as a method of diagnosing malignant hyperthermia susceptibility. *Can Anaesth Soc J* 23:624-635, 1976
8. Gronert GA: Muscle contractures and adenosine triphosphate depletion in porcine malignant hyperthermia. *Anesth Analg (Cleve)* 58:367-371, 1979
9. Nelson TE, Flewellen EH: Malignant hyperthermia: Diagnosis, treatment and investigations of a skeletal muscle lesion. *Tex Rep Biol Med* 38:105-120, 1979
10. Allen PD, Ryan JF, Sreter FA: Rigid vs. nonrigid MH, studies of  $Ca^{2+}$  uptake and actinomycin ATPase (abstracted). *ANESTHESIOLOGY* 53:S251, 1980
11. Solomons CC, McDermott N: Use of high pressure liquid chromatography and thin layer chromatography in the rapid detection of human cellular toxicity of environmental substances, *Trace Substances in Environmental Health—XII*. Edited by Hemphill D. Columbia, Missouri, University of Missouri, 1978, pp 447-454
12. Okumura F, Crocker BD, Denborough MA: Identification of susceptibility to malignant hyperthermia in swine. *Br J Anaesth* 51:171-176, 1979
13. Moulds RFW, Denborough MA: Identification of susceptibility to malignant hyperpyrexia. *Br Med J* 2:245-247, 1974
14. Anderson IL, Lipicky RJ, Jones EW: Dantrolene sodium in porcine MH: Studies on isolated muscle strips, Second International Symposium on Malignant Hyperthermia. Edited by Aldrete JA, Britt BA. New York, Grune and Stratton, 1978, pp 509-534
15. Goetz U, de Prada M, Pletscher A: Adenine-guanine and uridine-5-phosphonucleotides in blood platelets and storage organelles of various species. *J Pharmacol Exp Ther* 178:210-215, 1971
16. Gordon JL, Drummond AH: A simple fluorimetric microassay for adenine compounds in platelets and plasma and its application to studies on the platelet release reaction. *Biochem J* 138:165-169, 1974
17. Holmsen H, Holmsen I, Bernhardsen A: Microdetermination of adenosine diphosphate and adenosine triphosphate in plasma with the Firefly Luciferase system. *Anal Biochem* 17:456-473, 1966
18. Mills DCB, Thomas DP: Blood platelet nucleotides in man and other species. *Nature* 222:991-992, 1969
19. Scholar EM, Brown PR, Parks RE, Calabresi P: Nucleotide profiles of the formed elements of human blood determined by high pressure liquid chromatography. *Blood* 41:927-936, 1973
20. Rao GHR, White JG, Jachimowicz AA, Witkop CJ: Nucleotide profiles of normal and abnormal platelets by high pressure liquid chromatography. *J Lab Clin Med* 84:839-850, 1974
21. Ueda I: The effects of volatile general anesthetics on adenosine diphosphate-induced platelet aggregation. *ANESTHESIOLOGY* 34:405-408, 1971
22. Dalsgaard-Nielsen J, Gormsen J: Effects of halothane on platelet function. *Thromb Haemost* 44:143-145, 1980
23. Walter F, Vulliamoz Y, Verosky M, Triner L: Effects of halothane on the cyclic 3',5'-adenosine monophosphate enzyme system in human platelets. *Anesth Analg (Cleve)* 59:856-861, 1980
24. Salzman EW, Weisenberger H: Role of cyclic AMP in platelet function. *Adv Cyclic Nucleotide Res* 1:231-247, 1972