

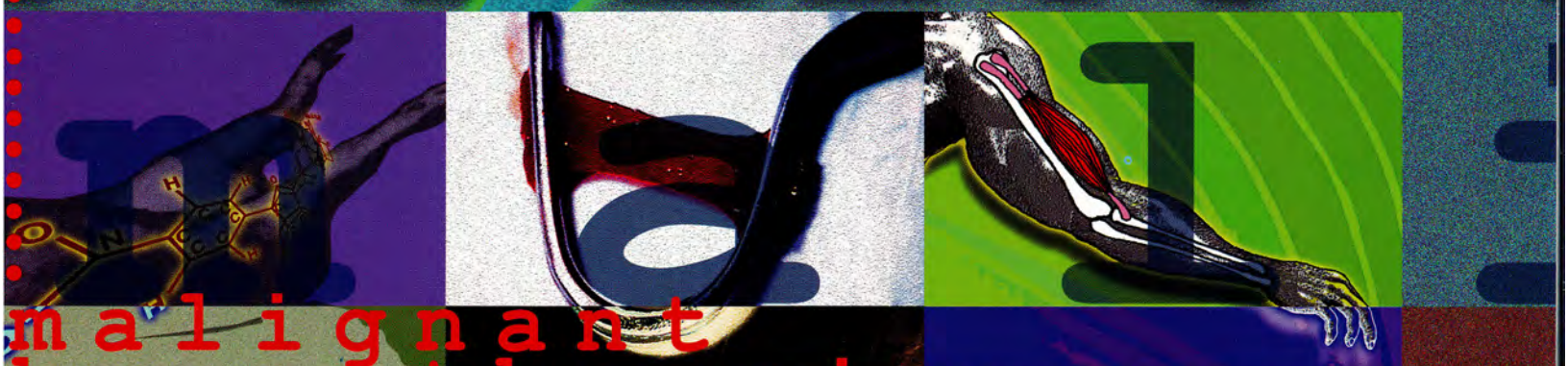
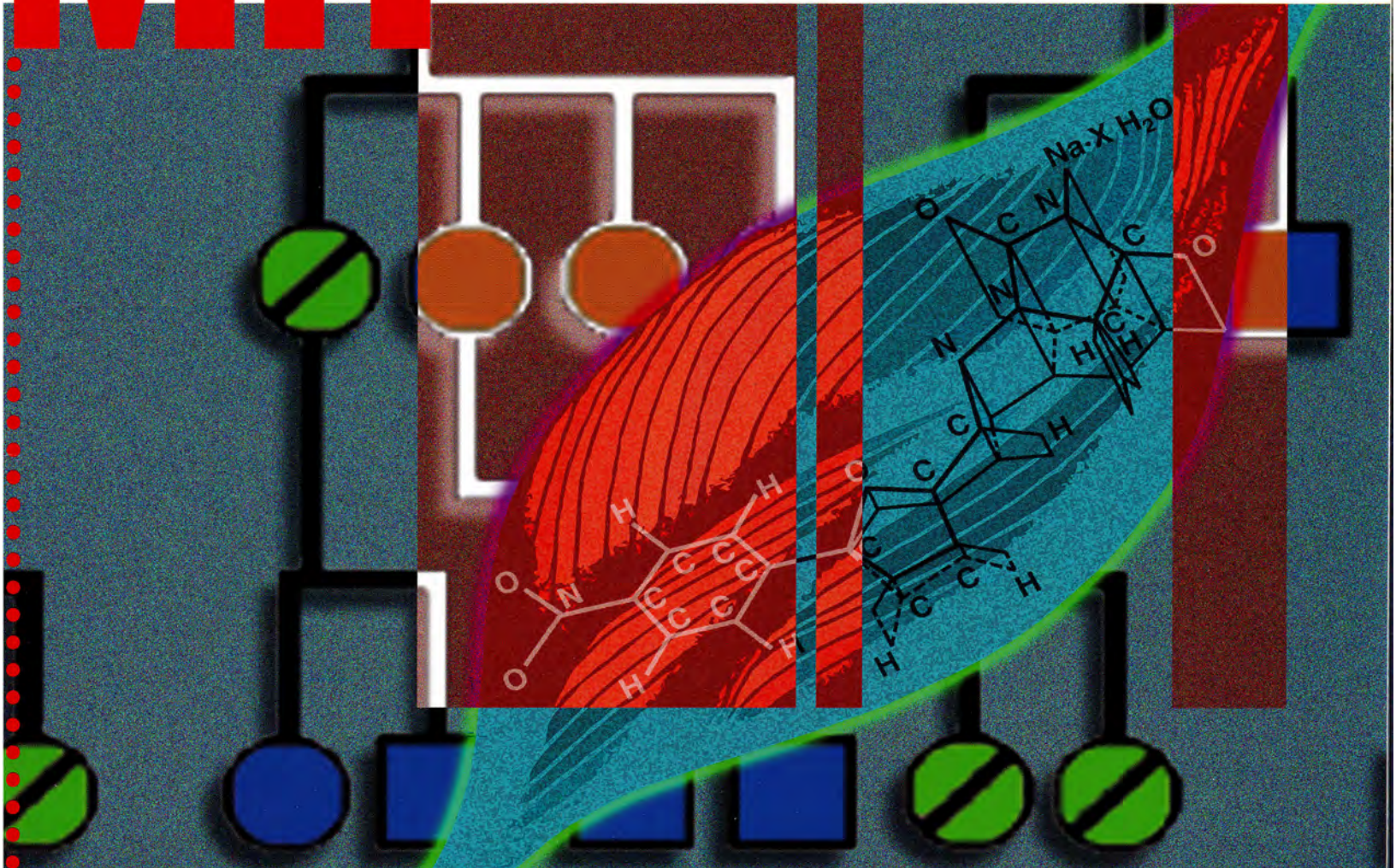
Breakthroughs in Bioscience

Developed by the Federation of American Societies for Experimental Biology (FASEB) to educate the general public about the benefits of fundamental biomedical research.

MMH

Making Anesthesia Safer: Unraveling the Malignant Hyperthermia Puzzle

Marilyn Green Larach, M.D., F.A.A.P.



malignant
hyperthermia

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Anesthesia triggers multiple-familial deaths

Melbourne, Australia, 1960. A young man breaks his leg but does not want anesthesia while it's being set. He is wary because 10 of his relatives have died after they received a general anesthetic for various simple surgeries. Physicians attending the young patient assure him that a newly developed anesthetic, halothane, should be safer than any of those previously given to his unfortunate relatives. Nevertheless, halothane causes the young man's heart to race, his blood pressure to fall, and turns his skin pale and blue.

The anesthesiologist stops administering the drug, but the patient falls into deep unconsciousness, his skin feels hot and sweaty. Ultimately, he recovers, only to return to the hospital with another medical problem that again requires anesthesia. This time, a spinal anesthetic, which numbs only the lower portion of the body, does him no harm.

Intrigued, an internist and his colleagues investigate the young man's family history and discover that this anesthetic problem appears to be inherited as a dominant trait (i.e., a disease transmitted by just one copy of a gene, if one parent has a problem with anesthesia, then each of his or her children will have a 50% chance of being similarly vulnerable; see figure 1). Two years later, the internist in a British journal publishes a seminal report on this patient and his family's difficulties with anesthesia. The authors acknowledge that, "...the nature of the inherited anomaly is not known."

What happened to the young man with the broken leg? As it turned out, he experienced malignant hyperthermia (MH), a syndrome that can afflict susceptible humans and animals that receive general anesthesia. Certain commonly used anesthetic medications may trigger deadly high fever and muscle rigidity and disturb blood chemistry as well.

The following story of MH illustrates the often-circuitous and interrelated nature of medical discoveries, a combination of serendipity and investigator insight.

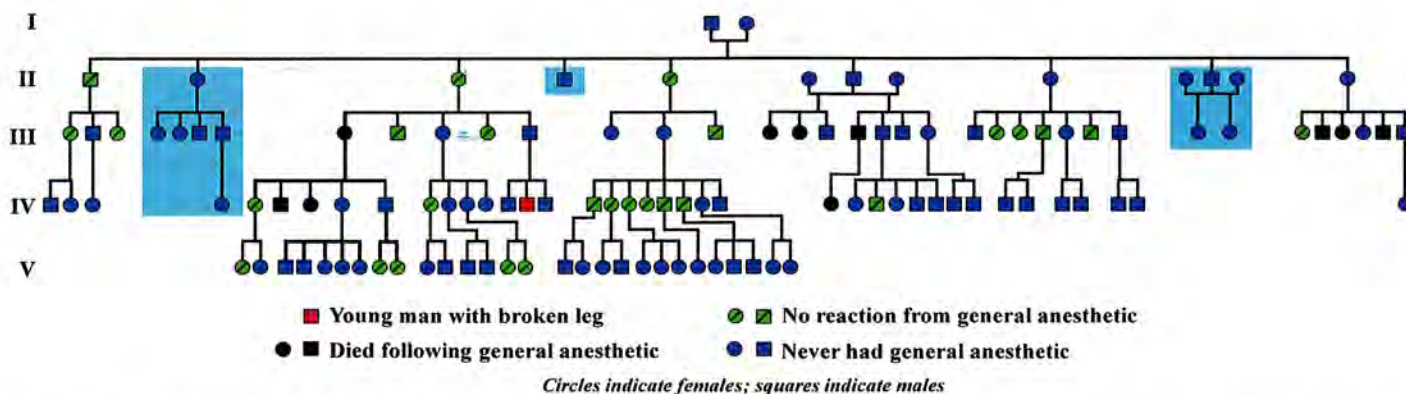


Figure 1. This is the family tree of the young man with the broken leg (red square). The color of the squares and circles shows the outcome of exposure to general anesthesia: black signifies death; green indicates survival. Blue circles and squares and pale blue family branches note lack of experience with general anesthetics. Of the six individuals in generation II whose descendants received anesthetics, deaths occurred in half; their MH crises were evenly divided between males and females indicating that MH susceptibility is a dominant trait carried by the non-sex-determining genes. (Modified with permission from Denborough, MA et al. *Anaesthetic Deaths in a Family*, British Journal of Anaesthesia 34:396, 1962.)

The first signs

It is still the 1960s, when major advances in anesthesia and surgery are allowing surgeons to perform operations that are more complex and to offer them to a wider range of patients. The death rate from anesthesia is dropping rapidly. Although older, sicker patients are sometimes denied surgical procedures because physicians think they're too fragile to undergo the rigors of anesthesia, younger healthy patients rarely die from being anesthetized.

However, anesthesiologists begin to report trouble in young, otherwise healthy patients who receive general anesthesia for surgical procedures that are usually low-risk. Frequently, trouble strikes before surgery even starts. At first, clinicians do not know whether their patients manifest a new disease or whether improved anesthetic safety enables clinicians to recognize a previously undetected disorder which is difficult to differentiate from other fatal illnesses that sometimes strike patients under anesthesia.

Malignant (extremely deadly) hyperthermia (very high temperature) aptly describes this nightmare syndrome, which afflicts some young patients and terrifies their physicians. Whereas anesthesia normally relaxes muscle, patients experiencing a malignant hyperthermia crisis develop stiff and board-like muscles. Slow, regular breathing—the usual condition under anesthesia—increases instead to rapid panting; slow, regular heartbeats degenerate into fast and irregular patterns; pink and warm skin becomes mottled with patches of blue; the body becomes hot to the touch, with temperatures rising by 6 degrees an hour, to peak readings sometimes exceeding 108 degrees Fahrenheit; stable blood pressure plummets dangerously.

As muscle cells deteriorate, massive amounts of potassium, acid, and proteins are released into the blood, deranging the body's chemical balance. The heart pumps poorly and irregularly, the kidneys clog and stop making urine, the liver fails to detoxify the body's waste products, the blood clots improperly, and the brain cells begin to die from lack of oxygen, fuel, and excessive heat. For eight of every ten patients suffering from malignant hyperthermia, routine anesthesia degenerates rapidly into a death spiral. Questions abound.

- Before an anesthetic is given, is there any way to predict which young, apparently healthy patients will develop malignant hyperthermia?

- Which anesthetic agents trigger malignant hyperthermia?
- If anesthesiologists diagnose malignant hyperthermia early enough, will they be able to treat it?
- What better treatments will increase survival once MH has struck?
- Why does routine anesthesia trigger prolonged muscle contraction and deranged body metabolism?

Using the affected patients' abnormal signs as a starting point, researchers realize they need to understand how muscles work, and the manner in which genes govern normal and abnormal muscular function. Ultimately, the search to find the best way to treat patients suffering from an MH crisis will reveal fundamental information on human body function. We shall see that most basic and clinical breakthroughs in the field of malignant hyperthermia will be achieved because academic and pharmaceutical research teams throughout the world worked together.

Serendipitous discovery of an experimental model

Now we're inside a laboratory in South Africa, 1968. Scientists here are developing an experimental model to study liver transplantation. ('Model' describes a non-human means used to study a human disease or procedure; for example, dogs made diabetic were the model used to test insulin in the 1920s, before humans were allowed to take the drug.) By chance, these transplant researchers choose the Landrace pig for their model. Much to the anesthesiologists' chagrin, the first experimental pig responds violently to the anesthetic, halothane, and dies before the liver transplant experiments can begin. Then, five more pigs receive halothane—and each dies (figure 2).

Why should halothane—in 1968 the most widely used anesthetic in the world—kill these pigs? The multidisciplinary research team of anesthesiologists, surgeons, internists, and laboratory technologists interrupt their liver transplantation research in order to find out.

They discover that the anesthetic agents halothane, chloroform, and succinylcholine will ignite "a veritable metabolic storm" in one out of every four pigs. The pigs develop rapid heart rates and breathing, abnormal muscle stiffening, blotchy blueness of the skin, and a rapid rise in temperature. Before they die with a 'rigor mortis' or

corpse-like muscle stiffening, pig muscles lose adenosine triphosphate (a muscle cell's fuel) and release a tremendous amount of acid and potassium, which helps to stop their hearts. Sound familiar? The scientists think so, too, and begin using the Landrace pig as an experimental model for investigating human malignant hyperthermia.

Why do they choose an animal model? Because MH occurs too rarely and unpredictably in humans (about one in 14,000 general anesthetics), researchers will have a difficult time trying to test different treatments on critically ill humans suffering from an acute crisis; hence, the pig. If scientists working with the pig model of MH, are able to diagnose the disease early in its progression and discontinue the triggering anesthetic agents immediately, some of the stricken pigs live. This discovery is significant at the time, because by using this approach, clinicians may be able to lower the 80% mortality rate suffered by humans afflicted with MH.

Working together to discover the triggers

By the 1970s, medical and veterinary researchers in South Africa, England, Canada, and the United States determine that many pigs bred for the attractive traits of lean, heavy muscle may also develop malignant hyperthermia when exposed to certain anesthetic agents that trigger the disease. Researchers still do not understand why these traits should be linked, but they use the susceptible pigs to test new anesthetic medications. When pigs known to be susceptible to MH fail to react adversely to the new anesthetic agents, scientists try the new drug on susceptible humans who are undergoing general anesthesia in the hope of avoiding lethal reactions. The pig model works: investigators find that the pigs' anesthetic experience predicts that of humans.

A Canadian anesthesiologist and her colleagues examine medical histories of U.S. and Canadian malignant hyperthermia susceptible families. They use this data to compile lists of anesthetic agents that appear to have triggered MH crises in these families. Research into pigs and humans determines that, other than nitrous oxide, most inhaled anesthetics can trigger MH in patients with an inherited susceptibility to this disorder, but most anesthetic drugs that patients receive by vein (intravenously) are safe. Medications that numb a part of the body are also safe (local, epidural, and spinal anesthetics).



*Figure 2. Top panel shows pig anesthetized with medications that do not trigger malignant hyperthermia (note relaxed rear legs). Bottom panel shows the same pig developing an MH reaction after receiving halothane. Please inspect the rigidly extended hind limbs. Modified with permission from Harrison GG. *The Discovery of Malignant Hyperthermia in Pigs—Some Personal Recollections*. Malignant Hyperthermia: A Genetic Membrane Disease (Ohnishi, ST and Ohnishi, T, eds) Boca Raton, FL: CRC Press, p. 32, 1994.*

In the 1970s, the pig model of malignant hyperthermia proves to be very successful for identifying triggers of the disease. In no case does an anesthetic drug induce MH in a human unless that drug also causes the disease in pigs. However, the converse is not true. Researchers find that factors other than anesthetic agents can also trigger malignant hyperthermia episodes in susceptible pigs. This shows that the pig is a useful but not a perfect model of human MH. Unexpectedly, the research into pig malignant hyperthermia leads to veterinary advances that have a major economic impact on farmers, meat-packers, and pork consumers.

Researchers learn that susceptible Landrace, Poland China, and Pietrain swine can die when exposed to the stresses of heat, coitus, birth, or a fight. When susceptible pigs are transported to the market for slaughter, they experience stress-induced malignant hyperthermia, which renders their meat inedible. The muscle becomes pale, soft, and oozy, and veterinarians frequently refer to pig malignant hyperthermia as the 'porcine stress syndrome'.

To stave off MH, veterinarians devise a barnyard test to detect it. Young pigs briefly anesthetized with halothane stiffen if they are susceptible. Unlike humans, pigs transmit malignant hyperthermia in a recessive fashion (i.e., two copies of a defective gene are required before the pigs become symptomatic). Researchers observe that generally one out of four pigs in a litter from a susceptible parent may develop MH. Today, early diagnosis combined with selective breeding reduces the frequency of MH in herds, although experts estimate that 1.5% may still carry two copies of the MH gene. The economic benefit of screening pig herds for malignant hyperthermia susceptibility approaches a value of several hundred million dollars per year worldwide.

Predicting susceptibility in humans

In the 1970s, scientists succeed in screening pigs with a barnyard halothane test, but how can they screen humans? How can we diagnose human susceptibility to MH before clinicians administer a potentially fatal anesthetic medication?

Multidisciplinary research teams, led by a pharmacologist and anesthesiologist in Canada in 1970 and an anesthesiologist and neuropathologist in England in 1971, develop a biopsy test that predicts susceptibility to MH. These research teams draw on knowledge generated by decades of basic research into the question of how the

skeletal muscles of our body perform work (see page 5).

For the biopsy test, researchers surgically remove a small piece of muscle and place it in a bath at normal body temperature with chemical concentrations similar to that of the bloodstream (see figure 3). They stimulate the muscle electrically to mimic the current acetylcholine normally generates in muscle, and then introduce halothane—with or without caffeine—to the bath. Scientists choose halothane because they already know that it triggers MH, but why caffeine? Swedish pharmacologists demonstrate that caffeine triggers contraction in frog muscle fibers independent of electrical stimulation even when caffeine only bathes the muscle fiber. They determine that small doses of caffeine produce small muscle contractions and large doses produce large muscle contractions. The ability of caffeine to activate the contractile mechanism within muscle makes it a good choice for studying a syndrome that produces abnormal muscle contraction.

Researchers then compare the force of contraction in muscle taken from patients who survived malignant hyperthermia episodes to the force generated by muscle from individuals who never experienced anesthetic difficulties. Muscle from the malignant hyperthermia patients generates excessive contraction.

While clinicians use the MH muscle biopsy to diagnose patients deemed at high risk for MH, the biopsy cannot be used widely. It cannot screen young patients before anesthesia, because the process requires a substantial amount of muscle that can be removed only under a general or regional anesthetic. In addition, patients must travel to one of the few specialized laboratories that perform this test, since diagnosticians analyze only freshly excised muscle. As a result, most patients still receive anesthesia without knowing whether they are susceptible to MH. Researchers still need to find a better way to identify susceptible individuals.

Developing a grading scale

In 1994, a pediatric anesthesiologist and a biostatistician lead prominent MH researchers and clinicians from the United States, Australia, Canada, Denmark and the United Kingdom in a collaborative effort to produce a malignant hyperthermia grading scale. Their work enables experts to clearly grade the likelihood that an anesthetic problem was MH. This clinical case definition helps researchers sort different anesthetic problems correctly and compare similar groups of patients.

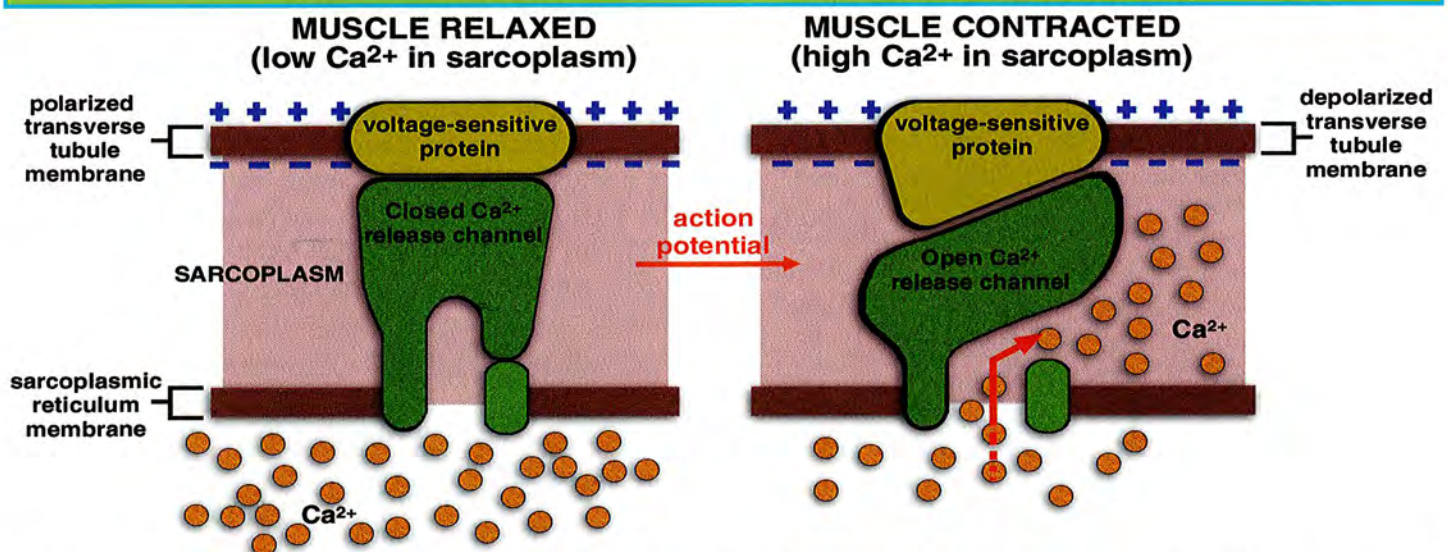
How skeletal muscle normally works

Our skeletal muscles attach to the body's bony skeleton that we consciously use to perform work. To activate one of these muscles, our brain sends an electrical signal to a nerve that ends close to the muscle cell at a place called the neuromuscular junction. The electrical signal helps release a chemical (named 'acetylcholine'), which travels across the junction to the muscle cell. This chemical attaches to special receivers (or 'receptors') on the muscle cell that spread current from the neuromuscular junction to the entire cell surface of the muscle. The current travels deep inside the muscle cell (via 'transverse tubules') to the calcium release channel (called the 'ryanodine receptor'), the gatekeeper for the muscle cell's internal storehouse of calcium (termed the 'sarcoplasmic reticulum').

The calcium release channel opens, allowing calcium to flow from the storehouse to the main part (or 'sarcoplasm') of the muscle cell. The rising concentration of calcium triggers a sliding interaction between the thin ('actin') and thick ('myosin') fibers (or 'filaments') contained within the muscle cell, so that they move towards each other, thereby shortening (contracting) the muscle cell. When the calcium release channel closes, a calcium pump returns the calcium to the storehouse—the sarcoplasmic reticulum. As the calcium concentration in the sarcoplasm surrounding the actin and myosin fibers falls, the filaments slide away from one another and the muscle cell gets longer or relaxes. (See figure 5 and diagram below.)

When the skeletal muscle calcium release channel malfunctions

If the calcium release channel were abnormal, it could flood a muscle cell with calcium and overpower the calcium pump. Excess calcium would prolong muscle cell contraction and cause rigidity, burn up oxygen and fuel supplies (ATP and glucose), and generate excess carbon dioxide, lactic acid, and heat from the work of contracting and as a byproduct of neutralizing the acid. Eventually, the muscle cell would be severely damaged and leak its contents including potassium and proteins normally contained with the muscle cell into the bloodstream. However, a high concentration of the MH treatment drug—dantrolene—inactivates the abnormal calcium release channel stemming the flood of excess calcium into the muscle cell.



This schematic diagram shows how a calcium release channel embedded in the sarcoplasmic reticulum membrane may open when current travels deep inside the muscle cell via the transverse tubules. The current or action potential changes the shape of a voltage sensitive protein in the transverse tubule that, in turn, helps to open the calcium release channel. Once the calcium channel opens, calcium flows from the internal storehouse of calcium into the main part of the muscle cell (or sarcoplasm) and triggers a muscle contraction. Abnormal functioning of the calcium release channel causes all known pig and some human malignant hyperthermia susceptibility. Redrawn with permission from Alberts B, Bray D, Lewis J, Raff M, Roberts K, and Watson JD, *Molecular Biology of the Cell* 3rd edition, Garland Publishing, 1994, New York, p. 853.



Figure 3. This is a small piece of muscle surgically removed from a patient's leg and held taut in a surgical clamp. Laboratory technicians will immerse the muscle in a bath, stimulate it electrically, and expose it to halothane and caffeine to test the patient's MH susceptibility. Reproduced with permission from Karan, SM, Lojeski, EW and Muldoon, SM. *Malignant Hyperthermia*, In *Atlas of Anesthesia: Principles of Anesthetic Techniques and Anesthetic Emergencies, IV.* (Tremper, JJ, ed) Philadelphia: Churchill Livingstone, 1998.

Using the MH grading scale to correctly classify patients who have various problems during anesthesia, MH researchers in Europe (1997) and in North America (1998) validate the muscle biopsy test. Scientists continue to use the biopsy to correctly diagnose individuals at high risk for malignant hyperthermia, because of their family or personal anesthetic experience. Investigators confirm the initial 1960 observation that transmission of MH susceptibility takes place in a dominant fashion.

Preventing death after onset

In 1971, five years after pharmacologists at a New York drug company synthesized a new class of chemicals with muscle relaxant properties, a pharmaceutical researcher collaborates with a pharmacologist at the University of Cincinnati to study the behavior of one of these newly created compounds, called dantrolene sodium. After studying goat skeletal muscle, they see that dantrolene (see figure 4) relaxes skeletal muscle directly, but does

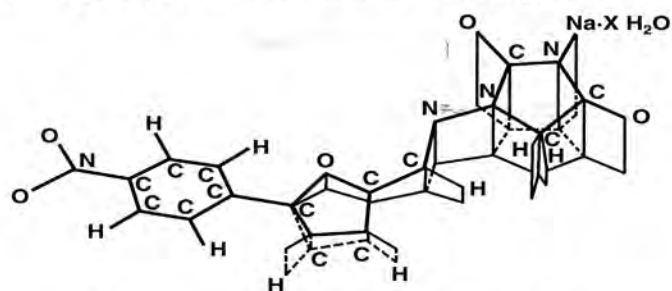


Figure 4. This shows the chemical formula for dantrolene. Reprinted with permission from GG Harrison, *Dantrolene—Dynamics and Kinetics*, In *the British Journal of Anaesthesia*, 60:280, 1988.

not interfere with the heart muscles or the smooth muscles of blood vessels, stomach, intestines, and air passages. The pharmaceutical company hopes that this basic

research will lead to a commercially successful product that will help relax the excessively tight muscles of stroke and cerebral palsy patients.

In 1975, the South African anesthesiologist uses his experimental pig model to test whether dantrolene will abort the metabolic storm produced by MH. Dantrolene reverses the malignant hyperthermia induced muscle rigidity, high muscle temperatures and acid production in seven of the eight pigs treated with dantrolene. The next year, in research partially supported by the National Institutes of Health, an anesthetic research team at the Mayo Clinic in Minnesota confirms and extends the South African findings. The Mayo team compares the outcome of the experimental treatment, dantrolene, to the outcome without the experimental treatment. Malignant hyperthermia-susceptible pigs receive halothane and succinylcholine in order to trigger an MH episode. Five control pigs receive supportive therapy without dantrolene and five pigs receive the same supportive therapy with dantrolene. All five pigs that receive only supportive therapy die; all five pigs that receive supportive therapy plus dantrolene live. Researchers in two different laboratories prove that dantrolene treats malignant hyperthermia successfully in pigs. More questions arise:

- Will dantrolene work for treating MH in humans?
- How can the safety of dantrolene be tested in humans?

The number of patients available to participate in drug tests will be low because malignant hyperthermia episodes occur infrequently. Although scientists know that a few specific families develop MH much more frequently than the general population, it is unethical to

expose members of these families to malignant hyperthermia-triggering anesthetic agents and risk their death in order to test whether a medicine that works in pigs will work in humans.

Of those patients who develop MH, most do so even though there is no known family history of anesthetic difficulty. In the 1970s, three of every ten patients with MH will die in spite of early diagnosis and rapid treatment. Conventional treatment is limited to stopping the anesthetic, administering medicines to neutralize the body's acid production, flooding body cavities with cold fluids, and immersing patients in cold baths. The high human death rate without dantrolene and the high rate of success with dantrolene treatment of pigs make it ethical to experiment on humans.

Because malignant hyperthermia occurs uncommonly and unpredictably, the manufacturer of dantrolene and its clinical research manager begin a multiple center study by enlisting anesthesiologists from 65 institutions in the United States and Canada. Appropriate institutional review boards approve the experimental plan for dantrolene treatment even though patient consent will not be obtained from unconscious patients during an MH emergency.

All the anesthesiologist-investigators commit themselves to a standard method or experimental protocol for recognizing, treating, and reporting cases of malignant hyperthermia. Sixty-five participating institutions stock 60 vials of dantrolene as the anesthesiologist-investigators educate their colleagues about recognizing malignant hyperthermia and treating it with the experimental dantrolene protocol.

Between 1977 and 1979, 11 patients with clear-cut or probable MH enter the study and receive dantrolene promptly. All 11 survive and appear to suffer no side effects. However, four other patients with clear-cut MH receive dantrolene more than 24 hours after the crisis begins, and three of them die. The study indicates that dantrolene, given early in the course of a malignant hyperthermia crisis, is both safe and effective. In 1980, the FDA approves the intravenous use of dantrolene for the treatment of malignant hyperthermia.

Dantrolene remains the only drug effective for the treatment of malignant hyperthermia; its development saves thousands of patients' lives. Through happenstance, and the efforts of many scientific investigators and clinicians, a manufacturer's search for a drug to treat

the common problem of muscle spasm in stroke and cerebral palsy patients produces a drug that saves the lives of those experiencing the uncommon crisis of malignant hyperthermia.

Disseminating what we know

MH organizations form as clinicians and researchers recognize that effective treatment for malignant hyperthermia crises must begin early before it is irreversible. MH-susceptible patients and their families work with clinicians to educate the public about a potentially fatal problem that must be recognized and treated immediately. In the developed world, malignant hyperthermia hotlines are established and staffed by anesthesiologists who volunteer their time to provide continuous expert medical advice to medical professionals confronted with malignant hyperthermia crises, which could occur anywhere that anesthetic agents are given. Calls to MH hotlines originate worldwide from the offices of dentists and surgeons, accident sites during resuscitations, local surgical centers and radiology suites, hospital emergency and intensive care units, and hospital delivery and operating suites. The 80% fatality rate from malignant hyperthermia that occurred in the 1960s plummets to near zero in the United States by the year 2000.

Why patients are still dying

Yet, one to two patients in the United States still die every year from malignant hyperthermia. Young, healthy patients often succumb because anesthesiologists or nurse anesthetists fail to diagnose MH early enough because of inadequate temperature monitoring, or because they diagnose MH appropriately but lack dantrolene to treat it. Many medical facilities outside of hospitals lack dantrolene, even as they continue to administer MH-triggering anesthetics. Patients receiving general anesthesia in surgical offices appear at highest risk. Surgeons and their office administrators sometimes fail to stock dantrolene because of the cost involved.

In many developing countries that use MH-triggering anesthetic agents, death rates remain high because of a lack of dantrolene. Even a medically sophisticated country such as Japan reports a death rate approaching 13% for all malignant hyperthermia cases.

In North America in 1987, clinicians, basic science researchers, and epidemiologists (scientists who study the pattern of how often disease occurs in groups of people) collaborate to develop a registry of this uncommon

disease. The Registry's standardized collection of detailed information on malignant hyperthermia reactions in many patients supports efforts to improve clinical diagnosis, to validate better laboratory tests for malignant hyperthermia susceptibility, and to improve the success of treatment with fewer side effects.

In addition, standardized data collection by the North American Malignant Hyperthermia Registry triggers the analysis of other uncommon anesthetic complications. Working together with the MH Hotline, investigators from the Registry show an association between the administration of anesthetic agents and cardiac arrest in young children and adults with undiagnosed muscle diseases such as muscular dystrophy. These cardiac arrests likely develop because anesthetic agents such as halothane and succinylcholine disturb abnormal muscle membranes. This scientific investigation changes common anesthetic practice, even though the cardiac arrests are due to high potassium levels and not malignant hyperthermia.

Understanding the biology of MH

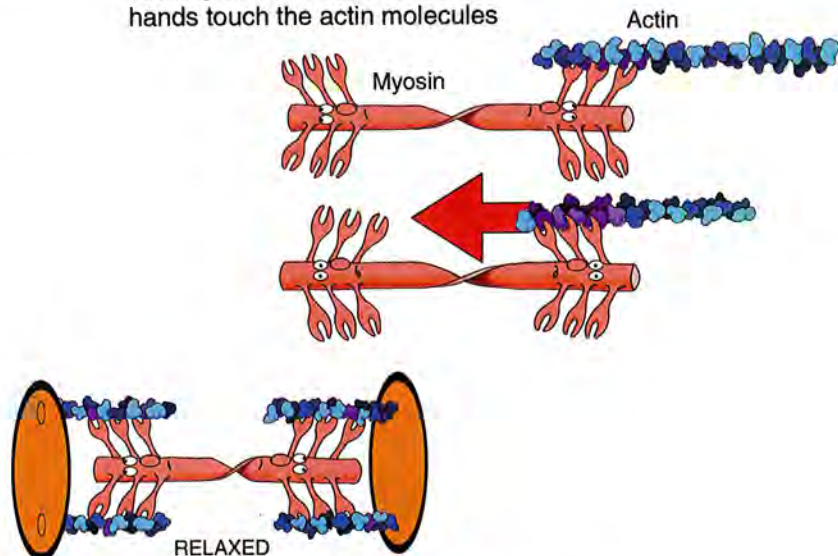
Clinical researchers and epidemiologists uncover a puzzle for the biochemists to study. As first described in the Australian family and confirmed by later investigators using the MH muscle biopsy, humans inherit malignant hyperthermia susceptibility in a dominant fashion. This means that the malignant hyperthermia trait should be distributed equally between sexes and experienced uniformly over all ages in families that carry that trait. Nevertheless, an MH Registry study confirms what earlier Canadian researchers described previously: young males (boys and young men) express their susceptibility with acute MH crises much more frequently than older men or females of any age.

The reported deaths of six Australian girls and women and five Australian boys and men in one MH family were truly exceptional. Ironically, if physi-

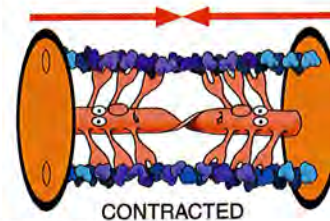
cians had encountered a more typical family in 1960, they would have concluded mistakenly that MH inheritance was linked to gender. Remember, no one had yet invented the muscle biopsy test to detect unexpressed susceptibility to the disease.

Besides genetic susceptibility and exposure to triggering anesthetic agents, what factors in boys and young men, play a role in initiating a malignant hyperthermia

1. Actin molecules are long and thin; myosin molecules are thicker and have many "arms" and "hands" sticking out from their sides. The hands touch the actin molecules



2. Each unit of contraction consists of two identical arrays of actins attached to discs and facing each other, connected by myosin. ATP binds to myosin's hands; in the process, phosphates break off, releasing energy. This causes the hands to grab the actin.



3. The subsequent release of the spent parts of ATP causes the myosin arms to make a stroke like an oar, pulling the actins with their attached discs toward each other; this causes contraction.

Figure 5. This is how skeletal muscle normally contracts and relaxes after our brain sends an electrical signal to a neuromuscular junction. ATP or adenosine triphosphate provides the energy for contraction. Adapted with permission from Hoagland M, Dodson, B, *The Way Life Works*, Times Books-Random House, 1995, United States, p. 106-107.

crisis? Why does male sex and young age increase the chances that a patient will have a bad experience with anesthesia? If we can answer this question, will we be better able to prevent MH episodes from happening? Will we be able to treat the episodes better? The solution to this piece of the MH puzzle awaits further basic research.

The next major breakthrough in understanding the biol-

ogy of MH and, thereby, improving its diagnosis, capitalizes on the development of sophisticated molecular genetics. Thirty years after the initial description of an inherited anesthetic anomaly, two molecular genetic research teams, composed of scientists originally trained as geneticists, biochemists, pharmacologists, anesthesiologists, and neurologists, elucidate a specific basis for this anomaly.

In 1990, two separate research teams simultaneously link the gene—or blueprint—for the skeletal muscle calcium release channel with malignant hyperthermia susceptibility in Canadian and Irish families. A year later, investigators from the University of Toronto and the Ontario Veterinary College isolate and identify a single mutation that codes for an incorrect amino acid located on a gene on pig chromosome 6 which corresponds to a gene on human chromosome 19. Mutations in this gene produce an abnormal calcium release channel. All pig malignant hyperthermia is linked to the presence of a cysteine rather than an arginine amino acid produced by the mistaken substitution of a C (cytosine) for a T (thymine) nucleic acid in the pig DNA. Investigators speculate that the abnormal calcium release channel stimulates spontaneous muscle contraction, which tones pig muscles and produces the heavily muscled and lean pig that animal breeders favor. Unfortunately, when these heavily muscled pigs undergo stress, their abnormal calcium release channels malfunction to inundate their muscle cells with calcium.

The inheritance of human malignant hyperthermia proves more complex. Researchers investigating MH families from many parts of the world analyze stored and freshly obtained blood and tissue specimens to discover more than two dozen additional mutations (errors) in the genes for the calcium release channel. These mutations may explain only half of all human MH susceptibility; the other half awaits discovery. Human complexity should not surprise us given the complicated nature of

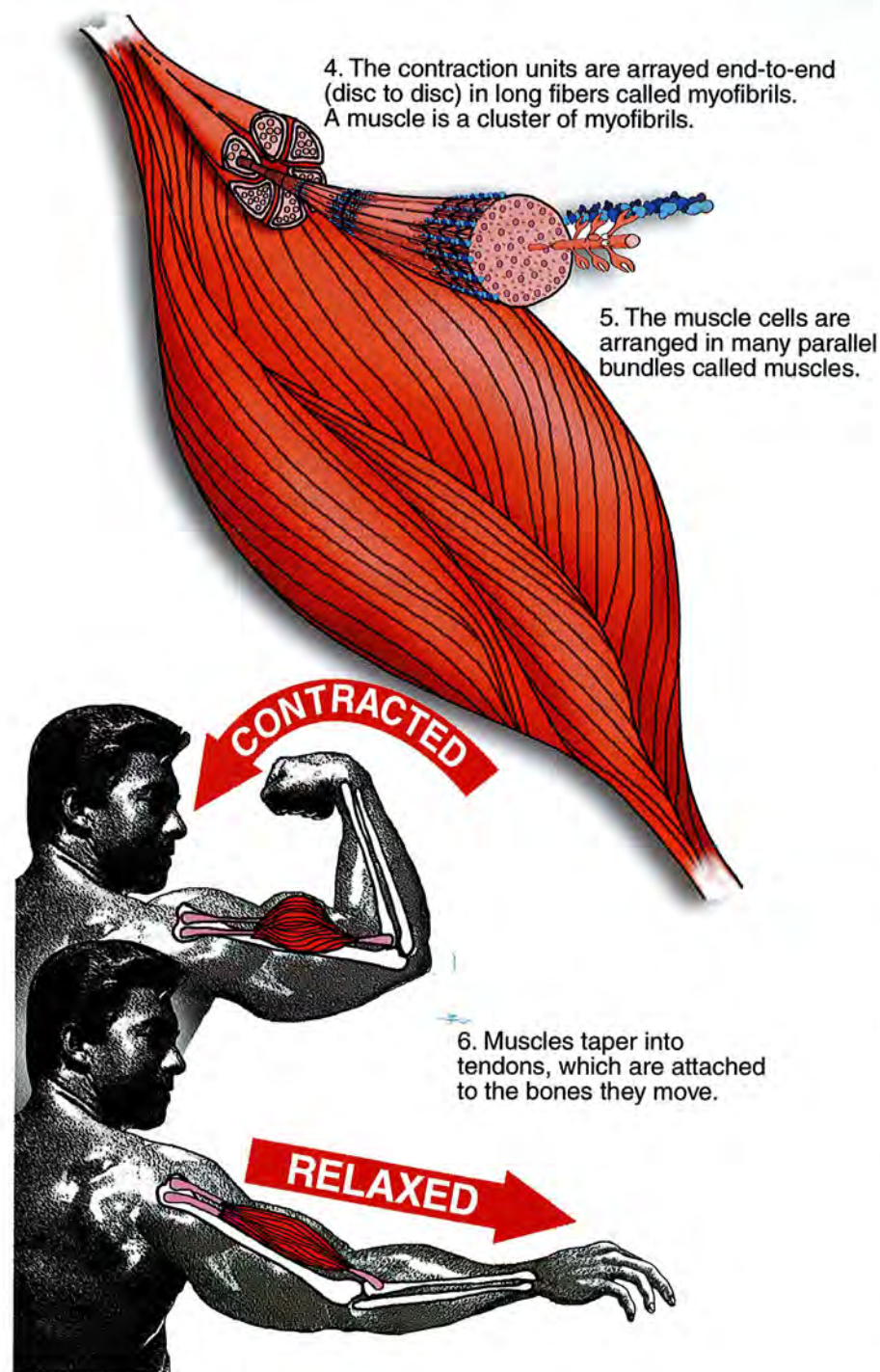


Figure 5, continued

This time line emphasizes the connection between early, basic findings and their effect on future discoveries in scientists' search for an answer to the MH puzzle

1780-1794

In Italy, the physician and physicist L. Galvani uses frogs to demonstrate that muscles contract when stimulated by electricity. By touching the exposed muscle of one frog with the nerve of another, he proves that electric forces exist within living tissue.

1863-1866

In an Austrian monastery garden, G. Mendel studies the manner in which pea plants inherit traits ('factors'). He demonstrates numerically that a pair of these factors controls characteristics (e.g., tallness, shortness) that can be inherited either dominantly or recessively. Despite publication in natural science society journals, Mendel's peers ignore his work during his lifetime. We now recognize Mendel as the father of genetics.

1907

In England, pharmacologist R. Hunt isolates acetylcholine from adrenal gland extracts.

1911

In England, physiologist and pharmacologist H. Dale proposes that acetylcholine transmits nerve impulses.

1921

In Germany, the physician and pharmacologist O. Loewi proves that acetylcholine is involved in transmitting impulses from one nerve cell to another and from the nerve cell to the muscle.

1922

German-American biochemist O. Meyerhof and British physiologist and biophysicist A.V. Hill share the Nobel Prize for Physiology or Medicine for their discoveries that muscles produce heat from the cyclic breakdown of sugars into lactic acid.

1929

With H. Dudley, H. Dale isolates acetylcholine from animal tissues.

1936

In England, H. Dale and his collaborators show that by electrically stimulating the nerve, it releases acetylcholine, which provokes skeletal muscle contraction.

1938-1956

In England, physiologist and biophysicist A.V. Hill and colleagues work with frogs to demonstrate how muscle contraction releases energy in the form of mechanical work and heat.

muscle contraction and the varied genetic background of humans. After all, no one carefully bred us to be lean and heavily muscled.

What's next?

When researchers succeed in identifying all of the genetic errors responsible for malignant hyperthermia in humans, they can develop an accurate screening test for MH. In the future, a small sample of blood will allow anesthesiologists to avoid the commonly used MH triggering anesthetics in patients identified as susceptible. In addition to saving lives, such a test will also reduce medical costs.

Further, as researchers discover each new genetic error, they will be able to explore how the altered gene contributes to muscle cell malfunction. Epidemiologists and molecular biologists will be able to study whether different genetic mutations produce different or identical malignant hyperthermia episodes. Parts of the malignant hyperthermia molecular genetic puzzle remain and await future collaborative scientific effort and insight. It is likely that these basic insights into calcium metabolism and muscle function will be important to our society. For example, heart failure is a very common and frequently fatal disease of heart muscle function. Because of the nature of the medical discovery process, knowledge about abnormalities in calcium metabolism gained from the study of malignant hyperthermia may someday produce therapies for other frequent threats to human life.

Resources

Websites

For a site maintained by the Malignant Hyperthermia Association of the United States for both the public as well as health care providers, see www.mhaus.org

For a website animation of the connection between the brain and muscular movement, see www.innerbody.com/htm/body.html and click on nerve/muscle connection.

Book

For marvelously inventive, easily accessible descriptions linked to brilliant cartoon depictions of skeletal muscle function (pages 106 and 107) and genetic mutation

1939-1946

In Hungary and America, biochemist A. Szent-Gyorgyi discovers actin, a muscle protein that, in combination with myosin, causes muscles to contract by using adenosine triphosphate (ATP) as the energy source.

1958

In Sweden, pharmacologists J. Axelsson and S. Thesslef show that a caffeine bath causes frog muscle fibers to contract even when electrical current has not affected the muscle membrane.

1960

In Australia, internist M.A. Denborough and colleagues determine the familial inheritance of deadly reactions to general anesthesia.

1967

In the United States, organic chemist H.R. Snyder and colleagues synthesize a new class of chemicals, including dantrolene, which appear to cause muscle relaxation in the anesthetized cat model.

1968

In South Africa, anesthesiologist G.G. Harrison and colleagues inadvertently discover that the pig can serve as an experimental model for malignant hyperthermia.

1969

In England, molecular biologist H.E. Huxley and physiologist A.F. Huxley propose the sliding-filament theory of muscle contraction: two muscle proteins, actin and myosin, arranged in partially overlapping filaments, slide past each other through the activity of the energy-rich compound, ATP, during muscle contraction.

1970

In Canada, anesthesiologist B.A. Britt and pharmacologist W. Kalow analyze the medical histories of U.S. and Canadian MH-susceptible families to help predict which anesthetic agents appear to trigger fatal episodes.

1970-1971

In Canada, W. Kalow, B.A. Britt, and colleagues develop a biopsy test to predict susceptibility to MH in humans. In Britain, anesthesiologist F.R. Ellis and neuropathologist D.G.F. Harriman suggest additional methods for performing this biopsy test.

1972-1973

In the United States, pharmacologists K.O. Ellis and S.H. Bryant determine that dantrolene relaxes goat skeletal muscle directly without affecting the other muscles of the body.

(pages 202 and 203), read Mahlon Hoagland's and Bert Dodson's book, *The Way Life Works*. (Times Books-Random House, United States, 1995). If you have time, consider reading the entire book, subtitled "...everything you need to know about the way all life grows, develops, reproduces, and gets along."

Original Scientific Publications

For the seminal report by Denborough of the young man with the broken leg and his family's anesthetic difficulties, see Denborough MA, Forster JFA, Lovell RRH, Maplestone PA, Villiers JD. Anaesthetic deaths in a family. *Br J Anaesth* 1962; 34:395-396.

For Harrison's description of the pig as a model of malignant hyperthermia, see Harrison GG, Saunders SJ, Biebuyck JF, Hickman R, Dent DM, Weaver V, et al. Anaesthetic-induced malignant hyperpyrexia and a method for its prediction. *Br J Anaesth* 1969; 41:844-855.

For Kolb's study of the human dantrolene trial, see Kolb ME, Horne ML, Martz R. Dantrolene in human malignant hyperthermia. *Anesthesiology* 1982; 56:254-262.

For the article describing the malignant hyperthermia clinical grading scale, see Larach MG, Localio AR, Allen GC, Denborough MA, Ellis FR, Gronert GA, et al. A clinical grading scale to predict malignant hyperthermia susceptibility. *Anesthesiology* 1994; 80:771-779.

For MacLennan and McCarthy's initial letters linking genetic defects in the skeletal muscle calcium release channel with malignant hyperthermia susceptibility, see MacLennan DH, Duff C, Zorzato F, Fujii J, Phillips M, Korneluk RG, et al. Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia (letter). *Nature* 1990; 343:559-561 and McCarthy TV, Healy JMS, Heffron JJA, Lehane M, Deufel T, Lehmann-Horn F, et al. Localization of the malignant hyperthermia susceptibility locus to human chromosome 19q12-13.2 (letter). *Nature* 1990; 343:562-564.

For the paper that identifies the amino acid error responsible for abnormal calcium release channels in pigs susceptible to malignant hyperthermia, see Fujii J, Otsu K, Zorzato F, De Leon S, Khanna VK, Weiler JE, et al. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science* 1991; 253:448-451.

1975-1976

In South Africa, G.G. Harrison demonstrates that dantrolene aborts MH in pigs. In the U.S., G. Gronert and colleagues confirm and extend Harrison's findings.

1977-1979

In the United States, clinical research manager M.E. Kolb leads a multi-center study that uses dantrolene to treat human MH crises.

1980

The U.S. Food and Drug Administration (FDA) approves dantrolene for treating human MH.

1985

In the U.S., toxicologist I. Pessah and colleagues identify calcium channel receptors (ryanodine receptors) in rabbit skeletal muscle whose function can be affected by caffeine and calcium. Molecular biologist S. Fleischer and co-investigators isolate these receptors at the ends of the skeletal muscle sarcoplasmic reticulum (storehouses for calcium).

1990

In Canada and Ireland, research teams led by D.H. MacLennan and T.V. McCarthy, simultaneously link the gene for the skeletal muscle calcium release channel with MH susceptibility in humans.

1991

In Canada, D.H. MacLennan and P.J. O'Brien determine that malignant hyperthermia in pigs arises from a single incorrect amino acid, which codes for an abnormal calcium release channel.

1994

Led by North American MH Registry director M.G. Larach and biostatistician, A.R. Localio, international experts from the U.S., Australia, Canada, Denmark and the United Kingdom collaborate to produce an MH clinical grading scale.

1997-1998


European and North American MH research groups validate the MH muscle biopsy tests for predicting human susceptibility to MH.

2000

International researchers describe more than two dozen mutations in the genes for calcium release channels and slow calcium channels that are linked to human MH susceptibility.

Marilyn Green Larach, M.D., F.A.A.P, authored this article. She was the founding director of The North American Malignant Hyperthermia Registry and currently serves as its Senior Research Associate. An associate professor of Anesthesiology at Pennsylvania State University College of Medicine and on the faculty of the Uniformed Services University of the Health Sciences, she uses the registry's extensive database to conduct collaborative epidemiologic studies of malignant hyperthermia.

David H. MacLennan, Ph.D., F.R.S., University Professor in the Banting and Best Department of Medical Research at the University of Toronto, served as science advisor for this article. A biochemist and molecular biologist, Dr. MacLennan investigates skeletal muscle cell calcium metabolism. His work has helped to reveal the molecular genetic basis of malignant hyperthermia in both human and pig.



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