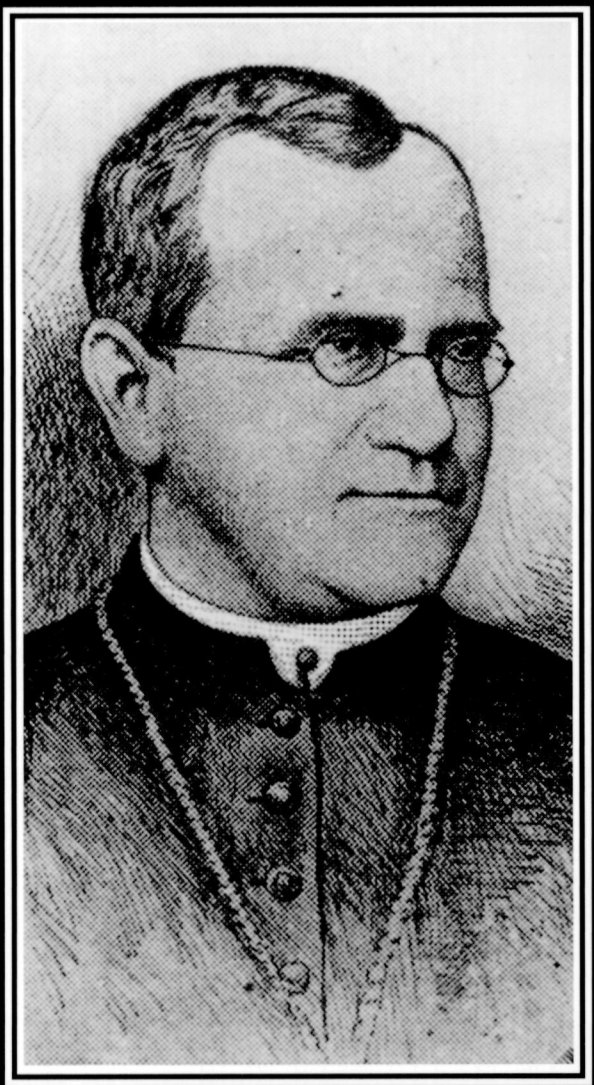
The History of Anesthesiology

Reprint Series: Volume Thirty

Genetic Aspects of the Practice of Anesthesiology



Gregor Mendel (1822-1884)

Although not the very first to experiment on genetic inheritance, Gregor Johann Mendel, a Moravian monk, is given the major credit for his seminal observations in that field. During the latter half of the 19th century, his contributions in that domain consisted of statistical analyses (collectively known as Mendel's law) of the manner in which the leguminous pea (Pisum sativum) reproduces during cross-fertilization. Those features (seed color for example) which were always evident in hybrids were designated as dominant and those not present as recessive, either transmitted as a matter of chance. Ultimately (as the chromosomal theory of inheritance in animals was promulgated) a distinction was made between the genotype (genetic constitution) and phenotype (influence of the environment).

THE HISTORY OF ANESTHESIOLOGY

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Introduction

The multitude of genes along the spiral of the DNA molecule in our chromosomes carry the recipe for an almost infinite variety of proteins that may affect the course of anesthesia. Even at the present stage of knowledge some of these possible effects are only dimly defined as, for example, an individual's susceptibility to narcosis, or the role of anesthesia in the immune response. One category of these relationships has appropriately been designated as Pharmacogenetics wherein genetically established differences are revealed solely by the administration of anesthetic agents. In this realm, over the preceding half century the malignant hyperthermia syndrome has been elucidated. In still another phenomenon, a group of genetically influenced pseudocholinesterases have been implicated in the hydrolysis of succinyldicholine.

The term Pharmacogenetics does not include those genetically transmitted diseases wherein symptoms are precipitated by anesthetic administration as in porphyria variegata or sickle cell disease. On the other hand, Pharmacokinetics concerns the genetics inherent in drug metabolism. Do halothane hepatotoxicity or the metabolism of inhalation anesthetics fall under this rubric, possibly methoxyflurane nephrotoxicity or the chloroform hepatotoxicity of yore? Surely, enzyme induction may be listed under this category. Finally and again only vaguely perceived is a putative genetic influence on Pharmacodynamics.

In this selective collection of reprinted articles we have only begun to scratch the surface of the myriad of genetic interactions involved, part of a major consideration in medicine at large today.

Leroy D. Vandam, M.D.
B. Raymond Fink, M.D.
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GENETIC ASPECTS OF THE PRACTICE OF ANESTHESIOLOGY

Selected Papers

1. Dundee JW, McCleery WNC, McLaughlin G. The hazard of thiopental anaesthesia in porphyria. *Anesth Analg* 1962; 41:567-574

2. Bunker JP, Blumenfeld CM. Liver necrosis after halothane anesthesia. *N Eng J Med* 1963; 268: 531-534

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4. Gronert GA. Malignant hyperthermia. *Anesthesiology* 1980; 53:395-423

5. Whitaker M. Plasmacholinesterase variants and the anaesthetist. *Anaesthesia* 1980; 174-197

6. Morgan PG, Sedensky MM, Meneely PM, Cascorbi HF. The effect of two genes on anesthetic response in the nematode Caenorhabditis elegans. *Anesthesiology* 1988: 69:246-251

7. Evans WE, Relling MV. Pharmacogenomics: Translating functional genomics into rational therapeutics. *Science* 1999; 286:487-491

THE HAZARD OF THIOPENTAL ANAESTHESIA IN PORPHYRIA

Dundee JW, McCleery WNC, McLaughlin G.

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Courtesy of the International Anesthesia Research Society

the hazard of Thiopental Anaesthesia in Porphyria

JOHN W. DUNDEE, M.D., Ph.D., F.F.A.R.C.S.*
W. N. C. McCLEERY, M.B., F.F.A.R.C.S.†
GEORGE McLOUGHLIN, M.D., F.F.A.R.C.S.‡

INTRODUCTION

WHILE INCREASING knowledge of anaesthesiology has reduced fatalities directly attributable to anaesthesia, deaths still occur in cases where obscure or rare pharmacologic contraindications are to blame. One such instance is the use of barbiturates in porphyria. In this communication we describe three illustrative cases and discuss the clinical aspects of the type of porphyria commonly affected by the barbiturates.

Barbiturates are used so commonly in anaesthetic practice today that any disease in which these drugs can precipitate paralysis—often with a fatal outcome — deserves the anaesthesiologist's closest attention. It is felt that a consideration of some of the more important aspects of acute intermittent porphyria warrants publication.

The barbiturates were introduced into clinical medicine in 1903; in 1906, a typical case of acute porphyria was described in a patient after the prolonged administration of diethylbarbituric acid.[1] Numerous clinicians have observed the association between barbiturates and acute intermittent porphyria, especially the occurrence of paralysis.[2-4] Dundee and Riding,[5] reviewing cases reported in the literature between 1948 and 1953, found that of 32 patients with acute intermittent porphyria, thiopental had been administered to 13, all of whom developed paralysis which was fatal in 5 cases.

While in some reported cases the barbiturate can be incriminated only by supposition, in the following 3 cases it seems certain that thiopental given in the presence of haematoporphyria was

*Department of Anaesthetics, The Queen's University of Belfast, Belfast, Northern Ireland.
†Neurological Institute, Montreal, Quebec, Canada.
‡United Liverpool Hospitals, Liverpool, England.

the precipitating cause of unfavourable results.

Case 1. A young woman attended the outpatient department complaining of "haematuria." Preliminary investigations proving negative, she was admitted to hospital for cystoscopy, by which time discolouration of the urine had ceased. The examination was carried out under thiopental sodium (550 mg. in all), nitrous oxide and oxygen anaesthesia. No abnormality was observed, the anaesthesia was uneventful, and recovery was prompt.

Following this, she passed reddish coloured urine and complained of acute abdominal pain. Two days later, a second cystoscopy, under thiopental sodium-nitrous oxide-oxygen anaesthesia again revealed no vesical abnormality. However, recovery was not so prompt on this occasion.

Forty-eight hours later she presented with a clinical picture of an acute abdominal emergency and at this stage the possibility of acute porphyria was considered, as she was still passing dark red coloured urine; later this diagnosis was confirmed by biochemical tests. Unfortunately, paralysis occurred and resulted in a fatal outcome.

Case 2. A woman of 32 years was admitted to a maternity hospital during the eighteenth week of her pregnancy on account of hypertension and "haematuria" of 2 weeks duration.

A cystoscopy was performed under general anaesthesia, but an attempt to pass ureteric catheters had to be abandoned on account of technical difficulties. The anaesthetic on this occasion was nitrous oxide, oxygen and ether. The patient made an excellent recovery from this anaesthetic and after transfer to a general hospital, cystoscopy and ureteric catheterization for retrograde pyelograph was carried out successfully 5 days later. Anaesthesia was induced on this occasion with 350 mg. of thiopental sodium and maintained with cyclopropane and oxygen. Induction, maintenance, and recovery were without incident.

Pyelography revealing a left hypoplastic kidney, it was decided to proceed to nephrectomy at a later date, but on the day following the second cystoscopy the patient developed an evening temperature and was somewhat disorientated. This state of affairs continued with pyrexia for 2 or 3 days, and it was presumed that she was developing a urinary infection. On the fourth day, she became garrulous, had periods of dementia, twitching of the mouth

About the Authors

Dr. Dundee

★ JOHN WHARRY DUNDEE, M.D., is Head of the Department of Anaesthetics at The Queen's University of Belfast. He attended The Queen's University of Belfast, receiving the M.D. degree, and the University of Liverpool where he received his Ph.D. degree. For many years he worked with Professor Cecil Gray in the Anaesthesia Department at the University of Liverpool. Dr. Dundee is a member of the Board of Faculty of Anaesthetists, the Royal College of Surgeons in Ireland, and Council Member and Fellow of the Association of Anaesthetists of Great Britain and Ireland.

★ W. N. C. MCCLEERY, M.B. is on a one-year appointment at the Neurological Institute and McGill University, Montreal, Quebec, Canada, after which he will return to the Department of Anaesthetics at Queen's University, Belfast, Ireland. He holds the degrees of M.B., B.Ch. and B.A.O. from Queen's University, Belfast, and F.F.A.R.C.S. (London).

★ GEORGE MCLOUGHLIN, M.D. is Consultant Anaesthetist to the United Liverpool Hospitals, Liverpool, England, and Clinical Teacher in Anaesthesia at the University of Liverpool. He holds the degrees of M.D. and F.F.A.R.C.S. (London).

and right arm, and complained of weakness of the arms and legs.

Examination showed a general muscular weakness and hypotonia associated with a general hypoaesthesia to pinprick. A consultant psychiatrist diagnosed her case as polyneuritis of toxic origin. There was little change in her condition until the tenth postoperative day, when she developed a cough with diminished air entry, diminished percussion note, and crepitant rales at the right base. Her muscular weakness made coughing ineffective and on this day porphobilinogen was found in the urine and a diagnosis of acute porphyria made.

In spite of active treatment, the chest condition deteriorated and by the thirteenth day, consolidation of the right lower and middle lobes was well advanced. The patient died the next day, 14 days following anaesthesia.

Case 3. A married woman aged 23 was admitted to hospital complaining of abdominal pain and vomiting of 10 days duration; the only other symptoms were loss of appetite and constipation for 2 weeks prior to admission. On examination she was an ill-looking woman with a temperature of 100.4° F.; her tongue was furred, and she had gross halitosis. Splinting and tenderness were present over the right side of the abdomen, being maximal in the right iliac fossae; rebound tenderness was not present. Respiratory, cardiovascular, and central nervous systems were normal. The patient was observed for 24 hours, during which time the abdominal symptoms remained unchanged and the pyrexia continued. Laboratory investigations showed a marked leukocytosis. A laparotomy was performed forthwith, but there was no evidence of intra-abdominal pathology. Anaesthesia consisted of thiopental sodium, gallamine triethiodide, nitrous oxide, and oxygen. The anaesthesia and recovery were uneventful.

After operation the abdominal pain persisted and on the third day she complained of coldness and numbness of her legs and great difficulty rising to the sitting position. On examination there was weakness of the extensors of the knee and of the shoulder girdle muscles and she was unable to maintain her arms outstretched. Biochemical tests confirmed the diagnosis of acute intermittent porphyria.

Over the next week the loss of power in these muscle masses became more marked. She was unable to raise her right leg from the bed; the left leg was less severely involved but walking or standing was impossible; muscle wasting became more obvious as the days passed. However, with intensive physiotherapy, muscle power gradually improved; after 3 months she was able to walk unassisted and the improvement continued over the next year.

COMMENT

In these cases two apparently healthy young women were anaesthetized for relatively simple diagnostic procedures; the barbiturate administered for the induction triggered an acute exacerbation of acute porphyria and led to a fatal outcome. In case three, the patient presented as an acute abdominal emergency, the type of case where prolonged investigation is usually out of place, but in which the differential diagnosis of porphyria is important. Once again, the barbiturate precipitated or accelerated an acute exacerbation of the disease resulting in paralysis of upper and lower limb. Fortunately, the respiratory muscles escaped and over the next year the patient made a good recovery.

PORPHYRIA

Porphyria (also called haematoporphyria) is the generic name given to a group of diseases in which excess porphyrin is formed in the tissues. Although some overlapping occurs, it can be divided into three distinct diseases:[6] (1) porphyria congenita, (2) porphyria cutanea tarda, (3) acute intermittent porphyria.

The relative frequency of the different types of porphyria in America is illustrated by reference to a series of 275 cases collected by Watson.[7] Acute intermittent porphyria formed the largest group (199 cases) which included 39 patients in whom the disease was in the latent form. Porphyria cutanea tarda was present in 55 patients, while there were only 7 of the congenital form. The remaining 14 cases were of the mixed acute intermittent and cutaneous type.

Porphyria Congenita — The basic defect of porphyria congenita probably occurs at an early stage of foetal development. The condition, more common in males, is characterized by an excessive tissue and skeletal content of porphyrins, causing pronounced photosensitivity as a result of which scarring and mutilations occur. There is no disturbance of the nervous system nor do abdominal symptoms occur. Porphobilinogen is not present in the urine and barbiturates do not affect the course of the disease.

Porphyria Cutanea Tarda — This occurs in later life and is not hereditary. It is characterized by photosensitivity and excretion of porphyrins in the urine. A tendency to scleroderma is present, but the disease is distinguished from the congenital type by the absence of gross mutilations. While periodic attacks of colic may occur, the disease differs from the acute intermittent type by the absence of serious nervous disorders. Barbiturates have not been found to have any effect on the condition. Porphobilinogen is not excreted in the urine when the attack is of the cutaneous type.[8]

Table

MANIFESTATIONS OF ACUTE PORPHYRIA IN 119 CASES

Presenting symptoms	Per cent
Abdominal pain	80
Abdominal pain associated with	
Constipation	35
Vomiting	20
Diarrhoea	10
Sensory disturbances	10
Mental changes	10
Entirely neurologic symptoms	20
Symptoms during illness	
Abdominal pain	90
Vomiting	65
Constipation	60
Diarrhoea	10
Nonabdominal pain	40
Paresis	75
Mental changes (hallucination, depression, hysteria)	60
Epilepsy	10
Signs during illness	
Tachycardia	60
Hypertension	50
Pyrexia	30
Diminished or absent reflexes	50
Cranial nerve involvement	30
(bulbar palsy)	10
Sensory abnormalities	50
Laboratory findings during disease	
Leukocytosis (10,000 to 31,000 WBC)	50
Red-coloured urine	60
Abnormal C.S.F.	20
Porphobilinogen in urine	100

A mixed form of this disease has been described by Dean and Barnes[9] (porphyria veriegata). This is a hereditary disease in which cutaneous lesions are associated with abdominal and nervous symptoms. This form is probably very rare in Northern Europe but occurs frequently in South Africa and the Baltic states. In America it accounts for about 5 per cent of the total cases of porphyria. Porphobilinogen is present in the urine only during the acute stage of the disease and the administration of barbiturates may lead to a precipitation of neurologic symptoms, as in cases of Dean and Barnes.

Acute Intermittent Porphyria — This is the condition in which the anaesthesiologist is primarily interested. It is an intermittent illness affecting persons in whom the synthesis of porphyrin pigment is persistently deranged and who generally excrete porphobilinogen in the urine. It is distinguished from the other main forms of porphyria by the absence of photosensitivity and the presence of porphobilinogen. Acute intermittent porphyria is not as rare as is commonly supposed. However, there is a marked geographical distribution in its frequency and it is more common in the Scandanavian countries and in South Africa.

Aetiology — The deranged synthesis of porphyrin pigment in acute porphyria is an inborn disorder, transmission being of the mendelian-dominant type and not sex-linked. Barker and Estes[10] first reported in 1912 the disease occurring in several members of the same family; in a recent series of 50 cases, 38 per cent had one or more relatives similarly affected.[11] In considering these figures, it must be remembered that in the latent phase of the disease, diagnosis is very difficult. There are no symptoms or signs and porphobilinogen may be detected only occasionally in any one case, even by such sensitive tests as paper chromography. These cases may, however, begin excreting porphobilinogen again if provoked and may go on to present gross intestinal or neurologic symptoms. Goldberg[11] describes 18 cases with previous gastrointestinal or neuro-

logic symptoms; in 5 cases, the excretion of porphobilinogen had ceased on re-examination during remissions. Thus, the absence of porphobilinogen in the urine on one occasion is inconclusive.

Pathology — Gibson and Goldberg[12] have shown that the significant lesions of the disease are multiple, segmental, scattered areas of demyelination of peripheral nerves, autonomic as well as sensory and motor but, if the reaction is very severe, the axon itself may be damaged. Demyelination of the autonomic nerves will lead to symptoms of abdominal pain and bowel upset; while the involvement of the peripheral motor and sensory nerves leads to paralysis and sensory loss; involvement of the cranial nerves, especially the vagus, is common. The pathology of bulbar palsy and respiratory failure is thus easily understood.

The central nervous system does not escape. Chromatolysis of the motor nerve cells in the spinal cord and medulla is frequent, while in the cerebellum, foci of demyelination of the white matter with slight shrinking of the Purkinjae cells is seen. In the cerebrum, areas of demyelination, especially centered around blood vessels, are present most often in the parietal lobes, but the temporal and occipital lobes do not escape. From these lesions psychiatric symptoms occur in 60 per cent of cases.

Porphobilinogen or porphyrins in general are not increased around these areas of degeneration, so the demyelination is probably not directly due to these substances. Peters and his colleagues[13] have suggested that the symptoms are due to zinc, copper, or other cationic block of several metallo-enzyme systems. This results in exhaustion of certain portions of the body's natural chelative defenses, subsequently leading to depletion of porphyrins for purposes of chelation, thus impoverishing metabolism and affecting the maintenance of myelin and the cytochrome systems that interrelate with the porphyrin pool. They suggest the use of BAL (dimercaprol) for chelation therapy.

The liver is probably the site of the deranged synthesis of the porphyrins. During acute phases, the liver shows central lobular necrosis, but liver failure is not common.[14]

Biochemistry — While uroporphyrins are often present in the urine in acute porphyria, they may be found in large quantities in other conditions also, or may be absent during acute exacerbations of this disease; thus, the diagnostic importance of uroporphyrine in the past has been overrated. Porphobilinogen, on the other hand, is always present during acute exacerbations. During latent periods the excretion may be intermittent and the urine must be tested on several occasions before a positive result is obtained. Porphobilinogen is probably a normal intermediary in haemopoiesis, consisting of one pyrrole ring. It is colourless in the urine but this may be coloured by other porphyrin derivatives, mainly uroporphyrins.

When first voided, the urine containing porphobilinogen is usually normal in colour, and darkens only when left in daylight for a few hours. This gives a simple test which can be used in the absence of laboratory facilities:[4]

Leave the urine in daylight for a few hours; there is a definite deepening of colour compared with the same specimen kept in the dark. A more refined version of this test is to heat some of the urine in a water bath for 10 minutes and compare with on unheated specimen. This simple test demonstrates the presence of derivatives of porphrobilinogen, not porphobilinogen itself. After blood transfusion a false positive may be obtained.

The modified Watson-Schwartz[15] test for the presence of porphobilinogen is generally used in the laboratory:

Take 2 ml. of urine and 2 ml. of Erlich's aldehyde reagent (0.7 gm. of P-dimethyl-amino benzaldehyde in 250 ml. of 6N HCl.). Mix and allow to stand for 2 minutes and compare with a mixture of 2 ml. of urine and 2 ml. of 6N HCl. (This safeguards against artifacts

due to certain ingested foodstuffs.) A red colour develops in 2 to 3 minutes in the presence of porphobilinogen or urobilinogen. These can be distinguished by the addition of 4 ml. of amyl alcohol; urobilinogen dissolves in the alcohol while porphobilinogen remains in the aqueous solution. (Amyl alcohol is a better solvent than the more commonly used chloroform as urobilinogen is more soluble in it.)[16]

Clinical Picture — Waldenstrom[6] divides acute intermittent porphyria into 5 clinical entities: (1) Latent—no symptoms or signs, but porphobilinogen has been demonstrated in the urine on at least one occasion. (2) Purely abdominal symptoms with no involvement of the nervous system. (3) Purely neurologic with no symptoms referrable to the abdomen. (4) Mixed abdominal and neurologic symptoms. (5) Terminal comatosed form.

The disease in any one patient may change from one group to the other in different episodes or even during a single acute exacerbation.

The signs and symptoms of acute intermittent porphyria will depend on exactly which nerves become demyelinated. The classical case presents with colicy abdominal pain, muscular weakness, and paralysis, psychiatric manifestations, and red-coloured urine. This description is often of little assistance in diagnosing the early stages of the disease, since the onset of an acute attack is frequently gradual, unless hastened by the administration of barbiturates. The patient may complain of nothing more than feeling "off colour," and objective sypmtoms may be absent apart from indefinite mental changes. Insomnia is a frequent symptom and may lead to the administration of a barbiturate.

As this disease has been called "The Little Simulator," it is important for the anaesthesiologist to know the frequency of the common presenting symptoms. Their incidence in two large published series[11, 17] is shown in the table. In our cases 1 and 2 it simulated renal disease, while in case 3, an abdominal emergency was suspected.

The abdominal pain when present is predominantly colicy, but there may be periods of hours or days when it is constant. It may occur in any part of the abdomen but it is most often in the right iliac fossa, or in the epigastrium. It may be deep-seated and cause great distress. All patients show some abdominal tenderness on examination but less than would be expected from the severity of the pain. Rigidity is generally absent and vomiting may occur either before or after the onset of abdominal pain. Constipation is severe and impaction of faeces common. Of the 50 cases described by Goldberg[11] 6 were subjected to laparotomy. Of the 12 deaths in his series (25 per cent mortality), all but one died of general or respiratory paralysis.

TREATMENT OF AN ACUTE EXACERBATION OF ACUTE PORPHYRIA

Prophylaxis: Avoidance of Barbiturates — In one series, 77 per cent of the patients with paralysis had received barbiturates, while only 35 per cent of the patients without paralysis had received the drug.[11] The question arises as to whether acute porphyria can be caused by the ingestion of barbiturates in a previously normal patient. Animal work[18] has shown that only barbiturates with an allyl group caused an increase in the porphyrins in the urine, and this occurred only after prolonged administration of sublethal dosage. Porphobilinogen could not be demonstrated except in very small quantities of doubtful significance and only after diallylbarbituric acid.

In man, With[19] examined the urine of 25 cases of acute and 25 cases of chronic barbiturate poisoning and found no rise in the level of the porphyrins in the urine. Porphobilinogen was never demonstrated.

It would appear that barbiturates per se do not cause acute porphyria, but that barbiturates, especially those containing the allyl group, given over a long period

to a person with a genetic defect, may precipitate an attack. There also seems to be an association between the administration of barbiturates during an attack and the onset of paralysis.

Sulphonamides, alcohol, heavy metals, and exposure to oil-based paints or paint solvents also have been incriminated in the production of exacerbation on occasions. All these precipitating causes should be avoided in a known porphyric.

Treatment of the Acute Attack — The damage to the myelin sheath is reversible and, as in tetanus, provided the patient can be kept alive during the acute stage (which may last 2 to 3 months), the prognosis should not be as bad as the literature suggests. It will be remembered that 11 of the 12 deaths reported by Goldberg[11] were due to general or respiratory paralysis. These cases require intensive care, preferably in a respiratory-failure unit.

In the treatment of the abdominal pain, chlorpromazine[20] is of value; but if the pain is severe, pethidine (meperidine) or similar drugs should not be withheld through fear of addiction. The pain may be due to changes in the motor innervation of the gut, leading to irregular peristalsis and disturbed mobility; thus, in 1922 Gunther[21] recommended vagotomy in severe cases. More recently, neostigmine and ganglionic blocking drugs have been used with good results on occasion.[22, 23]

In the specific treatment of the disease cortisone and ACTH[17] have been tried, in a few instances with apparently good results but on the whole disappointing. Chelation therapy has shown promise; in one reported series[13] 31 of 37 cases showed a marked improvement; 50 to 1200 mg. of BAL were given daily in divided doses. Another chelation agent (E.D.T.A., tetrasodium salt) has given similar results. Confirmation of this work will be awaited with interest.

ANAESTHESIA IN ACUTE INTERMITTENT PORPHYRIA

On occasion, known porphyrics present for anaesthesia; all the inhalation anaesthetic agents and the analgesic agents appear quite safe and can be used with impunity. Barbiturates are, of course, contraindicated. This disease is possibly the only absolute contraindication to the use of thiopental sodium as an induction agent.

All the relaxants have been used without any reported complications, but it has been suggested that, as certain synthetic anticholinesterase drugs which are used as insecticides can produce demyelination themselves, related drugs, such as neostigmine, should be avoided. Succinylcholine has been recommended for the production of relaxation in preference to the use of the nondepolarizing relaxants which might require use of neostigmine as an antidote.[24]

From the medicolegal point of view, spinal, epidural, and regional nerve blocks are probably best avoided, lest the blocks be blamed for neurogenic lesions which subsequently occur during the course of the disease.

CONCLUSIONS

The diagnosis of acute intermittent porphyria is difficult. Syphilis ("The Great Simulator") can usually be excluded by examination of pupils and knee-jerks. No such simple clinical examination will exclude the "Little Simulator." Only an awareness of the disease and an open mind, especially in emergency abdominal surgery and in cases of "haematuria" will suggest the possibility of this condition and lead to the testing of the urine for porphobilinogen. Once the diagnosis is made, the patient should be warned of the danger of barbiturates, and his relatives investigated and also suitably advised.

The role of the anaesthesiologist in porphyria is not limited to suspecting the condition and avoiding the use of barbiturates. He has a major role in the treatment of this illness, when severe neurologic involvement is present. Perhaps the mortality in the future can be reduced from the 25 per cent reported by Goldberg[11] and the 30 per cent reported by Dundee and Riding,[5] when the

anaesthesiologist and the respiratory failure team cooperate with the physician in the treatment of this disease.

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LIVER NECROSIS AFTER HALOTHANE ANESTHESIA

John P. Bunker and Charles M. Blumenfeld

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Courtesy of the Massachusetts Medical Society

LIVER NECROSIS AFTER HALOTHANE ANESTHESIA
Cause or Coincidence?

John P. Bunker, M.D., and Charles M. Blumenfeld, M.D.

IN 1956, after extensive pharmacologic studies, a new, potent and nonexplosive volatile anesthetic agent, halothane (Fluothane), or 2-bromo-2-chloro-1,1,1-trifluoroethane, was introduced into clinical practice.[1,2] Today, it is one of the most popular and widely used of all inhalation anesthetics. The danger that this compound, in common with other halogenated hydrocarbons such as chloroform, tribromoethanol and carbon tetrachloride, might damage the liver has been recognized, but to date only a handful of cases of possible halothane-induced toxic hepatitis have been reported.[3-7] Two additional cases occurring in the practice of one of us (C.M.B.) are the subject of this report.

Case Reports

Case 1. N.R., a 16-year-old girl, was admitted to the hospital on June 1, 1961, for repair of a deep laceration of the right wrist, sustained when she fell through the glass window of a door 55 minutes previously. Routine history and physical examination were unremarkable. Surgical repair was performed on June 2, 5 hours after admission. Anesthesia was induced with thiopental (Pentothal) and maintained with halothane and oxygen, lasting from 1:15 a.m. to 5:55 a.m. Respirations were controlled or assisted initially but were spontaneous during most of the procedure. The blood pressure was well maintained throughout. The postoperative course was uneventful until the 6th day. On June 8 the temperature rose to 100°F., and the pulse to 100. On the next 2 days the temperature rose to 104.5°F., and the pulse to 160 or higher. The temperature was 104.4°F. on June 11 and thereafter gradually fell to below normal. The white-cell count, which was 8450 on June 10, dropped to 3610 on June 11, but rose to 21,800 on June 13, with a marked neutrophilic leukocytosis and a shift to the left. Renal output began to fall on June 12, and thereafter the patient became virtually anuric. A tendency to bleed was noted, and a prothrombin determination on June 13 was less than 10 per cent of normal. A severe nosebleed developed on June 14; bleeding originated in the left anterior nasal septum and was controlled by cauterization. Cerebral symptoms, ranging from listlessness to yelling and screaming, appeared during the last few days of life. She died on June 15, 13 days after surgery.

At post-mortem examination major findings were limited to the liver and kidney. Sections from the right and left lobes of the liver showed profound necrosis (Fig. 1) that was estimated to have destroyed more than 4/5 of the liver. Liver cells in the vicinity of portal canals, still showing stainable nuclei, were in general altered by marked marginal vacuolization of the cytoplasm. The necrotic cells were hyaline and also indicated extensive vacuolization. Sheaves of greenish-brown crystals, consistent in appearance with tyrosine, were present in abundance. Occasional bile canaliculi with small plugs of bile were observed. The portal canals contained an infiltrate of lymphocytes, occasional eosinophils and rare neutrophils.

Sections of the kidneys showed degenerative changes commonly associated with liver necrosis, involving primarily the convoluted portions of the collecting tubules. Other changes included slight focal myocarditis, acute hyperemia of the spleen and aspirated blood and vegetable matter in the respiratory tree.

Case 2. W.W., a 67-year-old man, was admitted to the hospital January 23 and died on February 11, 1962. His chief complaint on admission was severe pain in the upper abdomen of about 4 hours' duration. Illness had begun 2 weeks previously, with intermittent pain in the upper abdomen. There was a past history of alcoholism, although not during the last few years, of duodenal ulcer and of rectal polyps. On admission he was ashen and dyspneic and, according to 1 observer, cyanotic. The temperature on the day of admission was recorded as varying between 97.6 and 99°F. by mouth, and the pulse between 60 and 90, and the respirations were 20. The blood pressure on the day of admission varied between 90/60 and 130/80. The chest was described as emphysematous. The lung fields were reported clear by 1 physician; another described a few rales, dullness and diminished breath sounds over the left lower lobe. The abdomen was boardlike, and no peristalsis was heard. The prostate was twice the normal size. The hemoglobin was 17.9 gm. per 100 ml., the hematocrit 53 per cent, and the white-cell count 9700; the coagulation time (Lee–White method) was 13 minutes, and the bleeding time 2 minutes. Laparotomy was performed 8 hours after admission. Anesthesia was induced with methohexital (Brevital)

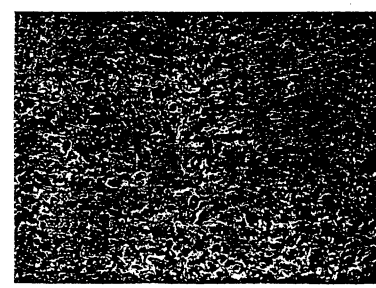

Figure 1. *Massive Midzonal and Central Necrosis of the Liver Lobules, with Preservation of Cells Surrounding the Portal Canal, in Case 1 (Hematoxylin and Eosin Stain X90).*

and maintained with halothane, nitrous oxide and oxygen, with succinylcholine (Anectine) for relaxation. Respirations were controlled throughout, and the blood pressure was well maintained. The duration of anesthesia was 1 hour and 25 minutes. A perforated ulcer of the anterior wall of the duodenum was found and repaired. The liver was described as normal in size and appearance. After operation there was progressive improvement except for some continued respiratory difficulty. On February 2 hematemesis and melena with gross blood occurred. After a transfusion a 2d laparotomy was performed. On this occasion anesthesia was induced with thiopental and maintained with endotracheally administered halothane, nitrous oxide and oxy-

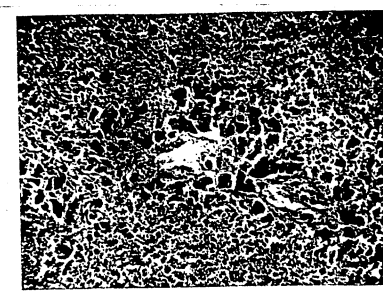

Figure 2. *Massive Central Necrosis of the Liver Lobules, Including Marked Damage of a Few Surviving Cells Surrounding the Portal Canal, in Case 2 (Hematoxylin and Eosin Stain X120).*

gen, with succinylcholine for muscle relaxation. The blood pressure was approximately 80/60 mm. during the 1st ½ hour of surgery, but gradually rose to 120/80 as additional

transfusions were administered during surgery. The duration of anesthesia was 4 hours and 45 minutes. An ulcer of the posterior duodenal wall, opposite the perforated one in the anterior wall, was found, with an eroded large artery in its base. The artery was sutured, and gastric resection was done. The liver again appeared to be normal. The early postoperative course was uneventful, and after 4 days the patient was taking fluids normally. The temperature rose to 101.2°F. on the evening of February 6, the 4th day after the 2d operation, and to 103°F. on the following day. On February 8 the temperature was 104.4°F. by rectum, the pulse 108 and the respirations 26. Jaundice became apparent, and was verified by an elevated serum bilirubin. Renal output decreased, and the blood urea nitrogen rose to 96 mg. per 100 ml. On February 11 the serum bilirubin rose to 10 mg. per 100 ml., and renal output ceased. The patient died that evening, 19 days after the 1st and 9 days after the 2d operation.

At post-mortem examination the liver showed severe acute necrosis (Fig. 2). Small islands of still recognizable liver cells were present around the portal canals. These demonstrated considerable damage, characterized by a dense granular cytoplasm, marked variation in size of nuclei and hyperchromatism of many of the nuclei. Occasionally, scattered liver cells were seen somewhat more within the interior of a lobule, but in a majority of places coarsely granular necrotic debris and hemorrhage were all that was left of the remainder of each lobule. Cellular reaction was sparse and was mainly of lymphocytes or macrophages. The debris of necrotic cells was often acidophilic and homogeneous. There was no evidence of cirrhosis or of interstitial hepatitis to suggest pre-existing liver disease.

Renal changes similar to those in Case 1, including necrosis of the epithelial lining of the convoluted tubules, were observed. Large, homogeneous casts occurred in the convoluted and collecting tubules, and there were refractile crystals consistent with leucine. In addition there was marked coronary-artery sclerosis and evidence of recent coronary occlusion, with infarction, estimated to be 2 or 3 weeks old, and bilateral bronchopneumonia.

Discussion

There is a striking similarity between the fatal outcome in the 2 cases presented above and liver death after chloroform anesthesia. The clinical picture of hepatic failure developing a week or less after anesthesia and the pathologic picture of central necrosis of the liver are precisely those that in a bygone era became recognized as a major hazard of chloroform. The appearance of this pattern in a patient who has received a new halogenated anesthetic strongly suggests that the anesthetic may be responsible.

However, it is clearly not possible to determine the cause of liver necrosis in either of these patients. Case 1 received, in addition to the anesthetic, a wide variety of drugs* that might conceivably be implicated. Brunson et al.,[8] Popper and Schaffner[9] and others have pointed out the increasing opportunities for toxic necrosis as vast numbers of new drugs are introduced clinically. Case 2 was known to be alcoholic and was suspected of having preoperative liver damage, although no evidence of this was found at surgery or post-mortem examination. Passive hyperemia of the liver secondary to impaired cardiac activity because of the recent infarct may have rendered the liver more susceptible to damage, and the severe postoperative bronchopneumonia, with accompanying hypoxia, may have contributed to liver damage.

Although one might suspect the principal anesthetic agent, halothane, in each of these cases, it is not possible to draw a firm conclusion; nor is it possible to determine the role of halothane in the small scattering of similar cases recently reported.[3-6] But the appearance of such sporadic reports, although difficult to evaluate individually, serves as a warning that halothane may, after all, damage the liver, and certainly is an indication for continuing study of the possibility of this serious complication.

The possibility that halothane can, in common with chloroform and other halogenated hydrocarbons, damage the liver has been widely recognized, and careful studies of liver function have been carried out in man and in the laboratory animal. The incidence and severity of histologic liver damage in animals appears to be greater after anesthesia with halothane than cyclopropane or diethyl ether, but less than that after chloroform or vinyl ether.[10,11] On the other hand, Little, Barbour and Given,[12] in careful studies in normal man, have reported no greater incidence or severity of disturbances in liver function after halothane than after anesthesia with ether or cyclopropane. It would be comforting to be able to assume, as have many, that these or similar data satisfactorily exclude the possibility of liver damage from halothane. However, in the extensive studies of chloroform anesthesia at the University of Wisconsin, and with the use of similar tests of liver function, no differences were detected between chloroform and a wide variety of other anesthetic agents in normal man, at least if oxygenation and carbon dioxide excretion remained normal.[13] From such data one might suspect that the liver-function tests commonly used are not sufficiently sensitive, if one is to continue to assume, as surely most observers do, that chloroform is hepatotoxic. Another perfectly logical conclusion might be that if chloroform were reintroduced today into general use, with the added safety of careful metering of anesthetic concentration and improved methods for the support of ventilation, the clinical results could be expected to be as good as those currently achieved with halothane. To test this possibility, clinical comparisons of halothane and chloroform have recently been carried out at the universities of Washington and Wisconsin[14,15]; at the latter institution, the anesthetic agents were administered as unknowns. The clinical results in both studies were unremarkable: no differences between agents were demonstrated, and, in fact, in the Wisconsin "unknown" study, the anesthetist could not distinguish between agents. However, impressive evidence that chloroform does, in fact, and under modern methods of administration, carry a serious threat of liver damage has been presented in a separate publication from Wisconsin.[16] In a small series of 7 patients who received chloroform anesthesia for open-chest surgery 2 died in liver failure, with acute centrilobular necrosis demonstrated at autopsy, and an additional 2 suffered nonfatal postoperative hepatitis, presumably toxic in origin. By contrast, halothane has been and is being used in thousands of thoracic procedures. Whatever the absolute incidence of complications with halothane may be, one is struck by the paucity of reported cases of liver damage. It has been estimated that halothane anesthesia has been administered to at least 6,000,000 patients in this country. To our knowledge, there are 5 previously reported cases of liver death after halothane.[3-6] The 2 patients described above make 7. If halothane could be implicated in all 7 (which it cannot), this would suggest a remarkably low incidence of this complication, particularly in comparison to the incidence of anesthetic death of 1:1000 from all causes and with other agents reported by Beecher and Todd.[17]

*Including meperidine, amobarbital and secobarbital, atropine, penicillin, dihydrostreptomycin, promethazine, Periodon, acetylsalicylic acid, sulfadimethoxine, tetracycline, chloramphenicol and tetanus antitoxin.

An opposing argument suggests itself: that for every reported case many may remain unreported. The 5 cases reported by Vourc'h and his co-workers,[5] Burnap et al.,[3] Virtue and Payne[4] and Temple and his associates[6] came from large teaching centers, which might be more apt to detect or to report such complications. How many similar cases may lie buried in the archives of the record room in other hospitals? Clearly, a great many additional data are needed to resolve, conclusively, such questions. And the simple reporting of isolated cases in which halothane may have been responsible for fatal liver failure will not be enough, since the finger of suspicion is apt to be raised if a drug such as halothane is given, but other cases with a similar outcome may be overlooked if no drug under suspicion has been administered.

Ideal data could probably be obtained only in a very large series of carefully matched surgical patients, the anesthetic (halothane as compared with one or a group of other drugs) chosen in a random fashion and the study planned in advance. Short of such a monumental task, much can be learned from careful review of all surgical patients in whom the post-mortem diagnosis of acute yellow atrophy or central necrosis of the liver has been made. If the incidence of liver death should turn out to be the same for halothane and nonhalothane cases, presumably one need look no further. In the event of significant differences it would be essential, of course, to make certain that other factors, such as other drugs with possible effects on the liver and operative procedures involving the liver or biliary tree, were balanced.

Finally, in the attempt to identify the cause of fatal or nonfatal postoperative liver failure, it is important to bear in mind the possibility of causes other than anesthetics or other drugs. The possibility of jaundice secondary to hemolysis of transfused whole blood is well recognized. Homologous-serum hepatitis from transfused blood or plasma, administered during surgery, should appear between thirty and ninety days after operation and can hardly be confused with toxic hepatitis appearing in the early postoperative period. But, of course, surgery may be performed in the incubation period of infectious hepatitis (or of homologous-serum hepatitis), and the appearance of clinical hepatitis might then be coincidental. At first thought, perhaps, this does not appear likely. However, one might consider that approximately 75,000 new cases of hepatitis were reported to the Public Health Service in 1961, a national incidence of 1:2500; currently an estimated 2,500,000 surgical patients receive halothane anesthesia in this country each year, and so, by rough calculation, one might assume that in some 1000 patients who receive halothane anesthesia in a given year hepatitis will develop in the following year, and, again by rough approximation, some 15 to 20 patients a year might be expected to be stricken by infectious hepatitis within five to ten days of surgery. Although the mortality of uncomplicated infectious hepatitis is low (approximately 1 per cent), this might account for an occasional postoperative death from liver necrosis, particularly in light of a reported mortality of over 20 per cent from homologous-serum hepatitis after transfusion in medical and surgical patients over the age of forty.[18] It might also be considered that the insult of surgery in preclinical or subclinical (anicteric) hepatitis might well lead to an increase in morbidity and mortality. Careful pathological study can often distinguish between the classical central necrosis of toxic hepatitis, as in the 2 cases reported above, and the inflammatory reaction of viral hepatitis. However, in many cases it is suspected that these differences may not be sought out; furthermore, in the event of massive liver necrosis, even careful pathological examination may fail to distinguish between the possible etiologic factors.

Summary and Conclusions

Two patients who died of liver necrosis after surgery under halothane anesthesia are described. That the anesthetic agent was responsible is suggested by the similarity of clinical course and pathological findings to the liver necrosis that may follow chloroform anesthesia, and by the chemical similarity of halothane to chloroform. However, other possible causes of hepatic damage were present in both cases, and it is not possible to implicate the anesthetic in either. Clinical experience to date suggests that liver damage after halothane is rare, and laboratory tests of liver function have failed to demonstrate differences between halothane and other commonly used anesthetics. Difficulties in the interpretation of currently available clinical and laboratory evidence and the need for further data are discussed.

Since this paper was submitted for publication 3 additional deaths after halothane anesthesia have been reported.[19]

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PHARMACOGENETICS AND ANESTHESIA

Werner Kalow

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Review Article

Pharmacogenetics and Anesthesia

Werner Kalow, M.D.

PHARMACOGENETICS deals with hereditary modifications of the response to drugs. The word pharmacogenetics is of recent origin [1] but already has been used by several authors to head reviews and monographs.[2-6] Interest in the subject has been initiated by the special medical and legal problems that arise when a hereditary defect causes a patient to be harmed by a conventional application of a conventional drug. On the other hand, observations in pharmacogenetics are a challenge and may become points of departure for diverse investigations.[4] The potential scope of pharmacogenetics is wide since all forms of life exhibit genetic variation and are able to respond to drugs or chemicals. The following review, however, is confined to drugs used in man for or during anesthesia.[7, 8]

Of particular concern are hereditary characteristics which alter pharmacologic responsiveness but which are hidden and unrecognized prior to drug administration. Obvious hereditary diseases which may also cause abnormal pharmacologic responses pose different problems. Nevertheless, there are transitions since individual patients suffering from, say, porphyria, may come to the anesthetist with or without diagnosis. Included in the following discussion are, therefore, some comments on genes with ordinarily concealed effects and those with usually visible effects. However, there will be some bias in emphasis; relatively much space will be devoted to a description of the cholinesterase variants because of the author's special familiarity with this topic.

The Cholinesterase Variants

Many cases of "prolonged apnea" after succinylcholine can be explained by the existence of an atypical form of pseudocholinesterase. The topic has been reviewed several times [4, 9-15] but the present survey is justified by a number of new observations. Furthermore, the occurrence of atypical esterase is still an occasional cause of anesthetic death.

At present, there are four genes recognized which control human pseudocholinesterase, not counting the gene which determines the usual esterase type. A few introductory paragraphs will therefore deal with biochemistry and means of determination of the esterase variants, to be followed by an outline of genetic aspects and a discussion of the clinical significance.

Classification of Esterase Types: Biochemical Characteristics and Means of Determination

Atypical Esterase. (1) Nature: Liddel *et al.*[16] have described a means of physical separation of usual and atypical esterase by chromatography on a DEAE column.* The ability to separate the two esterase types indicates that they are structurally different. Since the two enzyme types seem to contain the same amount of sialic acid,[17, 18] the difference is most likely in the content of amino acid.

As judged from the Michaelis constants, atypical esterase has a relatively low affinity for all investigated substrates.[21] In addition, indirect evidence suggests that atypical esterase has mostly lower turnover rates than does the usual enzyme.[21] These two functional defects render atypical esterase a very inefficient enzyme. The affinity of atypical esterase for succinylcholine is about 100 fold lower than that of usual esterase which appears to be

Dr. Kalow is Professor of Pharmacology, Department of Pharmacology, University of Toronto, Toronto, Canada.

* Liddel *et al.*[16] have also reported a separation of esterase types by paper electrophoresis. In spite of considerable effort, the electrophoretic separation on paper could not be reproduced.[18, 20] There is certainly no electrophoretic separation on starch gel at any *p*H [17] unless a small amount of decamethonium is incorporated into the gel.[11]

TABLE 1. Genotypes in Regard to Pseudocholinesterase, Drawn Under the Assumption that the Four Genes are Alleles

$E_1^uE_1^u$			
$E_1^uE_1^a$	$E_1^aE_1^a$		
$E_1^uE_1^f$	$E_1^aE_1^f$	$E_1^fE_1^f$	
$E_1^uE_1^s$	$E_1^aE_1^s$	$E_1^fE_1^s$	$E_1^sE_1^s$

See text for reservations to this assumption. Underlining indicates susceptibility of the phenotypes to succinylcholine.

E_1^u = gene for usual esterase; E_1^a = gene for atypical esterase; E_1^f = gene for fluoride type; E_1^s = silent gene.

the main reason for the failure of some patients to metabolize this drug.[11] For procaine, both affinity and turnover rates are moderately decreased but this double decrease may cause the hydrolysis of procaine to be very slow.[19]

The relative affinity of atypical esterase for numerous inhibitors is even lower than that for substrates so that atypical esterase tends to be less inhibited than is the usual enzyme.[22] Recently found exceptions are chloride [23] and urea [20] which, in high concentrations, inhibit atypical esterase slightly more than they block the usual esterase.

(2) Methods of Determination: Several methods of distinguishing atypical from usual esterase have been published. The first method [24] utilizes the fact that atypical esterase shows diminished inhibition by the local anesthetic dibucaine (nupercaine, chinchocaine, percaine), using benzoylcholine as the substrate. Percentage inhibition observed under standard conditions is called Dibucaine Number (DN). Esterase activity is determined by ultraviolet spectrophotometry which requires relatively expensive equipment but permits one to keep track of the substrate itself and not only of a secondary function like *p*H. Also, the biochemical test system is very simple. The measurements are therefore inherently accurate. The method is fast enough to permit determination of esterase type while a patient is apneic in the operating room.

Steinitz *et al.*[25] have determined DN using a colorimeter. Esterase activity is assessed by following changes of *p*H with the aid of color changes of an indicator.

Rubinstein and Deitz [26] have recently described a method which is based on a comparison of the hydrolysis velocities of acetylcholine and benzoylcholine. The hydrolyses are measured either in a titrimeter or in a colorimeter.

For screening of samples in the spectrophotometer, a method has been devised [12] which utilizes the neostigmine derivative R02-0683 instead of dibucaine. With this method, 50 sera have been classified routinely per two hours.

Harris and Robson [27] have described two tests for screening large numbers of sera. The preferred test requires agar plates in which there are small circular wells for sera. Esterase activity determines the diameter of a stained circular field around the well. Staining is produced by a histochemical method using alpha-naphthylacetate as a substrate. Sera with atypical esterase can be spotted by adding R02-0683 or dibucaine to the agar. The agar plates require overnight incubation.

Since the kinetics of atypical esterase differ coarsely and in many ways from that of usual esterase,[4] any of the cited methods would enable one to recognize persons homozygous (see below) for atypical esterase, and numerous additional methods could be devised. Problems of discrimination exist, and the choice of method may be important, if one wishes to recognize the heterozygotes who have a mixture of usual and atypical esterase, particularly if it happens that the normal esterase predominates in this mixture. The ability to recognize such a heterozygote is not vital for many diagnostic purposes but it is essential for family studies and is a prerequisite for most other work on esterase types.

Cholinesterase Anenzymia. There is a gene which causes lack of pseudocholinesterase activity.[11] Lehman *et al.*[28] called this a silent gene when they found a complete lack of esterase activity in a woman and a reduced esterase activity in her offspring and in her mother.

(1) Nature of the Deficiency: Lack of esterase activity is apparently not caused by enzyme inhibition. Thus, the lack could be either due to absence of the esterase protein or due to an alteration of the enzyme protein which renders it inactive. Hodgkin and associates [29] have searched in vain by immuno-

logical means for an inactive enzyme protein, but details have not been published.

(2) Methods of Determination: Persons with complete lack of esterase activity are easily recognized but are very rare. Otherwise, only family studies can reveal the silent gene. When testing DN in relatives of persons with atypical esterase, the silent gene shows up by the pattern of inheritance of atypical esterase.[11, 30]

The "Fluoride Type." (1) Nature: The name "fluoride type" refers to a cholinesterase variant discovered by Harris and Whittaker with the aid of sodium fluoride as enzyme inhibitor.[31, 32]

By starch gel electrophoresis at various *p*H, with and without prior removal of sialic acid from the esterase, the fluoride type is indistinguishable from both usual and atypical esterase.[17] The variant represents presumably an enzyme alteration.[33] Detailed kinetic data do not exist, but the affinity of the fluoride type for succinylcholine is at least somewhat reduced.[20] This statement is based on *in vitro* tests and is consistent with observations on patients who have received succinylcholine.[34, 35]

(2) Methods of Determination: The determination is based on the fact that the cholinesterase activity of some sera is relatively resistant to inhibition by sodium fluoride. The inhibition test is performed with sodium fluoride instead of dibucaine. In analogy to DN, per cent inhibition by fluoride under standard conditions is termed FN for Fluoride Number.[31, 32] At present, the determination of FN is the only means of identifying the variant. Unfortunately, the test is unsatisfactory for several reasons. First, atypical esterase is more resistant to fluoride inhibition than is the fluoride type itself. This increases the chances of error since any interpretation of FN must take into account the DN. Second, there is a peculiar dependence of FN on benzoylcholine concentration (to be published) so that FN, unlike DN, is influenced by any error affecting the substrate. Further uncontrolled variables seem to affect FN but addition of chelating agents did not help in stabilizing the action of fluoride.[20]

The Electrophoretically Identified C_5 Variant. (1) Nature: After two-dimensional electrophoresis, Harris and co-workers[36, 37] have found a variant which shows up as a spot of esterase activity which is not present in all sera. In the test system employed, every serum shows at least four spots with esterase activity designed as C_1 to C_4 with most of the activity being in C_4.[37, 38] Distinguishable from these is spot C_5 which appears in some sera and represents an additional pseudocholinesterase. Sera with this additional enzyme hydrolyze benzoylcholine about 30 per cent faster on the average than does usual serum. Dibucaine and fluoride inhibition is not affected by the presence of the C_5 variant.[37] Hence, there is no reason to expect any adverse pharmacological reactions in the presence of this variant, so that it can be dealt with very briefly.

(2) Methods of Determination: So far, the variant can be identified only by the electrophoretic means of Harris and his coworkers.[36, 37]

TABLE 2. Approximate Prevalence in a Canadian Population of Genotypes in Respect to Pseudocholinesterase

Genotype	Prevalence
$E_1^uE_1^u$	1:1
$E_1^uE_1^a$	1:25
$E_1^aE_1^a$	1:2,500
$E_1^uE_1^s$	1:200
$E_1^aE_1^s$	1:8,000
$E_1^sE_1^s$	1:100,000

See table 1 for symbols. No estimates available for E_1^f gene.

GENETICS

Since terminology threatened to become confusing, a group of interested investigators † have devised and agreed to the symbols and nomenclature used in the following description.

The letter E will be used to designate the gene for cholinesterase, and subscript 1 indicates the first found locus. Then, E_1^u is the gene causing formation of the usual type of pseudocholinesterase; E_1^a is the gene for atypical esterase, and E_1^s causes lack of esterase activity. These three genes are most likely alleles.[30] There are then three homo-

† Discussions have been initiated at the Second International Meeting on Human Genetics, The Hague, September 1963. The complete information will be published.

TABLE 3. Prevalence of Heterozygotes for Atypical Esterase (Genotype $E_1^uE_1^a$) in Various Populations

Population	Number Tested	Number Heterozygotes ($E_1^uE_1^a$	Percentage	Reference
Canadian (white)	2,017	74	3.8	39
British	703	27	3.8	40
German	118	4	3.4	41
Czechoslovakian	87	7	8.5	41
Greek	360	13	3.6	40
Portugese	179	6	3.4	40
Moroccan-Jewish	51	1	2	40
Berber	55	2	4	40
Mexican Indian	264	7	2.6	43
Australian aborigines	98	1	1	43

zygotes $E_1^uE_1^u$, $E_1^aE_1^a$, and $E_1^sE_1^s$. The first of these has usual esterase, the second has atypical esterase, and the third has no esterase activity. The persons are of phenotype U, A, and S, respectively.

There are the three heterozygotes $E_1^uE_1^s$, $E_1^uE_1^a$, and $E_1^aE_1^s$. The first of these has usual esterase but a reduced amount on the average. The second has a mixture of usual and atypical esterase, and the third has atypical esterase but a reduced amount on the average. The persons are of phenotype *U, I,* and *A,* respectively.

It has been suggested [4,32,33] that the gene giving rise to the fluoride type is an allele of E_1^u, E_1^a, and E_1^s. The gene is called E_1^f and is entered as such in table 1. However, some family data to be published make it seem advisable to consider the hypothesis of allelism as not completely established; if the fluoride gene should turn out not to be allelic, it would be E_2^f, and there would be 18 genotypes instead of the 10 shown in table 1. The poor method of testing makes it difficult to arrive at definite conclusions, both in regard to mode of inheritance and frequency of occurrence. The heterozygotes $E_1^uE_1^f$ and $E_1^aE_1^f$ led to the recognition for the E_1^f gene [31]; $E_1^fE_1^s$ is hypothetical in that the genotype has not been encountered. The homozygote $E_1^fE_1^f$ has been described recently.[33]

As indicated in table 1, six genotypes would suffer prolonged effects of succinylcholine. All heterozygotes who have some usual esterase show an almost normal rate of drug elimination. The reason can be understood on the basis of enzyme kinetics and drug distribution.[4] Any lowering of affinity between succinylcholine and cholinesterase will substantially reduce the hydrolysis rate of the drug, so that a reduction of affinity could be expected to have a much more serious effect than reduction of the amount of esterase.

Only estimates of the combined prevalence of the endangered genotypes can be given but their occurrence might be often as 1 per 1,500. Estimates of prevalence of some specific genotypes in a Canadian population [30, 39] are shown in table 2. As can be seen, $E_1^uE_1^a$ is one of the most frequently occurring genotypes. Estimates of this genotype have been made in various populations; the data are shown in table 3. For anesthesia, the listed genotype is not important *per se* but the figures suggest a relatively even distribution also of homozygotes with atypical esterase. The slightly elevated percentage of heterozygotes in the small sample from Czechoslovakia is insignificant but may deserve attention since a Slavic population studied in Canada [39] showed a similar trend.

As has been pointed out, the C_5 variant is not likely of great importance for anesthesia. The gene determining C is not allelic to E_1^u and E_1^a. Persons with the C_5 variant constitute about 5 per cent of the British population. Among the refugees from Tristan da Cunha, the prevalence of the variant was found to be 17 per cent.[37]

A study on twins and families indicated

that the level of esterase activity, *i.e.*, presumably esterase concentration, is ordinarily under environmental rather than genetic control.[44] That means, in spite of all the emphasis on the genetics of pseudocholinesterase, a worthwhile future investigation in this field may be a search for inducers or suppressors of the formation of this enzyme.

Clinical Significance

Two widely used drugs are destroyed by pseudocholinesterase, namely, succinylcholine and procaine. In the presence of atypical esterase, the *in vitro* hydrolysis of both agents is retarded.[11, 19] Nevertheless, atypical esterase is known to have given rise to fatal intoxication with succinylcholine but not with procaine. The main reason seems to be the different clinical application of these drugs.

Procaine is usually injected into tissues at the site of desired action. The most efficient means of extending the duration of action of procaine is to add vaso-constrictors to the procaine solution. Obviously, local absorption influences the duration of action more decisively than does procaine metabolism. Even a slow rate of metabolic conversion may be adequate as long as the drug enters the blood from the depot at a slow rate.

By contrast, succinylcholine is injected intravenously. The largest portion of the injected drug—say 90 per cent to 95 per cent—is destroyed within a minute after the injection.[4, 34, 35] It follows that only a fraction of the dose reaches the neuromuscular endplate where the drug acts and from where it is gradually dissipated by diffusion into surrounding fluid.[4] If there is a failure of esterase activity, the neuromuscular junction must be flooded with succinylcholine molecules. Dispersal of this high concentration at the endplate must take a relatively long time, and this explains the prolonged effect.

For persons with atypical esterase (genotype $E_1^a E_1^a$), full dose effect-curves for succinylcholine have been determined.[40] Complete apnea after 100 mg. of succinylcholine ranges from 50 to 65 minutes; double to triple that time may pass until spontaneous respiration is adequate. These apneas are manageable in a well-equipped hospital but real difficulties have been encountered if succinylcholine was given by continuous drip. When a gram or more is used, the apnea may be expected to last for many hours. Recently, two cases have come to my attention where irreversible cardiac failure occurred after several hours of artificial ventilation. Before succinylcholine is used by continuous drip, esterase function should be determined by any of the numerous biochemical methods. If this is not possible, a test dose of succinylcholine should be given, the response timed, and only then should large doses be used.

Vickers[47] has recently reviewed cases in which the apnea was apparently due to esterase abnormality and yet lasted very much longer than the one or two hours that are

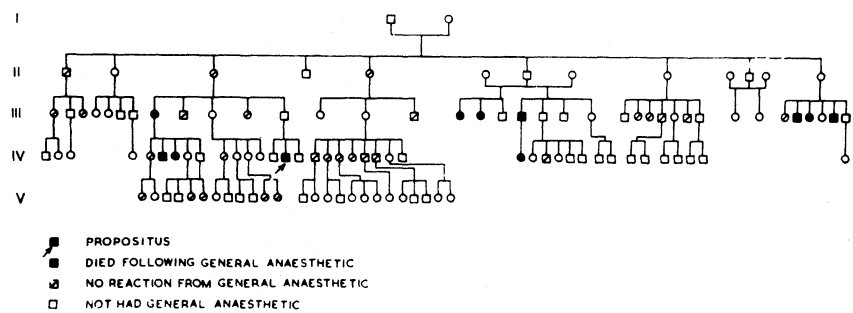

Fig. 1. Death with hyperthermia following general anesthesia. Family tree of affected persons. Circles indicate females, squares indicate males. (From Denborough, M. A., and others: Anaesthetic deaths in a family, Brit. J. Anaesth. 34: 395, 1962.)

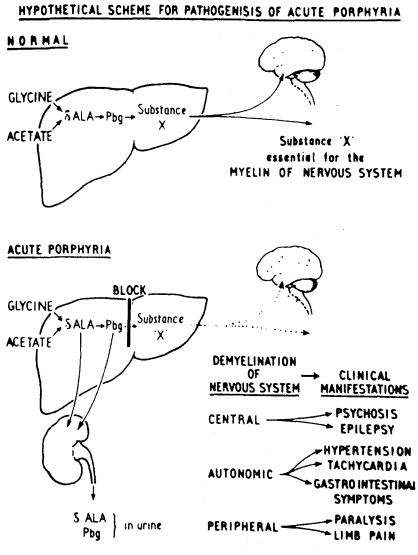

FIG. 2. Hypothetical scheme representing the pathogenesis of acute intermittent porphyria. (From Goldberg, A., and Rimington, C.: Diseases of Porphyrin Metabolism. Springfield, Ill., Charles C Thomas, 1962.)

expected after single injections.[40] He came to the conclusion that in all these cases neostigmine or some other anti-cholinesterase has been injected relatively soon after start of the apnea. The action of the anti-cholinesterase at the neuromuscular junction may have intensified the block. Only after the apnea has lasted for one and a half hours or more, the action of succinylcholine may convert into a dual block so that anti-cholinesterases may help to terminate the action.

General Anesthetics and Hyperthermia

Denborough and Lovell[48] in 1960 had to anesthetize a young man who came to the emergency ward with a compound fracture. The patient was frightened and stated that several relatives of his had died from ether anesthesia. Fortunately, the authors took the words of the young man seriously. They anesthetized him with particular care, using thiopental, nitrous oxide and halothane. After ten minutes, anesthesia had to be stopped prematurely because blood pressure started to fall. The patient remained unconscious, his skin felt very hot and sweaty. He received a blood transfusion and was packed with ice. He gradually recovered over a period of one and a half hours. In 1962, he came into the hospital for another operation. He withstood local anesthesia without difficulty.

Inquiry showed that of the 38 relatives of the young man who had had general anesthetics, 10 have died.[49] In all cases, the anesthetic agents were apparently ethyl chloride and ether. The family tree is shown in figure 1. It suggests dominant inheritance of the trait.

In all the cases of death following anesthesia, the course of events has been similar. In all except one, the operation has been minor and successful and so was unlikely to have been the cause of death. Three of the patients have been returned to the ward after operation in an apparently good condition only to die following convulsions about thirty minutes later. In two, the temperature was taken and found to be 43° and 42° C., respectively.

Speculative attempts at interpretations have been made.[4] The crucial finding in all these cases seems to be the elevated body temperature; if temperature is high, ether is known to produce convulsions, even in animals. Elevated temperatures after anesthesia do not appear to be extraordinarily rare; they evoke a guilt complex in the anesthetist who fears he has caused anoxic brain damage.[50] However, a rise of body temperature is by no means a regular or prominent consequence of cerebral anoxia.

Acute Intermittent Porphyria

The hazard of thiopental anesthesia in certain kinds of porphyric patients is well known, yet a series of fatal cases has been described recently.[51] One does not understand why thiopental is so dangerous to these patients; the fact is established on an empirical basis.

There are several distinct diseases called porphyria.[52, 53] Of these, the congenital porphyria erythropoietica and the porphyria

cutanea tarda hereditaria usually do not require the special attention of the anesthetist.

The disease important in this context is the acute intermittent porphyria. An instructive diagram from the book by Goldberg and Rimington [52] may be helpful for recalling the disease (fig. 2). The diagnostic biochemical feature is persistence of porphobilinogen in urine (fig. 3). Fresh urine is usually normal in color but darkens when left in daylight for a few hours. After standing, the urine might be red enough to suggest the presence of hematuria. Porphobilinogen can be detected easily with the Watson-Schwartz test.

Pathologically, the significant lesions are multiple, often segmental, scattered areas of demyelination. This involves mostly peripheral nerves, autonomic as well as sensory and motor. Demyelination of the autonomic nerves will lead to symptoms of abdominal pain and altered gastrointestinal function. Paralysis and sensory loss is explained by damage to motor and sensory nerves. Also, the central nervous system may be affected; psychiatric symptoms occur in a substantial number of cases.

The presenting symptom is most often abdominal pain. Neurologic symptoms of various kinds are the usual reasons to consult a physician in the remainder of cases.

Patients can be free of symptoms for years, perhaps throughout life. They are then usually considered highly strung and nervous individuals. Attacks of clinical symptoms may occur at any time. These attacks can be initiated by administration of thiopental. The most disturbing fact remains that attacks induced or aggravated by thiopental are frequently characterized by paralysis which may take weeks or months to subside or may lead to respiratory failure.

It would be a substantial achievement to understand the biochemical factors that lead a barbiturate to cause persistent paralysis. Solving this question would require full understanding of the mode of action of barbiturates and of the fundamental biochemistry of intermittent porphyria.

Myotonic Syndrome

Abnormal reactions of myasthenic patients to various drugs are well known.[54, 55] However, myasthenia, as well as several other diseases affecting muscle function, is not usually a hereditary disease and is, therefore, not discussed. The largest group of genetic disorders are the muscular dystrophies, most of which do not seem to show a special contraindication for anesthetics.[54] However, dystrophia myotonica and the similar myotonia congenita have been singled out for special attention of the anesthetist.[56] These diseases are characterized by a persistence of contraction after a voluntary effort has ceased; for instance, a patient may have difficulty in releasing the grasp of his hand.

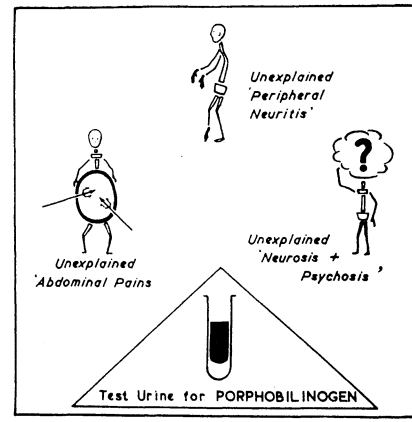

FIG. 3. An aid to the diagnosis of acute intermittent porphyria. (From Goldberg, A., and Rimington, C.: Diseases of Porphyrin Metabolism. Springfield, Ill., Charles C Thomas, 1962.)

Kaufman [57] carefully reviewed anesthesia in dystrophia myotonica. He came to the conclusion that muscular function may be so impaired in advanced disease that respiration is inadequate causing carbon dioxide concentration to increase. Dangers arising from the use of respiratory depressant drugs in patients with marginal respiratory function are obvious. There is no specific susceptibility to thiopental as had been thought [56] but respiration may cease when any depressant is administered; perhaps opiates constitute the greatest danger.

Kaufman [57] mentioned a personal communication to the effect that decamethonium had initiated a generalized attack of myotonia which ended fatally. A dramatic case recently reported by Paterson [58] illustrates the effects of

succinylcholine in a patient who suffered from myotonia congenita of mild degree. Succinylcholine in ordinary doses (0.66 mg./kg.) produced generalized myotonia of all skeletal muscles. The effect lasted for two to three minutes and was not followed by any muscle paralysis. Muscular contraction was so pronounced that intubation or artificial respiration were not possible. The patient recovered without damage.

Familial Dysautonomia

Familial dysautonomia [59] was recognized as a distinct clinical entity in 1949. It is a congenital condition manifested by specific disturbances of the central nervous system, particularly affecting autonomic function. Diagnosis is frequently made when children fail to produce tears when crying. The diastolic blood pressure of children with dysautonomia paradoxically falls when they stand up from a lying position. Other regularly expected features are cold hands and feet; excessive perspiration; disturbed swallowing reflex; poor motor coordination; dysarthria, relative indifference to pain; and emotional lability. So far, the condition has been observed almost exclusively in Jewish children. Family data are compatible with the assumption that dysautonomia is a recessive trait. Cause of the disease might be a metabolic defect. Some failure of catecholamine formation has been found [60] but it is doubtful whether this is the primary defect.

The problems of anesthesia in patients with familial dysautonomia have been described in some detail by Kritchman and associates.[61] The most frequent and serious complication was hypotension, often precipitated by a change of position. Among six cases with severe hypotension during anesthesia, cardiac arrest occurred twice. The experiences suggested that patients with dysautonomia do not tolerate thiopental or tribromoethanol. The authors recommended use of local anesthesia after premedication with chlorpromazine, otherwise use of a volatile anesthetic.

The cyclic vomiting may lead to aspiration and its disastrous consequences. Bronchopneumonia occurred frequently as a postoperative complication.

Sickle-Cell Crisis and Anesthesia

The presence of sickle-cell disease grossly increases the hazards of anesthesia.[62, 63]

Sickle-cell disease is due to replacement of the normal hemoglobin A by hemoglobin S. The difference between these two forms of hemoglobin resides in a difference of one amino acid in each of the two beta-chains of the hemoglobin molecule. This small change decreases the solubility of reduced hemoglobin S. Hence, hemoglobin S tends to precipitate within the erythrocyte if there is any anoxia. This leads to distortion of the red cell ("sickling") and often massive rupture of erythrocytes. This crisis may lead to sudden unexpected death from vascular occlusion by thrombosis or infarction, or there may be hemorrhage in vital organs due to increased fragility of the red cells. Massive hemolysis may cause renal failure and anuria with death following a few days after the crisis.

Sickle-cell disease occurs if there are two genes for hemoglobin S, in other words, it is a recessive disease. It is likely a fatal disease but nevertheless may remain undiagnosed for some time. Carriers of the disease are said to have sickle-cell trait. This trait is usually not noticed but in some cases may lead to sickle-cell crises with all the serious consequences that are commonly seen in patients with sickle-cell disease.[64]

A sickle-cell crisis may be precipitated by anesthesia since even a minor procedure is often accompanied [65] or followed [66] by hypoxia. As approximately 7 per cent of American negroes have the sickle-cell trait, the proportion of surgical patients exposed to this special risk is significant.

Miscellaneous Observations

A number of pertinent observations have been previously reviewed [4] so that brief reference must suffice. Other observations in pharmacogenetics are new but can be mentioned only because they are of marginal concern for anesthesia. The following two items are in the latter category:

It had been known for many years that people can be divided into rapid and slow inactivators of isoniazid.[3, 4] Price Evans has

recently shown that the difference is due to lack of a specific transacetylase in the liver of some persons.[67] As a consequence, some people are not able to acetylate such diverse drugs as the chemotherapeutic agents isoniazid and sulfadimidine, the antihypertensive drug hydralazine (Apresoline), and the psychic energizer phenelzine (Nardil). The observation that all these different agents are affected by this metabolic individuality is still too new to permit full evaluation of the clinical significance.

In a condition called "idiopathic ventricular septal hypertrophy," the septum between right and left cardiac ventricles is so thick that it tends to block the aortic valve.[68, 69] Cardiac enlargement due to incipient failure may under these conditions actually improve the circulation. A dose of digitalis might be fatal if it decreases cardiac size, thus cutting off the blood flow.

Mydriatic agents are generally less effective in dark than in light eyes which has been thought to be due to local factors. It seems, however, that atropine is less active in negroes than in whites, not only on the eye but also upon cardiac rate and on salivation.[4]

Epinephrine reduces intraocular pressure in most eyes but causes an increase if there is a narrow angle between cornea and iris. Since many anesthetics liberate adrenalin, one should therefore expect that intraocular pressure should be elevated for this reason in only some persons and not in others.[4] Also, atropine constitutes a danger only in persons with a narrow angle. However, in the opinion of Dr. Luke, there is a further restriction[70] in that ordinary doses of atropine will be of danger only to the rare persons who are especially sensitive to atropine and have a narrow angle in addition.

In the rare condition of hyperkalemic familial periodic parlysis (to be distinguished from the much more usual hypokalemic disease), Egan and Klein[71] observed extensive paralysis after thiopental. Local anesthetics were used without harm.

Differences in the ability to withstand the stresses of surgery and anesthesia may be associated with differences in cortical size and function that occur between individuals and between ethnic groups.[4, 8]

Summary

Pharmacogenetics deals with hereditary modifications of the response to drugs. Since there are numerous examples concerning anesthesia, emphasis in this review has been arbitrary. Recent work on the five cholinesterase variants has been surveyed in some detail; effects on metabolism of succinylcholine and of procaine have been compared and consequences of mismanagement discussed. The review includes sections on general anesthetics and hyperthermia, acute intermittent porphyria, the myotonic syndrome, familial dysautonomia, and sickle-cell crisis. Some further conditions have been commented upon in a section on miscellaneous observations.

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MALIGNANT HYPERTHERMIA

Gerald A. Gronert

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Malignant Hyperthermia

Gerald A. Gronert, M.D.*

CONTENTS

History

The first publication describing malignant hyperthermia (MH) concerned a 21-year-old student who had a compound fracture of the right leg. The young man was less concerned about his leg than the risk of general anesthesia, for, since 1922, ten of his relatives had died as a direct consequence of ether anesthesia. He subsequently survived an episode of malignant hyperthermia, and Denborough and Lovell's brief report of accelerated metabolism during anesthesia culminated in a world-wide awareness of the risks of genetic susceptibility to certain drugs and stress.[96] While there had been earlier reports of perioperative hyperthermia, these had been preoccupied with environmental causes and lacked sufficient information to relate these episodes to a specific pathophysiologic entity such as MH.[66,156,290]

Interest in MH in North America began with Locher, in Wausau, Wisconsin,[190] and with Britt, Kalow, and Gordon, in Toronto.[50,51,56,137] Between 1955 and 1958 Locher became involved with the anesthetic care of a family in which 30 members had died in conjunction with general anesthesia. His quest for an understanding of these bizarre incidents led to a relationship with Britt and Kalow, who had experienced several cases of MH in Toronto and were investigating the overall problem. The Wausau amd Marathon County, Wisconsin, area provided a fertile gene pool for Britt and Kalow's systematic human study of MH.[56] Other investigators also contributed to the growing recognition of intraoperative hyperthermia and its possible etiology.[70,349,352,401,415] Evaluation of susceptibility was aided by the recognition of abnormal creatine phosphokinase (CPK) levels by Isaacs and Barlow,[204] and the identification of low-threshold skeletal muscle contracture responses by Kalow and Britt.[227] These pioneering efforts and the unquestioning cooperation of the involved families have made it possible to trace the epidemiology of human MH, to study its pathogenesis and pathophysiology, and to provide safe anesthesia for susceptible patients.

The porcine model of malignant hyperthermia evolved from a report describing pork that was unsuitable for making sausage.[192] Ludvigsen later described a muscular degeneration in pigs, in 1953,[266] and subsequently demonstrated its genetic relationship.[267] This entity became important to swine breeders because the stresses related to slaughter resulted in accelerated metabolism and deterioration of the muscle of susceptible pigs, with the resulting production of pale soft exudative (PSE) pork.[35,106] Unsuitable pork was seldom obtained from normal swine because the time required for slaughter, cooling and processing of the animal was not long enough, nor the me-

* Professor of Anesthesiology, Department of Anesthesiology, Mayo Medical School, Rochester, Minnesota 55901.

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Address reprint requests to Dr. Gronert.

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tabolism sufficient, to produce the marked acidosis and higher temperatures peculiar to the susceptible animals.[35,309] The incidence of PSE animals increased with breeding patterns designed to produce pigs with rapid growth rates, good feed efficiency, and superior muscling.[69] This increased incidence led to the term "porcine stress syndrome" (PSS).[219,309,393] Any stress to which these pigs were subjected, *e.g.*, separation, shipping, weaning, fighting, coitus, or slaughter, might lead to increased metabolism, acidosis, muscle rigidity, high temperature, and death. In 1966, Hall reported MH induced by halothane and succinylcholine in stress-susceptible swine.[167] The descriptions of the biochemical processes in PSE[35,309] and PSS[124,181,309,382,393] animals leave little doubt that MH in swine is an anesthetic manifestation of a generalized susceptibility to stress. The porcine models of malignant hyperthermia have provided an ideal means for investigation of the pathophysiology and identification of susceptible individuals.[181,382] Investigations of animal models have helped pinpoint the major metabolic defect, now recognized as being in skeletal muscle.[23,143,181,382] They led to the early therapeutic trials with procaine[176,381] and dantrolene,[178] as well as to the identification of various triggering agents.[181]

While stress and its associated sympathetic responses were known to play a role in porcine MH in the absence of anesthesia,[35,219,309,393] direct sympathetic involvement in inducing human MH was suggested by Wingard[418] at a symposium in 1974. Based upon the higher incidence of sudden death in susceptible families,[417,418] he proposed human myocardial and sympathetic malfunction during the stresses of everyday life. These concepts have led to a closer examination of the role of the sympathetic nervous system in MH in general.[149,265,408]

The Syndrome of Malignant Hyperthermia

While one cannot say that the intracellular or membrane defects responsible for MH are identical in swine and human beings, the extracellular manifestations—changes in vital signs, metabolism, acid–base balance, and temperature—are remarkably similar. Both swine[5,23,24,147,164,181,217,218,264,382,395] and people[50,51,337,349,352,377,415] respond to certain anesthetic agents and drugs with a striking increase in metabolism, both aerobic and anaerobic, resulting in intense production of heat, carbon dioxide,[256] and lactate. Malignant hyperthermia can be triggered by any potent volatile agent,[45,71,94,137,151,166,181,318,415] but the onset is usually more abrupt when succinylcholine is used as the trigger, either by itself[100,152,264,311] or in conjunction with volatile agents.[100,147,164,166,181,264] Once initiated, the response cascades into a fulminant and vicious circle in which body temperature may increase 1 degree C every 5 minutes. It is not uncommon to see a temperature greater than 43 C (109.4 F), an arterial blood carbon dioxide tension greater than 100 torr, and an arterial blood *p*H less than 7.00. This syndrome is generally accompanied by tachycardia and other signs of circulatory and metabolic stress. Almost all pigs[5,23,24,147,151,152,181,217,218,264,382] and about 75 per cent of people[50,137,413] in whom MH is developing show signs of muscle rigidity, which is a contracture rather than a contraction.[51,151,195] A contraction is contractile activity associated with a propagated wave of depolarization; it is brief and reversible. A contracture is nonpropagated, prolonged, and reversible.[150] Active MH results in increased permeability of muscle: increased serum potassium,[23,24,36,50,93,147,151,152,164,217,218,264,327,395] ionized calcium,[12,149,153] CPK,[50,93,217,218,277,318,395] myoglobin,[50,349] serum sodium,[23,24,147,151,152] decreased[36,50,327,352,371] or increased[23,24,36,395] total serum calcium, and marked muscle edema.[50,51] There is probable eventual metabolic exhaustion with a more generalized increase in permeability.[120] Should the episode of MH not be fatal, upon recovery the muscles may be edematous and tender,[23,50,51] and the CPK may take ten to 15 days to return to normal from levels as high as 100,000 units.[50,248] In human beings this syndrome was identified as MH because of the precipitous rise in temperature and because of mortality rates of 60–70 per cent despite symptomatic therapy.[50,241,248,415] Recently (1976) the mortality rate was estimated to be 28 per cent.[39]

The clinical and laboratory findings in human and porcine MH have led to the *theory* that control of intracellular ionized calcium levels[115,330] is abruptly lost, resulting in a rise in intracellular calcium. Increased aerobic and anaerobic metabolism result from attempts to reverse these increases in calcium.[22,26,36,38,51,306,380] Metabolic and respiratory acidosis are consequences of the increases in metabolism, as are the changes in cellular permeability with the associated movement of water, ions, CPK, and myoglobin. The evidence also suggests that MH may involve a generalized disorder of membrane permeability affecting calcium movements.[38,203,337] It is probable that this includes specific enzyme defects that vary in different species or in families within species. These intracellular defects could result in similar extracellular manifestations, *e.g.*, increased metabolism and temperature, acidosis, and sympathetic stress responses. Study of porcine isolated actomyosin suggests that the contractile proteins function normally in susceptible animals.[138] A similar theory explaining muscular dystrophies proposes a slower process, eventually

resulting in calcium accumulation in intracellular organelles, with ultimate dissolution and muscle damage.[102] Confirmation of increased intracellular free ionized calcium levels awaits mammalian application of various intracellular measurement techniques, such as that using aequorin.[27]

Occasional episodes of human MH occur in the absence of significant fever.[25,360] This may be due to the rapid onset of severe acidosis with accompanying acute circulatory failure, or to heat loss greater than production. The former would be associated with profound susceptibility and/or the use of a potent trigger, and the latter would be more commonly expected in young children. With or without increased temperature or acidosis, there must be evidence of increased aerobic or anaerobic metabolism to support the diagnosis of malignant hyperthermia. Increased temperature is usually benign,[126] does not necessarily produce acidosis,[16] and, for example, may be observed in patients with osteogenesis imperfecta with or without anesthesia.[369]

There are several *variant responses* to succinylcholine: 1) muscle contracture, as in myotonia,[83,201,322,332,391] in which the related increase in muscle metabolism is easily tolerated; 2) changes in cellular permeability in the absence of contracture, as observed with loss of CPK and myoglobin;[25,28,284,288,348] 3) an increase in metabolism, as in MH, resulting secondarily in contracture and increased permeability.[152,264] Myoglobinemia and increased CPK values induced by nonanesthetic drugs or stress that are not associated with changes of metabolism or temperature are considered[288] at most a variant unrelated to MH (an "abortive" form).[25,284] Even in normal patients, release of CPK and myoglobin from muscle cells results from the use of succinylcholine.[281,348,386] This is exaggerated in the presence of halothane[3,13,25,200,385] and attenuated by *d*-tubocurarine.[387] Loss of CPK and myoglobin may be pronounced in some individuals subjected to anesthesia,[13,201,284,288,355] other drugs,[235,366] or exercise stress.[199,244] While it is rare for anesthesia to produce such an uncontrolled state, other conditions that predispose to or resemble this loss of control include porphyria,[374] thyroid storm precipitated historically by surgery and anesthesia,[376] the catatonic state produced by ketamine,[374] and hyperkalemia following succinylcholine administration.[150]

Specific Tissues and Organs

Skeletal Muscle

Myopathy. Most MH-affected people and their families appear normal and lead an active life, and their myopathy is detectable only by specific testing;[54,56,92,171,202,204,206,207,209,211,232,294,337–339,375,392] however, some have obvious musculoskeletal abnormalities.[206,242,375,392] It has been suggested that some disorders of muscle, such as muscular dystrophy,[29,136,230,236,284,402] myotonia,[83,286,322,332,352,391] and central core disease,[91,116,125,208] may result in susceptibility to MH, but there is insufficient (and conflicting)[116,284] evidence to confirm their direct association with MH.[51,56,202] Central core disease is a benign congenital myopathy in which longitudinal foci devoid of mitochondria or of oxidative enzymatic activity extend virtually the entire length of the fiber.[125] These cores may resemble targetoid fibers, which are seen more frequently in MH muscle.[172] An extensive histologic study of susceptible families did not disclose evidence of central core disease.[170,172]

Muscle CPK values, when determined in a resting, fasting state without recent trauma, generally reflect muscle membrane stability and are elevated in about 70 per cent of affected people[1,41,47,93,112,171,185,204,205,207,425,426] and most swine.[6,106,187,217,395,404,423] Some prefer determinations of pyrophosphate in testing for myopathy, but data are insufficient to confirm its superiority over CPK.[4,388,396] Pyrophosphate levels may rise with hypermetabolism or increased production of cyclic adenosinemonophosphate (AMP),[388] but this reasoning does not account for its elevation at rest.

Susceptibility in swine is particularly associated with breeding for accentuation of certain muscle groups, as well as for rapid growth and development to marketable weight. This inbreeding has apparently altered muscle enzyme or membrane responses.[69,251,309,393]

Histologic examination of porcine and human susceptible muscle discloses either no abnormality or protean nonspecific pathologic changes that are so variable that one cannot determine whether they are primary or acquired.[170] The fact that they are not seen in children less than 5 years old,[170] or in swine,[316,397] which are marketed at a young age,[316] in general, suggests that the various morphologic changes are acquired.[92,170,171,202,204,209,210,316] A freeze-fracture study proposes that the initial changes may involve phospholipids.[358] Histologic changes include internal nuclei, moth-eaten fibers, supercontracted myofibrils, and less often, target fibers, marked variation in fiber caliber, necrosis and regeneration, mitochondrial rupture and inclusion bodies.[7,170–172,186,202,204,209,338,339,357,375]

Muscle *contracture* responses, determined in studies of biopsy specimens of muscle fragments,[8,9,14,40,45,48,54,113,114,131,140,224,226,227,254,293,295–297,300–304,312] intact tendon-to-tendon muscle fibers,[130,131] or skinned fibers,[384,422] confirm the presence of abnormal re-

sponses in skeletal muscle from susceptible people or swine. The amount of tension developed by fragment specimens is much less than that developed by intact fibers,[131,297,350] but the latter are rarely obtained from human beings,[350] and responses of the two are qualitatively similar for swine[131] and people.[297] However, responses may vary due to differing involvements of various muscles within the same pig.[131,251] Exposure to any of various drugs in a temperature-controlled bath demonstrates an increased sensitivity or decreased threshold in susceptible muscle. These drugs include caffeine, halothane, their combination, potassium, and succinylcholine. While these tests have been relatively standardized, there are differences in results from various laboratories throughout the world.[203] For example, muscle contractures with halothane occur reproducibly and reliably in several laboratories,[8,9,14,32,113,114,293,295,296,300–304,312] but inconstantly in others.[54,140,224,226,227] Similarly, there are differences in results with caffeine, or with the combination of caffeine and halothane, in that some laboratories have reproducible and reliable results[54,140,224,226,227,312] and some have greater variability.[8,9,14,113,114,293,295,296,300–304]

Britt *et al.*[48] suggest that this variability is associated with gradations of clinical MH. Thus, muscle from patients with severe episodes of MH developed contractures upon exposure to halothane alone, as well as to caffeine alone and to halothane–caffeine. Muscle from patients with moderate episodes developed contractures with caffeine alone and with halothane–caffeine, and muscle from patients with mild episodes developed contractures only when exposed to halothane–caffeine. They relate these variations to a genetic spectrum[48,223] of susceptibility. Unfortunately, they do not correlate specific contracture responses with the clinical MH data, admittedly difficult and tedious, but necessary for support of their proposal. Furthermore, episodes of MH in swine vary in relation to adjunctive drugs[151] or prior exercise,[21,395] suggesting that genetic predisposition may be considerably modified by environment.

The lower threshold to potassium-induced contractures in susceptible muscle[131,294,312] suggests a lower mechanical threshold as a part of the disorder of MH. Results with succinylcholine are puzzling. Denborough's laboratory is consistently able to identify susceptible muscle contracture responses using succinylcholine.[14,294,312] However, others do not observe contractures in biopsy specimens exposed solely to succinylcholine.[226] One would not expect chemical depolarization of a specimen dissected to several bundles and mounted in an organ bath, because of the absence of end-plate areas and the associated chemically sensitive membrane of the receptor sites.[150] Ellis' laboratory combines succinylcholine with caffeine to provide the graded response lacking in their halothane-induced contractures.[169]

Kalow and Britt differentiate MH into two types, rigid and nonrigid, based upon gross observation of skeletal muscle tone during MH in human beings,[50,227] and supported by differing contracture responses.[224,226] They correlate this distinction with caffeine- and caffeine–halothane-induced contracture threshold responses that are significantly less than (rigid) or greater than (nonrigid) the threshold responses of normal muscle.[226] Clinically, the nonrigid cases differed from the rigid ones, manifesting *e.g.*, an absence of tachypnea, tachycardia, dysrhythmias, cyanosis, and acidosis.[50] Kalow and Britt also base their expanded and more complex genetic analysis in part upon the variations in contracture responses.[48,225,226] Other investigators support the concept of decreased thresholds in people and swine for MH susceptibility in general, but have not observed a correlation with thresholds greater than normal in patients whose episodes of MH do not include rigidity. However, not many laboratories have examined as many caffeine-initiated contractures as have Kalow and Britt, and interlaboratory comparisons may therefore not be valid. Nonetheless, Kalow and Britt's large variability in normal threshold responses is inconsistent with their theory of nonrigid MH in association with above-normal contracture thresholds. They estimate the normal caffeine threshold as 8 mM,[226] but their thresholds for known normal muscle range from 2[226] to 32 mM[197] caffeine, or more. The latter value is greater than that specified in their definition of nonrigid (insensitive) caffeine-induced responses.[226] This degree of variability has not been found in other examinations of normal thresholds.[141, 295, 304] Rather than invoke additional genetic differences, the author believes that all MH is potentially rigid, but that there are modifying factors (also suggested by Kalow and Britt[223]), and that muscle tension is ultimately related to intracellular calcium levels,[115] energy stores,[87] genetically determined responses of specific enzyme systems or membranes, the potency and duration of the initiating agent, modification by treatment, and probably other environmental factors not yet identified. Metabolism can be greatly increased by intracellular free–ionized calcium levels that are insufficient to activate contractile mechanisms to the point of observable rigidity.[27,79,368] One could argue that the nonrigid episodes reported by Britt may not have been MH

at all, for the laboratory data suggest that metabolism was not increased and that there was no acidosis,[50] *i.e.*, the hyperthermia was due to some other factor.

Measurements of depletion of adenosinetriphosphate (ATP) as an adjunctive test of susceptibility were introduced by Harrison from study of swine,[181] based on the premise that the greater metabolism in the halothane-triggered MH muscle results in a greater reduction in energy stores as compared with normal halothane-exposed muscle.[23,24,81] Pieces of muscle weighing 100–300 mg are exposed to either carbogen or carbogen plus halothane in an organ bath at 37 C for 30–45 minutes, following which they are assayed for ATP. Some,[46,181,310] but not all,[140] laboratories have found that determination of the ATP depletion ratio is a valid test for differentiating susceptible from normal human beings or swine. There may be considerable variation in individual values, making interpretation difficult due to overlap of values between normal and susceptible individuals.[140] Since the bath provides substrates and oxygen, normal muscle should maintain energy stores better than should susceptible muscle. However, the central core of the muscle is probably poorly supplied with oxygen and other substrates, and the muscle bundles may be damaged during preparation. Assay values thus may be skewed downward a variable amount. Values of ATP depletion probably fluctuate more due to experimental than due to biologic variability.

The *metabolic responses* of susceptible skeletal muscle are also greater than those of normal muscle, in both *in-vivo* and *in-vitro* preparations. Virtually all of these studies are necessarily in swine, and the susceptible responses are equivalent in a variety of preparations. The responses include approximately threefold increases in oxygen consumption, increases in circulating lactate to as much as 30 μm/ml (15–20-fold) and associated acid–base imbalances.[5,81,143,147,149,151,152,264,395]

The earliest detectable changes in MH appear in the venous effluent from skeletal muscle, as decreases in pH or P_{O_2} or as increases in P_{CO_2}, lactate, potassium, or temperature.[133,151,264] These changes in muscle metabolism and acid–base balance have profound effects upon the systemic circulation, because skeletal muscle comprises about 40 per cent of body weight. Careful examination of the time courses of these changes has shown that the increase in lactate occurs before there are obvious signs of tissue hypoxia, as evidenced by decreases in venous effluent P_{O_2}. These presumed nonhypoxic increases in lactate indicate greatly increased energy demands, for a decrease in ATP tends to alter the balance between NAD and NADH (oxidized and reduced forms, respectively, of nicotinamide adenine dinucleotide), forcing an increase in lactate production.[22–24,151,228] The increase in aerobic and anaerobic metabolism has been shown to occur prior to the increases in temperature, heart rate, and circulating catecholamines.[151,259,264] When the increase in metabolism is halted (by specific treatment of MH in swine,[148] or following a period of severe exercise in a healthy person[30]), blood lactate slowly returns to normal over a period of approximately 30 minutes.[30,148] The metabolic acidosis during porcine MH is primarily due to lactate production,[23,24,146–149,151–153,264,395] and individual case reports indicate that lactate is also the main factor in the metabolic acidosis of human MH.† One study of isolated human muscle fibers did not demonstrate increased lactate production upon exposure to halothane, but the reasons for this difference are unknown.[20] Increases in aerobic metabolism of skeletal muscle that are extrapolated to the whole body agree with observed increases in whole-body oxygen consumption, and support the premise that these are due solely to increases in skeletal muscle oxygen consumption.[143] However, the increases in oxygen consumption are smaller than the maximal increases possible, such as those seen in exercise,[342] and are paradoxically small considering the measured acid–base and temperature aberrations. Possible explanations for this are discussed under Mitochondria.

Sources of *heat* during active MH include aerobic metabolism, glycolysis, hydrolysis of high-energy phosphates involved in ion transport and contraction–relaxation, and neutralization of hydrogen ion.[23] Heat production in porcine MH is initially accounted for by increased aerobic metabolism and later, as aerobic metabolism decreases, by lactate production.[23,24,158] Precise calculations are difficult,[87] in part due to unsteady metabolic and circulatory states, measurements in only a few animals, variable and uncontrolled heat losses, and production of heat by neutralization of acid.

In examining *mitochondria* isolated from skeletal muscle, investigators have found various differences in mitochondrial function in susceptible people or pigs as compared with normal individuals. Some of these differences may be due to technique: study of pigs[24,43,62,75–78,95,108,142] or human subjects[52,54]; temperature of 25 C,[52,54,62,75,108] or 37 C[62,76–78,142]; measurement of calcium release in the absence of oxy-

† Kolb ME: Norwich Laboratory evaluation of intravenous dantrolene in humans (personal communication).

gen[75,77,78]; measurement of the concentration of halothane in liquid medium.[142] Several investigators have compared values between susceptible and known healthy subjects,[52,54,76,108,142] but others have not mentioned values from healthy subjects,[24] have not identified screening methods,[43,62,75,95] or have compared values with those obtained from unrelated swine rather than littermate normals.[43,62,75,78]

In human subjects, Britt *et al.*[52,54] found no difference between normal and MH mitochondria in regard to respiratory function, and halothane decreased state 3 respiration (oxygen consumption in the presence of a phosphate acceptor, *i.e.*, adenosinediphosphate, ADP) similarly in both. In contrast, the same group[43] reported increased respiratory function in mitochondria from MH pigs and decreased capacity for calcium uptake. Denborough *et al.*[95] found no difference in mitochondrial respiratory function between normal and MH-susceptible Landrace pigs, but, in comparison with unrelated pigs, exaggerated depression by halothane. Several groups[24,62,76,108,142] have in general found reduced respiratory and calcium-binding activities of MH porcine mitochondria. Some of these investigators also saw depressant effects of halothane upon NAD-dependent respiratory function,[24,62,108] without evidence for calcium release by halothane.[142] Cheah and Cheah reported that halothane enhanced calcium efflux from MH mitochondria under anaerobic conditions[75,77,78]; this is probably not related to initiation of MH, but could be a factor in the full-blown syndrome.

These reduced mitochondrial functions do not explain the functional and metabolic derangements observed in MH, but they are consistent with the proposal that MH is a myopathy. Reduced mitochondrial respiration is a feature of muscular dystrophy[324] and of experimental dystrophy such as that due to vitamin E.[273] Also, abnormal mitochondria have been observed in ultrastructural examination of human MH muscle.[186,198,202,210,357] The mitochondrial alterations are probably nonspecific manifestations of the disease process. Mitochondrial uncoupling had initially been proposed as a cause of MH,[416] but this has been theoretically[401] and empirically[51,142] discounted. Animal models based on uncoupling are discussed on page 408.

The diminished aerobic response[23,24,143,146,147,151,152,264] in MH, in the face of apparent marked demands for energy, is not explained by the mitochondrial deficiencies. Differences in muscle type also do not explain this aerobic deficiency. White (fast) and red (slow) muscle differ in regard to capillary structure and aerobic capability: white muscle is more dependent upon glycolysis for increased energy demands, and accordingly has a more sparse capillary network.[123,282,334] However, these muscle types are apparently equally affected in porcine MH, and histologic evidence does not support the involvement of white (fast) muscle in MH.[7,84,353] The porcine longissimus dorsi muscle lumbar segments[251] and forelimb extensor muscles,[131] respectively, show greater MH abnormalities than do thoracic segments[251] and intercostal muscles.[131]

Evaluation of the function of the *sarcoplasmic reticulum* (SR) is complicated by the use of a variety of techniques examining a physiologic process the mechanism of which is not yet known, and which involves release and reaccumulation of calcium so rapidly as to make difficult accurate measurement.[115] Also, calcium binding to SR vesicles isolated from skeletal muscle is assumed to reflect the calcium binding affinity of intact SR, although isolation itself may somehow alter function.[115] Nonetheless, SR binds calcium at accepted physiologic concentrations more effectively than do mitochondria, suggesting that the latter have at most a reserve function in this regard.[115,142,144] Calcium binding by SR is estimated by the rate and capacity of calcium accumulation in the absence of oxalate. However, the performance of these vesicles does not approach that of intact SR; this deficiency is corrected in part by oxalate.[115,144] When oxalate is added to the medium, the duration of binding, as well as the capacity, is greatly increased, and it is called calcium uptake for isolated vesicles of SR under these conditions. It bears no relation to the mechanism or character of calcium "uptake" by SR *in situ*.[144] Calcium release is difficult to measure because of problems inherent in loading SR with sufficient labeled calcium.[144]

As with mitochondrial data, varying results have been reported for SR functions.[24,44,54,63,95,98,100,144,186,210,211,227,280,303,310,347,378] This may be in part because most investigations have used calcium concentrations above the estimated physiologic range. In most studies, SR transport functions for calcium appear to be diminished in both human and porcine susceptible subjects, but, as with mitochondria, the differences from normal may not be large. Calcium binding and uptake are diminished about a third in susceptible pigs compared with normal pigs,[144,186,303] and halothane stimulates calcium binding in both normal and susceptible isolated SR in concentrations of about 0.5 to 1 per cent.[144] Halothane progressively depresses binding of both types of SR as the concentration is increased above clinical levels. Release of calcium by MH and normal SR is minimal with clinical concentrations of halothane, and marked with concentrations above clinical levels.[98,144]

These findings in regard to intracellular organelles are consistent with the diagnosis of a myopathy;

however, they do not reflect changes severe enough to account for the MH syndrome, nor does the effect of halothane explain its triggering action.[144] The specific action of dantrolene in preventing or reversing MH suggests that the lesion in MH is in part located at the link between the transverse tubule and SR, the terminal cisterna of the SR, or both.[60,289,300]

Examination of function of porcine *muscle cell membranes* also discloses differences between normal and susceptible muscle.[130,155] Halothane acts beyond the neuromuscular junction[300]; it produces contractures via mechanisms apparently involving surface membrane calcium equilibria.[155] It produces a depolarization, 5–10 mV, of susceptible skeletal muscle, but not of normal muscle (with one reported exception in the rat),[234] and this depolarization is returned towards the resting potential by dantrolene.[130] This finding suggests a common mode—depolarization—of initiating MH by volatile agents and succinylcholine. One would not expect a small depolarization, in contrast to the greater depolarization by succinylcholine, to reach mechanical threshold (fig. 1) and start the chain of events leading ultimately to contractile activity. However, susceptible porcine muscle has a lower mechanical threshold than does normal muscle,[64,294,312] implying that slight depolarization is sufficient to initiate contractile activity. I cannot speculate as to the mechanism by which this abnormal response triggers MH at the level of the SR.

In most evaluations of MH or of the metabolism of stress responses, the muscle is a "black box," and one cannot infer where specific enzymatic disorders may be located. These could involve calcium pumps or adenylate-cyclase and cyclic AMP mechanisms.[38,327,380,414] Variations from normal may not be functional and detectable unless MH has been triggered,[121] and measurements of muscle metabolites provide valid clues[87] only when the muscle biopsy specimen can be frozen rapidly, *e.g.*, using supercooled clamp forceps.[245] Several investigators have examined mechanisms of abnormal metabolism. In a family with a history of two deaths due to MH, Schmitt *et al.* demonstrated adenylate kinase deficiency within skeletal muscle biopsy specimens, but not within erythrocytes, of two of four close relatives.[361] Adenylate kinase reversibly catalyzes the reaction 2 ADP ↔ ATP + AMP.[407] If its action is adequate, increased muscle metabolism should result in increased tissue levels of AMP; if inadequate, increased levels of ADP. While intracellular pathophysiologic mechanisms in susceptible human beings and swine may differ, the only data that define adenylate kinase function, albeit indirectly, are porcine. Neither ADP nor AMP levels were elevated during MH,[81,228] leaving the question unanswered. These porcine studies also demonstrated

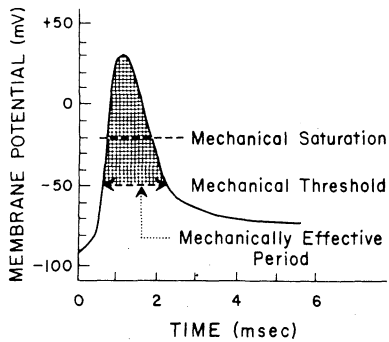

FIG. 1. Mechanical threshold is the membrane potential at which just-visible muscle contraction occurs. In normal muscle this requires a depolarization from the resting potential of approximately −90 mV to −50 mV. In susceptible porcine muscle the mechanical threshold is decreased, perhaps to −70 or −80 mV. A modest depolarization by halothane of about 10 mV approaches the mechanical threshold and can stimulate muscle metabolism. (Reprinted from Fed Proc 24:1116–1123, 1965, with permission.)

stimulation of glycolysis, in particular accelerated substrate cycling, as a possible mechanism for markedly increased heat production.[81] This occurs normally in bumblebee flight muscle, whereby metabolic recycling of fructose-6-phosphate warms muscles to temperatures efficient for flight.[80]

All of the above-mentioned responses of skeletal muscle, while decidedly greater in susceptible than in normal swine, probably represent an exaggeration of normal, rather than different, responses.[291] They occur more readily with greater environmental stress, *e.g.*, the incidence of slaughter PSE muscle is greater in summer than in winter[35]; halothane produces more marked or quicker changes after exercise[21,395]; PSE muscle develops more rapidly when the animal is exercised prior to slaughter.[35] Similar reactions occur in the so-called capture myopathy (overstraining disease) of wild animals after prolonged chase.[183,184]

Other Tissues and Organs

Heart. Myocardial function is altered during human and porcine MH, as evidenced by the early appearance of tachycardia and dysrhythmias and, later, by hypotension, declining output, and eventual cardiac arrest. Myocardial physiologic abnormalities during porcine MH include a fivefold increase in myocardial oxygen consumption and an eightfold decrease in myocardial efficiency, as measured using a right-

heart-bypass preparation.[153] These changes were mediated by beta-sympathetic agonists, as they were blocked by continuous infusion of propranolol, 40 μg/kg/min. This study did not evaluate comparative responses to beta stimulation of heart muscle specimens from normal and susceptible animals, and therefore one cannot state whether these beta-mediated changes were a normal adrenergic response to marked stress or an exaggerated adrenergic effect due to a myocardial abnormality. However, the heart did not show evidence of active MH, as there was neither myocardial lactate production nor potassium loss during whole-body MH.

Specific myocardial abnormalities have been found in three people who died during malignant hyperthermia.[119] The case histories are incomplete with reference to agents and drug therapy, but all apparently died during hyperthermia, acidosis, shock, and hyperkalemia. The combination of these factors is not often seen at death, and one wonders to what extent these account for the subsequent findings of fiber lysis, sarcolemmal disruption, and contraction bands adjacent to areas of over-stretching. Myocardial histologic features of other MH nonsurvivors have been reported to be normal.[337]

While the heart could be primarily involved in MH because of its similarities to skeletal muscle, cardiac function would be expected to be altered in MH because of activation of the sympathetic nervous system and the associated increase in circulating catecholamines.[147,151,152,259,264,395] Pigs and people maintain remarkable cardiovascular stability during active MH,[264] but episodes of sudden death in members of susceptible human families[418,421] and otherwise unexplained nonspecific cardiomyopathies and abnormal thallium scans have been suggested as evidence of direct myocardial involvement.[40,197] Cardiac abnormalities or dysfunction may occur during periods of emotional stress, or secondary to sympathetic hormones,[65] but one cannot differentiate primary changes in human myocardial function from those that might have occurred secondary to adrenergic stimulation during otherwise undiagnosed stress-induced MH in the absence of anesthetic drugs (see below for awake human MH). The associated sympathetic stimulation might alter cardiac function in any one of several ways,[65] *e.g.*, acute dysrhythmia, nonspecific cardiomyopathies or coronary vasospasm[193,255] in the event undiagnosed episodes occurred intermittently and repeatedly in awake human beings.

Central nervous system. Involvement of the central nervous system during human fulminant MH appears to be secondary to increased temperature, acidosis, hyperkalemia, and hypoxia.[36,50,248,249,337,352] The extreme clinical picture resembles acute cerebral edema with coma, areflexia, unresponsiveness, and fixed dilated pupils.[72,88,233,349] There is a variable recovery, which appears to be related to early diagnosis and the severity of the MH episode. A severe fever may result in a virtually flat electroencephalogram and coma, but recovery is still possible.[67] A most unusual response to succinylcholine, given during controlled observations, has been observed in a single susceptible child. It resulted in the acute onset of upper motor neuron lesions.[248,249] This patient was given 5 mg succinylcholine (0.1 mg/kg) injected into one arm isolated by a tourniquet: the succinylcholine produced apparently normal relaxation. When the tourniquet was deflated, fasciculation occurred, and the patient became apneic, with development of hypertonicity of the legs, hyperactive tendon reflexes, and extensor plantar reflexes. The latter neurologic findings gradually disappeared over the next 48 hours. Succinylcholine crosses the blood–brain barrier with difficulty, if at all, and it is therefore hard to explain these results.

Histologic examination of human muscle has disclosed signs of neurogenic atrophy in intramuscular axons with degenerating and regenerating fibers; other neural abnormalities are suggested by fiber-type grouping and targetoid fibers.[186,202,210,232,248,249,337–340] Although early data suggested that the increase in CPK was predominantly of the BB (brain) isoenzyme,[336,373,425,427] an indication of neural involvement, more recent reports fail to confirm this, and describe increases in CPK primarily of the muscle type.[1,185,277,283]

Britt *et al.* using the technique of motor unit counting in peripheral muscles, have found lower values in a high proportion of susceptible human subjects.[57] Unfortunately, the interpretation of these differences is complex. The technique of motor unit counting has yet to win general acceptance[325] and, in their paper, the actual data are blurred by statistical methods and difficult to evaluate accurately. Because of these studies suggesting motor nerve dysfunction that could include denervation of muscle, Moulds compared abnormal responses of denervated mice and human MH-susceptible muscle.[292] He found that the corresponding abnormalities were different, in that the effect of denervation was limited to the development of extrajunctional cholinergic receptors, while the abnormality in MH muscle occurred at a later step in the excitation–contraction coupling mechanism.

Kerr, Wingard and Gatz suggested that the nervous system plays a role in porcine MH because epidural anesthesia in susceptible swine prevented muscle

rigidity induced by halothane in the anesthetized limbs, but did not prevent rigidity in the unanesthetized limbs.[238] Other studies contradicted these data, in that conduction anesthesia did not prevent the metabolic changes induced by halothane.[149] Discussions with Wingard‡ have disclosed that mechanical stimulation, *e.g.*, incision or needle puncture, resulted in immediate rigidity in their pigs, suggesting that the prevention or delay of halothane-induced rigidity by epidural anesthesia did not signify blockade of MH responses.

Brain involvement during MH is unlikely, for *in-vivo* measurements of porcine cerebral oxygen consumption and lactate production show no increase during whole-body MH.[12] Other evidence against neural initiation of MH is provided by the lack of rigidity in a limb isolated by a tourniquet during episodes of human whole-body MH otherwise associated with rigidity.[101,354]

Sympathetic nervous system. Controversy exists as to whether sympathetic responses are abnormal in MH, and whether they help to initiate MH.[139,149,260,265,408,409,417,418,421] The sympathetic nervous system is obviously intimately involved with MH, as evidenced by the following: 1) MH develops in stress-susceptible pigs[35,69,309,393]; 2) the "fight, fright or flight reaction" can initiate an episode in swine in the absence of triggering anesthetic agents[35,309,393]; 3) typical signs of sympathetic stimulation are observed during active human and porcine MH.[35,353] Circulating epinephrine and norepinephrine increase markedly during MH from control levels of less than 1 ng/ml to levels as high as 30 ng/ml; however, these levels increase following the changes in metabolism and acid–base balance,[147,151,264,395] and their elevation is not essential to the development of halothane-induced MH.[149] They probably produce the hyperglycemia and the early,[164] but not the late, hyperkalemia; the later rise is apparently due to efflux from muscle.[151] Potassium efflux from muscle occurs much sooner when the course of MH is rapid, *e.g.*, with the use of succinylcholine.[147,152]

Under certain circumstances, sympathetic agonists trigger what appear to be legitimate episodes of MH in susceptible swine. Alpha-agonists were more effective as a trigger than were beta-agonists, and the single clear instance in which an alpha-agonist, phenylephrine, was demonstrated to initiate MH also gives a clue as to why the mechanism appears to be secondary.[162] Earlier studies from the Bristol laboratory had shown that the initiation of MH by succinylcholine resulted in an increase in lactate production prior to an increase in temperature.[264] In the study involving phenylephrine, the rise in muscle temperature preceded the increase in lactate.[162] This strongly suggests that the mechanism was due to muscle or cutaneous vasoconstriction, resulting in ischemia or decreased heat loss. Thus, hypoxia[262,353] or increased temperature[124,146,221,270] may have produced the MH response in susceptible muscle.

Williams also argues for sympathetic activation of MH, via vasoconstriction by norepinephrine.[408] The mechanism would be expected to be physical, that is, changes in blood vessel diameter and perfusion, with secondary effects upon muscle oxygen supplies and heat loss. This is supported by the recognition of the triggering action of hypoxia[262,353] or of increases in temperature *per se*.[124,146,221,270] Williams believes that awake susceptible pigs are hypertensive[412,413] and always produce more heat due to their constantly greater metabolism,[410,411] and that this is generally counterbalanced by greater heat loss. A vasoconstrictive event "tips the balance" in favor of heat retention,[299] followed by increased temperature, and progression into the cascade of events leading to fulminant MH.[299,309] Other investigators have not observed greater basal oxygen consumption in sedated susceptible swine as compared with normal swine,[147,151,152,264,383] suggesting that the unusual experience of being placed in a whole-body calorimeter while awake may evoke stress responses in the susceptible animal.[410]

Further examination of sympathetic initiation of MH involved the use of the alpha- and beta-agonists, phenylephrine and isoproterenol, in an isolated perfused preparation of porcine skeletal muscle.[146] Phenylephrine did not increase either oxygen consumption or glycolysis, but did result in tissue edema at roughly similar doses in both susceptible and normal muscle. Isoproterenol did not increase oxygen consumption of either susceptible or normal muscle, but did similarly increase lactate production in both. Others also observed that beta-agonists did not trigger MH[181] or result in PSE muscle.[35] There are thus no indications of differing responses to sympathetic agents.

The use of sympathetic antagonist drugs has suggested protection or amelioration during episodes of MH,[35,259,261–263,266,408,412] by lowering temperature and modifying the acid–base changes. However, not all studies have demonstrated this,[181,405] and, in those that did, large doses of alpha-antagonists and/or adrenalectomy were necessary to provide variable protection.[259,261,263,408,412] This improvement has been somewhat uniformly interpreted as an effect upon

‡ Personal communication.

"sympathetic-induced MH."[259,260,265] However, alpha-antagonists may increase heat loss and can potentially increase muscle perfusion by eliminating alpha-agonist-induced vasoconstriction, thus minimizing ischemic hypoxia. Beta-antagonists have attenuated metabolic and temperature responses, but insufficiently to improve survival.[181,261] In large doses, they completely block the stimulation of myocardial metabolism observed during porcine MH.[153] However, the mechanisms of these modifications appear to relate to recognized actions of propranolol in blocking beta-mediated stimulation of the myocardium and beta-induced vasodilation[18] or stimulation of glycolysis[146] in skeletal muscle. Effects upon muscle blood flow during MH are indirectly supported by the observation of decreased vascular resistance during isoproterenol infusion in perfused isolated skeletal muscle.[146]

Evidence against sympathetic initiation of porcine MH during anethesia is provided by a study utilizing total spinal blockade.[149] The accompanying sympathetic denervation failed to affect the onset, development, or characteristics of halothane-induced MH, while the increases in circulating epinephrine and norepinephrine were completely blocked.

Neurotransmitters and relaxants. Carbachol, a long-lasting laboratory equivalent of acetylcholine, has effects similar to those of halothane and increased temperature *per se* upon isolated perfused porcine skeletal muscle, *i.e.*, it increases oxygen consumption approximately threefold and stimulates lactate production markedly.[146] Succinylcholine is structurally related to acetylcholine and also has similar effects.[152] An earlier study did not demonstrate this stimulation by succinylcholine, but that was apparently due to measurement of muscle tone and temperature without associated measurements of metabolism and acid–base balance.[309] Nondepolarizing relaxants such as *d*-tubocurarine and pancuronium block the effects of succinylcholine or carbachol§ in triggering MH, but do not block the triggering effects of halothane.[160,173,176] Other studies in intact swine have suggested delay or attenuation of the effects of halothane by nondepolarizing relaxants,[160] but similar delay has been observed with thiopental[151] and the mechanisms are unknown. Pancuronium has been suggested as a trigger in porcine MH, but the concomitant use of halothane in that study makes that interpretation highly unlikely.[74] One case report[403] discusses the possible role of pancuronium in triggering human MH, but this seems unlikely, as it has been used in many susceptible patients without triggering MH. See below for discussion of curare as a trigger in MH.

§ Gronert GA: Unpublished data.

Splanchnic viscera. Increases in hepatic temperature in pigs had suggested that heat production in MH began in the liver, implying a direct involvement of hepatic metabolism.[23,24,36] Measurements of metabolism[58,164,177] of perfused liver *in vitro* and of splanchnic oxygen consumption and lactate production *in vivo*[143,164] contradict this initial impression. Stimulation of splanchnic or hepatic metabolism during exposure to MH triggers was not observed; splanchnic blood flow and metabolism decreased as whole-body MH progressed.[143] However, decreased function has been found in liver tissue from susceptible as compared with normal animals, whether the swine were stressed[99,117] or not[89] prior to death. Findings in the latter study rule out stress responses and the associated decrease in splanchnic blood flow as a cause of the diminished function. The relationship of structural differences in mitochondria of livers from susceptible and normal pigs[63] to these functional differences is not known.

Blood. Abnormal membranes of human or porcine blood cells have been inferred from findings showing variations in permeability[424] or fragility.[77,182,232,337,370] Normally, platelet ATP stores may be depleted during ADP release associated with aggregation.[406] Initial data suggest that halothane may increase ATP depletion in platelets from susceptible people but not in those from normal people,[61] an effect similar to ATP depletion in skeletal muscle. Present data are insufficient to permit estimation of the reliability of platelet ATP depletion as a screening test for susceptibility. Abnormal coagulation during fulminant MH is discussed under Treatment.

Pulmonary. Pulmonary changes during MH include tachypnea, hyperventilation, $\dot{V}/\dot{Q}$ abnormalities, increased arterial blood P_{CO_2}, decreased arterial blood P_{O_2}, and ultimately, pulmonary edema.[23,24,50,70,147,181,217,218,264,275,337,349,352,354,382,395,413] Increased Pa_{CO_2} is due less to pulmonary failure than to increased CO_2 production. In fact, when cardiac output decreases late in MH, $\dot{V}/\dot{Q}$ may be well matched with efficient CO_2 removal. This is demonstrated in figure 2, where mixed venous blood P_{CO_2} had reached 124 torr at a time when Pa_{CO_2} had decreased to 55 torr. In this situation, whole-body carbon dioxide stores are better reflected by mixed venous blood P_{CO_2} than by Pa_{CO_2}.[146,151]

Renal. Renal function during active MH has been evaluated only indirectly; the oliguria and anuria appear to be secondary to shock, ischemia, cardiac failure, myoglobinemia, and myoglobinuria.[349,352,354,413]

Endocrine. Data suggesting that altered human membrane responses may affect calcium transients are provided in the reports that susceptible patients showed a greater increase in plasma insulin concen-

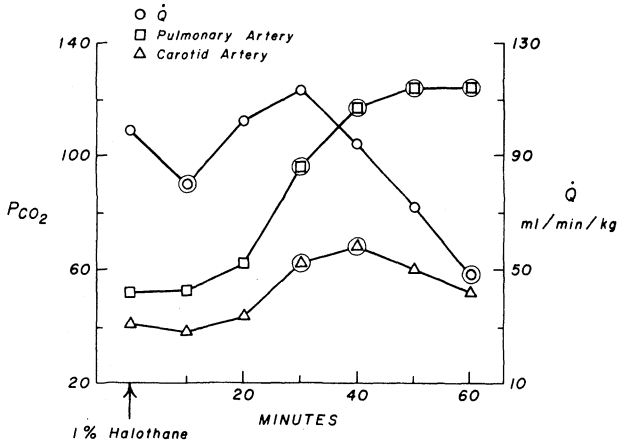

FIG. 2. Q and P_{CO_2} of MHS swine with halothane. During fulminant MH and incipient cardiac failure, arterial blood carbon dioxide decreases while mixed venous blood carbon dioxide (pulmonary artery) is still elevated.[151]

trations following a glucose load than did normal patients,[97] and that diabetic MH-susceptible patients may have diabetes that is more difficult to control.[321] While hyperglycemia occurs during active MH, probably due in part to catecholamine effects,[154,164,217,218,264,395] insulin levels remain low to slightly increased; this may be secondary to the associated increase in catecholamines.[164,165] Data from swine evaluating endocrine function initially suggested partial adrenocortical insufficiency.[219,220] Further studies demonstrated adequate adrenocortical function[165,269–272] with increased turnover of cortisone in susceptible animals.[271] This seems likely to be related to exaggerated responses to the stress of handling and sampling that would not be observed in stress-resistant animals. Thyroid function in susceptible animals has been reported as both increased[105] and diminished[35,219,220,258,266]; in one of these studies cautious administration of triiodothyronine was reported to increase survival in stress responses.[258] In these animals, MH was triggered solely by succinylcholine, there were no control animals, and triiodothyronine had a low margin of safety; therapeutic applications appear unfeasible. The catecholamine activity may also diminish thyroid function.[259]

Bone. The calcium content of bone, analyzed by neutron activation, is lower in some, but not all, susceptible patients.[49]

Overview

Alterations in calcium control result in obvious dysfunction in skeletal muscle exposed to appropriate stimuli. Altered calcium control in tissues with more subtle effects upon the whole body may be less apparent. Calcium ion affects the permeability and control of both excitable and inexcitable tissues, but mechanisms within the former may not be relevant to the latter.[330] A wide variety of multi-organ-system defects has been found in susceptible individuals, suggesting that there may be a generalized alteration in membrane properties or permeability. These have been discussed above, and include skeletal muscle membranes, enzyme systems, mitochondria and sarcoplasmic reticulum; heart; central nervous system; liver; blood cells including platelets; endocrine–pancreas, thyroid; bone.

Many of the changes in tissues and organs observed during fulminant MH may be due to blood flow insufficient for metabolic demands, resulting in breakdown of cell membranes, with resultant edema and further loss of perfusion. While not specifically due to MH *per se*, this failure of the peripheral circulation is probably produced by severe acidosis, vasoconstriction, hyperkalemia, decreased cardiac output, and hypotension.

Triggering of MH

The mechanism of triggering in human beings is difficult to examine; in swine it apparently *requires*, at least in part, depolarization of muscle membranes. This reasoning is supported by 1) the muscle end-plate depolarization (and stimulation) of metabolism by carbachol and succinylcholine that is prevented but not reversed by nondepolarizing relaxants, and 2)

the depolarization (and stimulation of metabolism) by halothane that is not blocked by non-depolarizing relaxants but is prevented and reversed by dantrolene.

In swine, increased metabolic responses result from environmental stress such as exercise,[69,221,309,393] heat stress,[124,146,221,270] anoxia,[262,353] apprehension or excitement,[69,309,393] the potent volatile anesthetics,[45,146,147,149,151,335,336,395] and succinylcholine[147,152,167,264] or decamethonium,[90] but not from lidocaine.[166,420] In people, triggering in the absence of anesthetic agents is not proven, but consider the following: 1) susceptible families may have an increased incidence of unexplained sudden deaths[418,421]; 2) susceptible individuals may develop a nonspecific cardiomyopathy[197] related either to unrecognized awake episodes or to primary myocardial abnormalities in MH; 3) (based upon contracture responses) one susceptible patient has had awake febrile episodes (40.6 C) for 10–15 years; these last several days, are related to fatigue or emotional upset, and respond to therapy with dantrolene, but not to aspirin, surface cooling, or other symptomatic treatment.[154,419]

In the absence of anesthetic drugs, the mechanism of triggering in swine is hypothesized from the laboratory findings as follows. Progression of MH results from a hypermetabolic response to the neurotransmitter in association with normal or unchanged responses to sympathetic stimulation. Muscular activity occurs during exercise, excitement, and sympathetic stimulation, *e.g.*, tail twitching, "jumpy." This muscle contractile activity, resulting from endplate effects of acetylcholine, apparently produces elevated uncontrolled levels of intracellular ionized calcium, the ultimate reasons for which are unknown, but which are related to exaggerated responses to this stimulation of susceptible muscle as compared with normal muscle. Thus, normal muscle undergoing the same degree of activation does not develop metabolic aberrations. The elevation in intracellular calcium results in greater than normal muscle oxygen consumption and lactate production. The beta-sympathetic stimulation accompanying excitement may further increase lactate production.[146] These combined metabolic effects result in respiratory and metabolic acidosis, increased temperature of the muscle venous effluent, and secondary adrenergic stress responses. Alpha-sympathetic stimulation produces vasoconstriction, resulting in decreased heat loss and, possibly, limited muscle perfusion. Increased temperature or relative ischemia resulting from blood flow that is inadequate for the increase in metabolism can exacerbate the metabolic changes and the combined acidosis. These may cascade into a vicious circle of fulminant metabolism, acidosis, and high temperature in association with metabolic exhaustion, failure of cellular membranes, further loss of control of calcium,[129] and cardiovascular collapse. While human beings could have similar responses, one would expect that they would tend to control their emotions and activities more effectively than do swine, and that episodes related to awake triggering might be more subtle.[154,419]

Anesthetic triggering of MH, first by depolarizing drugs such as succinylcholine or decamethonium, would be by effects in people and swine similar to, but more prolonged than, those of acetylcholine. Secondly, triggering by halothane would be due to an action beyond the end-plate.[130,300] There is no information to predict effects of volatile agents upon intact porcine sarcoplasmic reticulum, but at present the data regarding isolated SR suggest that volatile agents do not trigger MH via effects on intact SR. Data about intact human responses to volatile agents at the cellular or subcellular level are not available.

Nitrous oxide and *d*-tubocurarine have been separately incriminated as weak triggers in human MH; the former because it twice produced hyperthermia (blood-gas values not reported) in an 11-year-old susceptible girl who needed dental care,[111] and the latter because it produced hyperthermia in two susceptible children, 8 and 13 years of age (blood-gas values support the diagnosis in one of these cases).[59] These questions cannot be settled conclusively by animal experiments because of possible species differences. At present, few believe that nitrous oxide is a real trigger of MH, because it has been used repeatedly as the basic anesthetic agent for MH-susceptible patients.[37,50,55,68] The hyperthermic episodes have been attributed to residual halothane in the breathing tubes,[247] light anesthesia with multiple reflexes stimulating skeletal muscle,[152] or the belief that nitrous oxide may indeed be a weak trigger easily overridden by barbiturate or opiate depressants.[50,68] *d*-Tubocurarine and metocurine are unlikely MH triggers, based upon known pharmacologic responses in MH, unless this is a side effect unrelated to their action as nondepolarizing relaxants. *d*-Tubocurarine has been associated with greater lactate production in susceptible pigs exposed to environmental stress,[353] but it is not a trigger in susceptible swine.[176] Its effect in these two cases may be attributed to the use of other possible triggering drugs (nitrous oxide or chlorpromazine[344]), or to light anesthesia. At present, pancuronium is the only nondepolarizing relaxant with sufficient use in susceptible patients to confirm its safety (see above in section on neurotransmitters and relaxants regarding pancuronium as a trigger).

Drugs or conditions other than the volatile agents or succinylcholine that have produced human responses remarkably similar to malignant hyperthermia include vitamin E[213] (later retracted when two of the patient's three children had positive contracture responses to halothane),[214] ketamine, phencyclidine, viral infections,[356] lymphomas,[394] some of the tranquilizers,[183,212,298,344] tricyclic antidepressants and monoamine oxidase (MAO) inhibitors.[31,240,246,268,326,372] Ketamine and phencyclidine are structurally related and can increase temperatures of healthy people.[215,314,343] Ketamine has occasionally,[189,287,346,363] but not always,[257,364,399] increased the temperatures of MH-susceptible individuals, but the difficulties in differentiating a benign fever from MH preclude its general use.[252] In large doses MAO inhibitors can produce hyperexcitability and exaggerated motor activity, and, used with diazepam, they have produced heat stroke.[240] While it is likely that these adverse effects could occur in both normal and MH-susceptible people, the risk of these is probably greater in the latter.

Genetics

Initially, human inheritance seemed to be autosomal dominant[51,56,94,205,206,222,242,250,320] with reduced penetrance, *i.e.*, fewer affected offspring than predicted by dominant patterns,[118] and with variable expressivity, *i.e.*, differing susceptibility between families with little variation within a given family.[56,222,320] Some investigators have not seen evidence of reduced penetrance manifested as generation skipping.[110] Others felt that the human pattern of inheritance fit no known genetic system.[232,233] It has recently been proposed that humans inherit susceptibility to MH via more than one gene or more than one allele, and that the pattern of inheritance may thus range from recessive to dominant, with graded variations in between. This is discussed below.

Several years ago, Kalow and Britt[224–226] suggested that caffeine and halothane–caffeine measured different features of muscle responses. Halothane–caffeine thresholds seemed to separate a group of biopsy specimens into control responses (threshold greater than 1.3 mM caffeine) and susceptible responses. The responses to caffeine then divided the susceptible group into a spectrum of susceptibilities. Kalow and Britt further analyzed genetic variation by examining the ratio (threshold to caffeine)/(threshold to halothane–caffeine). High ratios, 17–27, suggested a separate phenotype of nonrigid MH susceptibility.[226] More recently, Kalow and Britt correlated clinical severity of MH with graded contracture responses—the occurrence of halothane-induced contractures as the correlate of greatest susceptibility and severest clinical episode, that of caffeine-induced contractures as somewhere in between, and that of halothane–caffeine-induced contractures as the correlate of least susceptibility and clinical severity.[223] From this they inferred a graded inheritance, and they tended to discount other factors modifying MH episodes. There are not, at present, sufficient clinical data with clear genetic implications because of a lack of control of drug or environmental modifying factors in clinical MH. The theories of Kalow and Britt are somewhat clouded by the variation in contracture responses of normal subjects, whose caffeine thresholds range from 4 to 32 mM or more, and whose estimated threshold ratios range from 4 to 27. However, despite these contradictions, all of their data together suggest genetic complexities, and it seems likely that there is a multifactorial inheritance with a range of susceptibilities.

Ellis and Harriman,[109,110] after extensive examination of histologic features of muscle and contracture responses to halothane, proposed two independent indicators of susceptibility: the histologic presence of a myopathy, and the demonstration of a halothane-induced contracture *in vitro*. However, pathologic features varied, even among siblings, and a diagnostic pattern was not observed. Furthermore, since structural changes may surface later,[170] it may be inappropriate to use histologic changes for genetic interpretations. Since either of these changes or both could be present in susceptible individuals, these investigators also felt that MH is a multifactorial genetic disorder.

With multifactorial inheritance, the offspring tend to be the average of the parents, and there may be gradations of susceptibility among members of a given family. Nonetheless, some families would be expected to have dominant patterns of inheritance. Other myopathies also show patterns of variable inheritance.[317]

Porcine inheritance of MH also originally appeared to be autosomal dominant.[6,217,411,412] Others have proposed a recessive pattern of inheritance with high or complete penetrance,[11,107,313,367,383,404] or a multifactorial inheritance involving at least two abnormal genes or alleles.[53,367] Many of these investigators relied on screening with halothane to identify susceptible animals.[11,107,313,331,367,383,404] This method identifies only those pigs that are most susceptible, and may miss animals with graded and lesser susceptibility.[140,411,412] The latter can be detected by screening with both halothane and succinylcholine,[140,411,412] or by additional screening of muscle biopsy specimens utilizing ATP depletion[217] or contracture studies.[53] These

less susceptible swine are decidedly different from normal swine, and their inclusion in data for genetic analysis could alter the interpretation. Blood groups and certain production traits become associated when swine are inbred.[2] Andresen[10] and Christian[331] have suggested that porcine inheritance is cross-linked with blood type, and Andresen's data further suggest that these may be cross-linked with the locus for 6-phosphogluconate dehydrogenase.[11] Assuming this also represents a multifactorial inheritance, there may be recombinant linkage of these specific factors leading to MH susceptibility in offspring of apparently normal but heterozygous parents. This may also account for sporadic cases of MH susceptibility in swine usually considered nonsusceptible.

Depending upon degree of susceptibility and environmental factors, the ease of initiating MH in human beings or swine could fluctuate. This concept might explain those situations in which known susceptible individuals have shown no sign of MH during exposure to triggering agents.[28,70,86,88,168,329,346] In the event muscle membrane depolarization and decreased mechanical threshold play a role in human MH, as they seem to do in swine, then drug or environmental factors might alter these characteristics, and there may be instances in which susceptibility could be acquired.[356,363,394] With this "acquired" state of susceptibility, exposure to the proper triggers could result in MH. Anesthetic-induced MH, and, in some instances, stress-induced MH, have been reported to occur in the horse,[243,279,400] cat,[90] dog,[15,364,365] deer,[323] birds,[191] and wild animals during capture.[183,184,191] It is not known whether these episodes occur in species that have some form of recombinant or other genetic susceptibility, or whether this is due to environmental or drug-related factors.

Other Animal Models

Animal models have been used to examine abnormal responses and the findings used to study different aspects of MH. Human and frog muscle respond to caffeine and halothane with contractures, and these drugs potentiate each other.[293,345,381] Lidocaine accentuates these contractures and procaine inhibits them,[293] although the latter drug is not effective once the contracture has developed.[381] This then becomes a model of human MH as mimicked by caffeine, and the different volatile anesthetic agents have different effectiveness in augmenting these caffeine-induced contractures.[333] Application of these effects of procaine and lidocaine to human MH is limited because the tissue concentrations of 2–5 mM cannot be achieved without administering large doses.[159,188,293] A similar preparation has been developed using rat rectus muscle,[175] and an *in-vivo* rabbit model closely mimicks MH by using caffeine to produce a response similar to MH upon challenge with halothane.[103]

Models of uncoupling, in general using dinitrophenol,[416] demonstrate lethal increases in temperature upon exposure to halothane at high (25 C) but not at low (20 C) environmental temperatures.[184] These have demonstrated that haloperidol pretreatment antagonizes the hyperpyrexic and lethal effects of dinitrophenol in the absence of anesthetic agents or caffeine.[134] Unfortunately, haloperidol alone may produce fever.[135] Gatz has reported higher temperatures in dinitrophenol-treated rats that breathed oxygen as compared with those that breathed room air.[132] On this basis, and because of oxygen's action in uncoupling nonbiologic systems, he proposed,[132] but could not demonstrate,[237] that treatment of MH using ventilation with air may be more efficacious than ventilation with oxygen. Ryanodine-induced contractures in mice, cats, and frogs have been proposed as an alternative to caffeine; however, dantrolene and procaine are not effective in counteracting this contracture.[73] While all of these models have acquired, rather than genetic, hypermetabolic responses, they nonetheless may help to increase our understanding of the genetic form.[51]

Human–Porcine Differences

Human and porcine MH have several differences: 1) histologic abnormalities are found in people, but seldom in swine; 2) MH can develop in swine in the absence of anesthetic drugs, but this occurs rarely in man; 3) total serum calcium increases in pigs,[36,51,395] while it more often decreases in people.[36,50,51,327,352] The first of these apparent differences may be explained by the observation that the histologic abnormalities may be acquired or secondary and may develop only with time, *e.g.*, they are not seen in young children; swine, because they are marketed at an early age, have not been examined when older. The second difference is more difficult to explain: perhaps people can generally manage their emotional responses so as to avoid prolonged or extreme agitation or excitement, once they realize the intense upset provoked by sympathetic discharges that occur during MH.[154] The third apparent difference is probably due to copious calcium-free intravenous therapy of human patients, and the general lack of therapy of swine. Muscle calcium contents of susceptible pigs[42,309] and people[19,42] are variable, and can be less than[42,309] or not different from[19] those of healthy individuals. Comparisons of blood or serum ionized calcium would probably be more meaningful than those in-

volving total calcium. In swine, ionized calcium increases during MH.[12,149,153] While the two species tend to have similar myopathic deficiencies in mitochondria and sarcoplasmic reticulum, intracellular functions in intact tissues could vary considerably without necessarily altering extracellular manifestations.

Diagnosis

During anesthesia suspicion of MH is aroused by symptoms or signs, including rigidity with succinylcholine,[100,379] that are extraordinary for that patient and procedure.[50,55,137] In susceptible subjects MH may be triggered even in the absence of triggering anesthetic agents when the level of anesthesia is very light and reflex responses are present.[152] In general, though, MH would not be expected to occur in any patient given barbiturate–nitrous oxide–opiate–pancuronium anesthesia.[37,55,68] Using potent volatile agents or succinylcholine, one would be suspicious if there were undue tachycardia, tachypnea, dysrhythmias, mottling of the skin, cyanosis, increased temperature, muscle rigidity, sweating or unstable blood pressure. In particular, rigidity following an adequate dose of succinylcholine correlates highly with susceptibility to MH.[100,379] If any of the preceding abnormalities is present, one must search for signs of increased metabolism, acidosis, or hyperkalemia. Arterial blood analysis should demonstrate metabolic acidosis, and may show respiratory acidosis if the patient is unable to increase ventilation as metabolism increases. In this regard, central venous blood levels of oxygen and carbon dioxide will change more markedly than will those of arterial blood.[133,151] Suggested limits for the diagnosis are a base excess of less than −5 mEq/l and an arterial blood P_{CO_2} greater than 60 torr without reasonable explanation. Increased oxygen consumption is difficult to measure while the patient is anesthetized in the clinical situation. Although CO_2 production can be measured more easily,[256] one must generally rely more upon measurements of P_{CO_2} in relation to the estimated alveolar ventilation. In particular, when the temperature is rising and there are signs of muscle stiffness and acidosis, the diagnosis is established and treatment must be instituted.

Treatment

Malignant hyperthermia is triggered in proportion to the susceptibility of the subject and to the total dose of triggering agent (concentration × duration of administration). Following a brief administration, discontinuation of the agent may be adequate treatment.[55] Also, some drugs such as thiopental appear to slow the development of MH so that termination of anesthetic administration may again be sufficient therapy.[151] While these factors may account for the puzzling instances wherein patients have tolerated triggering agents during prior episodes of anesthesia without observed signs of MH and then experienced fulminant MH during subsequent anesthesia, there remain troubling cases wherein known susceptible patients tolerated prolonged exposure to (adequate doses of) triggering agents.[28,70,86,88,168,329,346]

When MH becomes fulminant—arterial blood P_{CO_2} greater than 60 torr and rising, mixed venous blood P_{CO_2} greater than 90 torr and rising, base excess less than −5 mEq/l and falling, temperature increasing at least 1 degree C per 15 min—adequate therapy is urgently needed for survival.[38,50,336] In some patients the metabolic and acid–base changes occur so rapidly that they markedly diminish cardiac output and result in minimal tissue perfusion and minimal increases in temperature, with rapid demise.

Dantrolene is the only known specific therapeutic drug, but it must be given while there is still adequate muscle perfusion. It is a lipid-soluble hydantoin derivative that acts distal to the end-plate within the muscle fiber, and is specific in preventing or halting porcine MH.[148,161,178,305] A few case reports have begun to confirm its effectiveness in human MH.[118,128,256¶] Its efficacy in both species suggests that the defect in MH may be located at or prior to its site of action. Dantrolene attenuates calcium release without affecting uptake, by an action upon the connections between the transverse tubules and the terminal cisternae of the sarcoplasmic reticulum, or upon the terminal cisternae directly, or both.[60,289,300] Dantrolene has been demonstrated to be effective in a variety of experimental porcine situations, including contracture studies of isolated muscle,[8,9,14,300,305] and in the intact pig.[122,148,161,178,180,239,305] It controls abnormal metabolic responses and the associated acid–base imbalances, ion fluxes, and sympathetic stimulation more predictably than does symptomatic therapy.[148] It is not useful in screening for susceptibility.[121] It is not associated with serious toxicity at effective doses.

Dantrolene, even in very high doses, does not produce muscle paralysis, although weakness may result.[148] Its one ill effect is hepatic dysfunction, which has not been seen with oral administration of less than three weeks' duration.[362] The porcine minimum effective dose is 3.5–5 mg/kg, intravenously, with attenuation at 1–3 mg/kg.[148,178,305] The human

¶ Kolb ME: Norwich Laboratory evaluation of IV dantrolene in humans (personal communication).

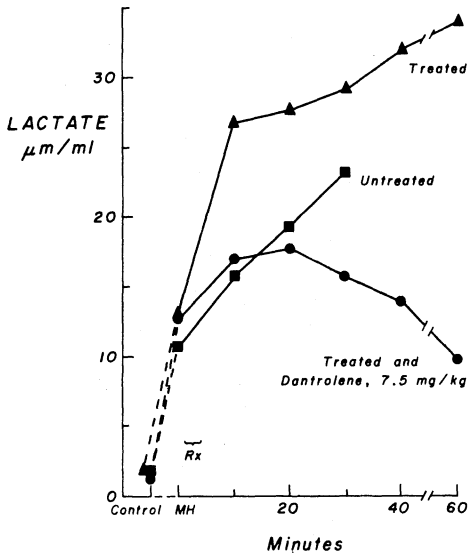

FIG. 3. Arterial blood lactate in MHS swine. During symptomatic treatment of MH, blood lactate continues to rise as a sign of unchecked fulminant metabolism (treated). When dantrolene is used in addition (treated and dantrolene), blood lactate decreases—a sign of dantrolene's specific inhibitory effect upon muscle.

effective dose intravenously is probably 1–2 mg/kg, which may be repeated each 5–10 min, to a total dose of 10 mg/kg.[118,128] The dose for effective oral pretreatment with dantrolene in human patients is not known, but is probably in the range of 4–7 mg/kg/day, given in divided doses and started at least 24 hours preoperatively.[127,319] Because dantrolene is poorly soluble, any provision for its rapid emergency use necessitates that the drug and solvents be immediately available in the anesthetic area.[104,145]

Procaine is theoretically effective in the treatment of MH, but is impractical clinically (see above under discussion of other animal models). Reports have both condemned[82,159,166,188] and praised[17,36,173,176,231,293,295,311,379] the use of procaine for the treatment of human MH. Two controlled evaluations of procaine and dantrolene in swine suggest that procaine is ineffective in treating the clinical syndrome and that dantrolene is highly effective.[147,148,304] Procaine may be useful in the treatment of dysrhythmias during the acute episode, and is safer than lidocaine, particularly with the higher blood levels associated with intravenous administration (see above under discussion of other animal models).

Symptomatic therapy for MH, while important, must be used in conjunction with dantrolene, because MH may continue to smolder or become fulminant despite control of temperature and acid–base disturbances (fig. 3). Ventilation should be increased two- to threefold[147,148,237] and sodium bicarbonate, 2–4 mEq/kg, given rapidly intravenously. Arterial blood-gas and acid–base values guide subsequent therapy. Cooling is necessary to lower temperature. Cooling should be aggressive for rapidly increasing temperatures and for those above 40.6 C: surface cooling with the patient packed in ice, gastric or peritoneal lavage, iced intravenous fluids, and pump bypass with a heat exchanger can be used.[347] Cooling should be halted when temperature falls below 38.3 C to prevent inadvertent production of hypothermia.

Urinary output should be measured and diuresis maintained with output approximately 2 ml/kg/hr, using volume loading or diuretics.[257] Initial volume loading would include 2–8 ml/kg balanced salt solution, depending upon the response of the patient. In the event large doses of sodium bicarbonate are necessary in controlling acidosis, furosemide will help to start excretion of the sodium load. Renal failure is primarily prevented by maintaining adequate urinary output. Forced diuresis utilizing either mannitol or furosemide may be necessary as additional therapy. Mannitol may be risky if urinary

output is low because high blood levels of mannitol can be associated with passage across the blood–brain barrier, particularly in association with coma or altered membrane permeability, *e.g.*, Reye's syndrome.[359]

Steroids have been recommended by Ellis for the treatment of human MH, based upon clinical results in nitrous oxide-induced MH and upon human contracture responses.[111] Their efficacy has not been confirmed in porcine MH,[163] and steroid use was associated with a higher mortality rate in a retrospective statistical examination of the treatment of human MH.[35] However, many factors contribute to MH-associated deaths, and steroids are probably helpful during these severe stresses and are unlikely to be specifically harmful.

Based upon retrospective statistical data, and theoretical considerations, Britt suggests that cardiac glycosides may worsen MH[55]; while they have been used in therapy of human MH without untoward effect,[85] their practical application is unknown, and could be hazardous. Deliberate reduction of plasma potassium concentration is at best slow, and the most effective means of lowering it is probably the reversal of the MH process, *i.e.*, effective doses of dantrolene. The administration of calcium to counteract hyperkalemia is risky. Few hospitals have the means to measure ionized calcium levels quickly, and the calcium could conceivably retrigger MH.

Treatment of pulmonary, cerebral, and muscle edema is not different from that used when these occur in association with other disorders. Neurologic sequelae (coma, paralysis) may occur in advanced cases, probably secondary to oxygenation and perfusion that are inadequate for the increased metabolism, as well as to fever, acidosis, and potassium release. Apparently satisfactory anesthesia care may be grossly inadequate in this situation. These neurologic sequelae may persist.

Disseminated intravascular coagulation or consumptive coagulopathy (DIC) may be caused by hemolysis, increased release of tissue thromboplastins due to increased permeability or overt tissue damage, shock secondary to inadequate capillary perfusion, or some rare mechanisms perhaps related to the increased permeabilities present in fulminant MH.[50,86,88,257,275,327,328,349,351,352,413,426] The best treatment is adequate therapy of MH to prevent stagnation of peripheral blood flow and to lower temperature. If the coagulopathy develops and persists despite treatment for DIC, heparinization may be successful in treating it.[285]

One cannot overemphasize the necessity for early diagnosis and early effective treatment to avoid complications.[277] They are all difficult to treat and are associated with serious and sometimes permanent sequelae.[275] Retriggering may occur,[50,55,274] even with dantrolene, as the initial dose of dantrolene is redistributed, metabolized or excreted. Dantrolene has a half-life of about five hours, and its administration should probably be continued for 12 to 24 hours following control of MH, and reinstituted with signs of increased metabolism or acidosis, Dantrolene can be given orally when the gastrointestinal system is functional. The recovering patient therefore needs close monitoring for approximately 24 to 48 hours postoperatively.[50] During this period there should be normal renal function, blood coagulation, bleeding time and blood gases; the neurologic status must include absence of rigidity, and temperature and EKG must be normal.

Lewis, of Children's Hospital in Los Angeles, has proposed that therapy of human MH should include cooling and reversal of acidosis, but that halothane administration should be continued, so as to aid in cooling by maintenance of vasodilation. His approach is based upon the lack of mortality in a series of patients treated for intraoperative hyperthermia at his institution[253]** (discussion following Zsigmond *et al.*[427]). This is not a rational approach to a disorder in which halothane is acknowledged to be a trigger, and animal data contradict its efficacy.[90] In the absence of published data that these patients and their families were susceptible to MH, the author speculates that these patients had fever, but not malignant hyperthermia. At least one other patient has survived the continued administration of halothane during symptomatic therapy for active MH,[276] but at present this is not an accepted mode of therapy. Morphine has been recommended for treatment of human MH despite its lack of effect in porcine MH, but the nonspecific depression by opiates is not likely to be as effective as dantrolene treatment, assuming it is effective at all.[33]

Evaluation of Susceptibility

Evaluation of susceptibility[37,113,203,211,296] to malignant hyperthermia includes a history and physical examination for detection of subclinical muscle weakness or abnormality. A genealogy going back two generations with specific information about anesthetic exposure and, if possible, the agents used, will help in estimating the likelihood of adequate exposure to triggering agents. Measurements of blood CPK are 70 per cent reliable in estimating susceptibility,

** Lewis GB: Current Problems in Pediatric Anesthesia, Review Course, International Anesthesia Research Society, San Francisco, March 1978, Audio-Digest, Vol 20, no. 10, May 22, 1978.

and provide a basic screening tool.[47,112] When CPK is elevated and the subject is a close relative of a known susceptible individual, then he may be considered susceptible to MH without further testing. When the patient is a close relative of a susceptible human and CPK is normal on three occasions, then muscle biopsy is necessary to determine susceptibility. Investigations of the biopsy specimens obtained to detect MH are performed in several centers around the world, and utilize exposures to halothane, caffeine, halothane and caffeine, potassium, or succinylcholine. Such investigations, in conjunction with histologic examinations of muscle and/or measurements of ATP depletion, are perhaps more than 90 per cent reliable in the evaluation of susceptibility, despite a number of differences among the investigators in regard to laboratory technique, preferred triggering agents, and normal values. Porcine data indicate that pretreatment with dantrolene does not alter muscle contracture responses *in vitro* from susceptible to normal, presumably because of washout of dantrolene in the bath.[307] Platelet ATP depletion,[61] skinned fiber responses,[40,422] and a tourniquet test of muscle ischemia and altered twitch responses[341] have yielded data inadequate for evaluation as screening tests. Porcine susceptibility is related at least in part to blood type,[331] but determination of blood type has not yet replaced halothane and succinylcholine challenges as a screening method.[140,412] There is no information regarding human susceptibility and blood type.

Anesthesia for Susceptible Patients

In the preoperative period it is important to avoid subjecting the patient to anxiety and stress, and to reassure the patient that you have confidence in monitoring for MH and in providing proper treatment should it develop.[21,37,55,287,417,421] The patient should be well premedicated, but phenothiazines should be avoided, as they may release calcium from sarcoplasmic reticulum.[344] Atropine is probably best not used unless needed during anesthesia, and then used in small intravenous doses only. It is preferable to give dantrolene orally the day before the surgical procedure, in divided doses that total 4–7 mg/kg/day.[127,180,216,239,319] The safe anesthetic agents[37,55,68] include nitrous oxide, thiopental and other barbiturates, althesin,[174,179,196,389] opiates, droperidol, and pancuronium.[68,335,336] Potent volatile agents, depolarizing relaxants, and ketamine should be avoided.

Stimulation *per se* during light anesthesia may trigger MH responses.[152] Monitoring should include temperature, electrocardiogram, and blood gas–acid–base capability, as well as the usual monitoring done for any patient undergoing anesthesia. Some patients have apparently had MH triggered in the postoperative period, and thus all should be followed for 24 hours.

Regional or conduction anesthesia avoids the use of volatile agents and relaxants,[94,426] but may yet be associated with increased temperatures in susceptible patients.[229,398] Amides such as lidocaine or mepivacaine are best avoided when large volumes are to be used, even though it is unlikely that blood concentrations will be high enough to release calcium from sarcoplasmic reticulum. Dental use of small volumes should not be risky. Esters such as chloroprocaine, procaine, piperocaine, and tetracaine do not diffuse through tissue as well as amides, but are safer for use in large volumes.

Legal Implications[278,315]

Awareness of MH is now generally widespread, as reports concerning it have been disseminated in a variety of journals. Ignorance is therefore not a valid defense against litigation. However, an episode of MH may be a fortuitous event, and one would not incur liability unless he had departed from a recognized standard of care.[390] This should include: family and personal anesthetic history, continuous monitoring of temperature during most anesthesias, avoidance of potent volatile agents and depolarizing relaxants in susceptible patients or in close relatives not evaluated for susceptibility, keeping immediately available resuscitation equipment and drugs appropriate for MH crises, and the use of diligent and due care in the treatment of any such crises. An estimated 1 per cent of cases of MH have involved litigation, but emotional responses of juries make it difficult to predict the outcomes.

Puzzles

1) Why do triggering agents sometimes fail to trigger human MH? Is this due to depression of the initiating process by other drugs? or graded susceptibility associated with complex inheritance?

2) Can triggering anesthetics be used for patients "proven" nonsusceptible by contracture tests of muscle biopsy specimens?

3) Should triggering agents ever be used for susceptible patients who have been pretreated with dantrolene?

4) A reliable screening test for MH that does not involve surgical intervention is needed.

5) The theory of uncontrolled intracellular free ionized calcium levels needs confirmation.

6) Sarcolemmal function in MH-susceptible individuals needs examination.

7) The transfer of membrane depolarization from transverse tubule to sarcoplasmic reticulum in normal muscle is not understood. It is probably involved in the pathophysiologic mechanism of MH.

Summary

In MH, skeletal muscle acutely and unexpectedly increases its oxygen consumption and lactate production, resulting in greater heat production, respiratory and metabolic acidosis, muscle rigidity, sympathetic stimulation, and increased cellular permeability. The best-accepted theory is that MH is due to an inability to control calcium concentrations within the muscle fiber, and may involve a generalized alteration in cellular or subcellular membrane permeability. Episodes are predictably initiated in susceptible people and swine by potent volatile anethetic agents or succinylcholine. In addition, in swine, MH is consistently triggered by excitement, apprehension, exercise, or environmental stress such as heat or hypoxia. Several genetic factors probably control the human and porcine inheritance of MH. Sympathetic involvement in MH, while controversial, is probably a response to stress that affects blood flow, heat loss, and myocardial function, rather than a direct sympathetic activation of susceptible muscle. Diagnosis is based upon extraordinary temperature and acid–base and muscle aberrations. Specific treatment is the action of dantrolene upon muscle calcium movements; symptomatic treatment is by reversal of acid–base and temperature changes. Evaluation of affected families is guided by measurements of circulating creatine phosphokinase and by analysis of drug-induced contractures in muscle biopsy specimens. Anesthesia for susceptible patients includes thiopental, opiates, droperidol, pancuronium, nitrous oxide, and preoperative oral doses of dantrolene.

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PLASMA CHOLINESTERASE VARIANTS AND THE ANAESTHETIST

Mary Whittaker

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REVIEW ARTICLE

Plasma cholinesterase variants and the anaesthetist

MARY WHITTAKER

Summary

Biochemical properties of plasma cholinesterase of significance to the anaesthetist are reviewed.

The role of the genetic variants of the enzyme in suxamethonium sensitivity and hyperthermia are discussed with emphasis on the pregnant patient. Altered gene frequencies of the enzyme variants in some mental disorders is commented upon.

Key words

Enzymes; plasma cholinesterase.

Genetic factors.

Neuromuscular relaxants; suxamethonium.

Hyperthermia.

Pregnancy.

Serum or plasma cholinesterase is synonymous with pseudocholinesterase, butyrylcholinesterase, non-specific cholinesterase or S-type cholinesterase. The nomenclature of the cholinesterases has been clarified by the Enzyme Commission.[1] Plasma cholinesterase (ChE) has been given the systematic name acylcholine acylhydrolase with the code number EC. 3.1.1.8. Acetylcholinesterase (AChE), found in erythrocytes and at the nuromuscular junction, becomes acetylcholine acetylhydrolase EC. 3.1.1.7. Human ChE hydrolyses many esters but its optimal substrate is butyrylcholine. Unlike AChE it hydrolyses benzoylcholine but not β-methyl-acetylcholine. The enzyme is found in plasma and in most tissues but not in human erythrocytes.

Some general properties

Biosynthesis

ChE, like albumin, is synthesised in the liver[2–5] and according to histochemical evidence only by functioning liver cells.[6] There appears to be a parallelism between the decrease in ChE activity and serum albumin levels in chronic liver disease.[7–11] Although the two proteins are synthesised in the liver they are not interdependent[12–14] and both have been used independently as an index of liver function. The nephrotic syndrome is, however, the only condition in which hypo-albuminaemia and a high ChE activity regularly coexist.[15,16] Some caution must be exercised in the case of ChE since the enzymic activity is affected by many

Mary Whittaker MSc, PhD, DSc, Chemistry Department, University of Exeter, Exeter, Devon EX4 4QD.

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Table 1. Some causes of decreased plasma cholinesterase activity

Type	Condition
Inherited deficiencies	Rare cholinesterase variants[17–19]
Physiological variance	Last trimester of pregnancy[20–22]
	Newborns and infants[23,24]
Acquired causes	Liver diseases (acute hepatitis and hepatic metastasis)[25,26]
	Myocardial infarction[25]
	Collagen diseases (progressive muscular dystrophy, congenital myotonia, dermatomyositis[27])
	Hyperpyrexia[28]
	Tuberculosis[29]
	Acute infections[30]
	Carcinomas[31–35]
	Chronic debilitating diseases[36]
	Surgical shock[11]
	Chronic anaemias[37,38]
	Uraemia[25,39–41]
	Malnutrition[42–44]
	Myxoedema[45]
Iatrogenic causes	X-ray therapy[38]
	Anti-cancer drugs[46,47]
	Monoamine oxidase inhibitors[48]
	Contraceptive pills[49]
	Ecothiopate iodide[50,51]
	Propanidid[52]
	Neostigmine[53]
	Chlorpromazine chloride[54]
	Pancuronium[55,56]
	Organophosphorus insecticides[57]
	Burned patients[58–60]
	Cyclophosphamide[61–63]
	Extracorporeal circulation[64]
	Rheumatic fever[28]
	Typhus[65]
	Tetanus[66]
	Kwashiorkor[67]
	Epilepsy[68]

other conditions shown in Tables 1[17–68] and 2.[15,45,69–79] Some of these conditions will be discussed later. The use of ChE activity as an index of liver function is not advocated.

Physiological role

Several schemes have been devised to give ChE a useful function. The most favoured are either that the enzyme plays an essential role in the transmission of slow nerve conduction processes[80] or that the enzyme is involved in lipid metabolism[81] or that it plays a regulatory role in conjunction with choline acetylase in choline homeostasis in plasma.[82] Other roles for the enzyme have been proposed by Ballantyne and co-workers[83–85] but although there is some experimental support for each, no unequivocal role has yet been assigned to ChE.

Half life

The half life of ChE has been estimated by measuring the fall of enzymic activity following the transfusion of plasma or injection of purified ChE into an anenzymic patient and the various results are shown in Table 3.[14,86–91] The short half life reported by Schuh[91] is surprising since ChE is known to be a very stable enzyme[92] and although he has suggested that the solvents used during the preparation of the ChE may have affected its half life, such

Table 2. Some causes of increased plasma cholinesterase activity

Type	Condition
Inherited	Electrophoretic variants C_{5+}[69]
	Nietlich or Cynthiana variant[70,71]
Acquired	Obesity[72]
	Hyperlipaemia[73]
	Nodular goiter[74]
	Psoriasis[75]
	Essential hypertension[76]
	Thyrotoxicosis[45]
	Nephrosis[15]
	Asthma[77]
	Anxiety states[78]
	Alcoholism[77]
	Schizophrenia[79]

an explanation seems unlikely because others[88–90] have used a similar preparation from the same laboratory. A half life in the range 8–12 days seems probable.

Stability

A study of 82 healthy adults has indicated that there is no appreciable variation in the ChE activity of a given individual when measured at irregular intervals over periods up to 5 years[93] and this has been confirmed by other workers.[94,95] No significant change in enzymic activity has been reported in random specimens of whole blood stored at 4°C for 30 days[96] but this procedure is not recommended as appreciable haemolysis can frequently occur. In separated plasma or serum, the enzyme is stable for several weeks when stored at 0–5°C.[97] Although it has been reported[98] that single freezing and thawing of plasma results in an average decrease of approximately 30% of the ChE activity, plasma or serum may be kept frozen at −20°C for several years without appreciable loss in enzymic activity provided that the samples are not repeatedly frozen and thawed. Furthermore outdated plasma from National Blood Banks can be useful sources for large scale studies of the enzyme. The ChE activity in plasma separated from autopsy blood up to 72 h after death did not differ appreciably from the activity of samples from live subjects.[99]

ChE activity in healthy individuals

Conflicting results have been reported for the influence of age and sex on the ChE activity in normal healthy adults. Whereas no age or sex effect was found in 247 adults[94] and in 120 adults,[100] others have found a conflicting correlation of enzymic activity with age—negative for 1200 Canadians[101] and positive for 1154 Australians.[102] The ethnic origin of the individuals in all the surveys was essentially Caucasian. The mean adult ChE activity using a variety of analytical procedures has been summarised.[93] Adult males appear to have a higher ChE activity than females.[93,101–104] The constancy of ChE activity in adults has already been referred to. ChE activity in the plasma of maternal blood is 16% higher than that found in the corresponding cord blood for ten pregnancies.[105] This contradicts observations by the author that the ChE activity of cord blood in 16 pregnancies is 17% higher than in maternal activity. All had the usual phenotype for ChE but similar results were found for the rare phenotypes.

Interesting changes in ChE activity occur in infancy and childhood. At birth the activity is low[106–109] compared with 50%[24] of non-pregnant adults. There is complete disagreement about ChE activity during the first 6 months of infancy. According to earlier workers there is a dramatic increase in the activity during the first 3 weeks of life to a value greater than that of the healthy adult and which persists until the

Table 3. Estimated half life of plasma cholinesterase

Half-life	Method
16 days	DFP_{32}[86]
10 days	Plasma transfusion of anenzymic patient[14]
3–4 days	Plasma transfusion of anenzymic patient[87]
12 days	Purified ChE therapy of anenzymic patient[88]
8–9 days	Purified ChE therapy of anenzymic patient[89]
8 days	Purified ChE therapy of anenzymic patient[90]
44·7 h	Purified ChE therapy of anenzymic patient[91]

third year.[110,111] The later workers[24] report that the activity remains at about 50% of the adult value until 6 months and no particular interest seems to have been paid to the next 2½ years of life. Between 3 and 6 years the mean ChE activity of 1024 children is about 30% above the adult level[111,112] but begins to decrease during the fifth year and continues to do so until the adult level is reached at puberty.[104,105,112]

Suxamethonium

Suxamethonium, the dicholine ester of succinic acid, is rapidly hydrolysed by plasma cholinesterase in two stages:[113]

Succinyldicholine + H_2O → Succinylmonocholine + Choline

↓ + H_2O

Succinic acid + Choline

Several workers have measured *in vitro* the rate of hydrolysis of the drug by ChE and their results are summarised in Table 4.[114–117] Not all the data are directly comparable since temperature and pH are not always identical. However, if the figures quoted by the last two groups,[116,117] are doubled to correct for these differences then the figures become roughly comparable and, within the spectrum of cholinesterase activity found in healthy adult populations, give fair agreement at the same concentration of suxamethonium. It may be that the pharmacological technique used by one group[114,115] is not as accurate as those used by others.[116,117]

When 100 mg suxamethonium is given to an adult with a plasma volume of 3·5 litres, 70 mg of the drug should be hydrolysed in 1 min[117] but later workers[114] have found that with such a dosage, a final plasma content of 64 mg is obtained following the distribution of the drug into extracellular fluid in the absence of hydrolysis or excretion. *In vivo*, the combination of these two processes will lead to a very rapid loss of the drug from the plasma during the first minute after injection.

It has been found *in vivo* that during constant infusion[118] about 4 mg/min of suxamethonium was required to maintain 90% twitch depression.[119] On this basis a maximum rate of hydrolysis of about 6 mg/l/min has been calculated.[118] The progressive loss of suxamethonium from blood after a single intravenous injection of the drug has been studied.[120] This was done by occluding one arm from the general circulation immediately after injection. The occlusion was relieved after 1–3 min and the neuromuscular effects of the drug on both arms were recorded and compared. The estimated rate of disappearance was 7·7 mg/l/min which includes not only the hydrolysis of the drug but diffusion out of the plasma, binding to plasma proteins and urinary excretion. The last two factors have been shown by radioactive techniques[121] to be unimportant but the relative contribution of hydrolysis and diffusion to the rate of disappearance of suxamethonium remains a matter of speculation. At the low concentrations of suxamethonium that are present after the first minute in the plasma, it is not surprising that the rates of hydrolysis of the drug *in vivo* are lower than might have been expected from the initial dosage given. Other explanations[117,122,123] of the observed slow rate of hydrolysis of the drug *in vivo* do not seem necessary.

Suxamethonium sensitivity

A few individuals are unable to hydrolyse the drug quickly and in consequence a prolonged

Table 4. *In vitro* hydrolysis of suxamethonium by ChE

Rate of hydrolysis (mg/min/l)	Concentration of suxamethonium (Mol)	Number of experiments	Temp (°C)	pH	Method
27	$2{\cdot}5\times10^{-5}$	13	37	7·4	Frog rectus abdominis preparation[114]
34	$2{\cdot}5\times10^{-5}$	6	37	7·4	Frog rectus abdominis preparation[115]
15·6	$3{\cdot}3\times10^{-4}$	1	26	7·4	Radioisotope and chromatography[116]
4	2×10^{-5}	1	30	7·0	pH stat[117]
18	10^{-4}	1	30	7·0	pH stat[117]
31	10^{-3}	1	30	7·0	pH stat[117]
40	10^{-2}	1	30	7·0	pH stat[117]

apnoea occurs after administration of suxamethonium. The study of the ChE from such individuals has established a new field of scientific investigation—pharmacogenetics.

Genetic variants

Although Stovner[124] had indicated that the ChE of suxamethonium-sensitive individuals appeared to be different from that of other patients, it was Kalow and his associates who first clearly demonstrated a qualitative difference between the ChE of suxamethonium-sensitive individuals and that of other patients.[125] The two types of enzyme hydrolyse the same substrate at different rates and show distinct inhibition profiles with varying concentrations of a suitable inhibitor such as dibucaine hydrochloride (Cinchocaine or Nupercaine).[126] This is shown in Fig. 1. Kalow & Genest introduced the term dibucaine number (DN) defined as the percentage inhibition by a fixed concentration of dibucaine of the rate of hydrolysis of benzoylcholine under standard conditions of temperature, pH, buffer and fixed concentration of substrate.[126] Earlier workers had shown that the sensitivity to suxamethonium appeared to be inherited.[127–129] This was proven when the sensitive individuals were found to have DN ~20 whereas other patients had DNs ~80 and some of the relatives of sensitive individuals had DNs ~60. These findings were the basis of the hypothesis[17] that the biosynthesis of ChE is controlled by two allelic genes, E_1^u and E_1^a. Individuals with DN ~80 are homozygotes with two usual genes $E_1^uE_1^u$ whereas the sensitive individuals (DN ~20) are homozygotes with two atypical genes $E_1^aE_1^a$ and the relatives (DN ~60) are heterozygotes with one of each gene $E_1^uE_1^a$. The hypothesis was in general established by family studies.[17,130] The advantage of DN determination as compared with the mere measurement of enzymic activity for the families of 11 sensitive individuals is shown in Fig. 2. A clear trimodal distribution indicating three distinct genotypes is present for the DN measurements which is totally lacking in the Gaussian distribution of activity.

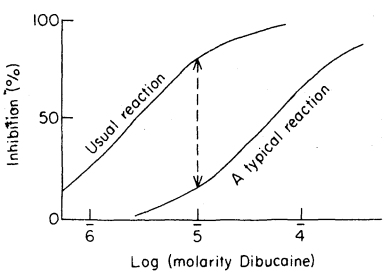

Fig. 1. Inhibition of cholinesterase activity by varying concentration of dibucaine hydrochloride in individuals with genotype $E_1^uE_1^u$ and $E_1^aE_1^a$. The dashed line indicates the region in which the results fall when a standard concentration of 10^{-5M} dibucaine is routinely used to determine dibucaine numbers in different sera. (After Kalow W. & Genest K. *Canadian Journal of Biochemistry and Physiology* 1957; **35**: 339.)

Silent gene

In the case of a homozygote $E_1^aE_1^a$ for simple

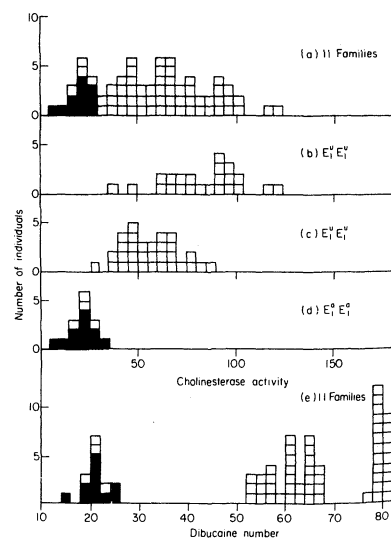

Fig. 2 Distribution of cholinesterase (ChE) in individuals in 11 families selected by sensitivity to suxamethonium. □ Relative; ■ Propositus. (a) enzymic activity (μmol acetyl choline/min/ml plasma of all individuals, (b), (c) and (d) enzymic activity of individuals $E_1^uE_1^u$, $E_1^uE_1^a$ and $E_1^aE_1^a$ respectively, (e) dibucaine numbers of individuals in eleven families). (After Harris H. *et al. Acta Genetica et Statistica Medica (Basel)* 1960; **10**: 1.)

Mendelian recessive inheritance both parents must have at least one E_1^a gene and moreover all the children of such an individual must have an E^a gene. But in one of Kalow's original families[117] and in one of the eleven families mentioned above,[130] it was found that this was not so as is illustrated by the family in Fig. 3. It

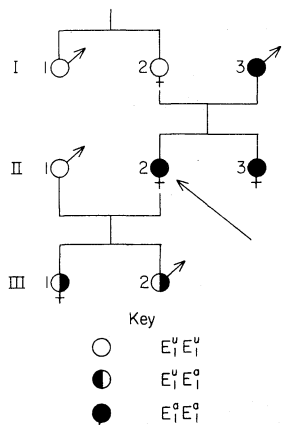

Fig. 3. Pedigree of family with unusual inheritance as determined by dibucaine numbers.

was fortunate that in both families a mother was the dissident member so that illegitimacy could be discounted. The presence of a silent gene (E_1^s) was proposed. Such a gene is not really silent since it does not imply non-production of a protein molecule but that it would biosynthesise ChE lacking the structure required to hydrolyse the choline–ester bond and have no enzymic activity and which could therefore not contribute to any of the parameters, such as dibucaine numbers, which were being measured. If this concept is applied to the individuals of the family shown in Fig. 3 then I_2 should be a heterozygote $E_1^u E_1^s$ and if this is so her DN remains ~80 whilst her children II_2 and II_3 become heterozygotes $E_1^a E_1^s$ with DNs ~20. This hypothesis of the silent gene was vindicated when two healthy individuals with absolutely no ChE activity were reported.[131,132] These were homozygotes $E_1^s E_1^s$. Many such anenzymic individuals have now been identified and moreover in some ethnic groups, e.g. Alaskan Eskimoes[133] and some Caucasian South Africans[134] there is a large incidence of the silent gene. Biochemists are now able to recognise at least two different types of silent gene.[135–138] It has been found that one type of cholinesterase deficiency is truly anenzymic with no ChE activity[139] while a second group has 2–8% of the normal average level of ChE activity[133,140,141] and the available evidence implies at least two or three different genetic defects.[138,142] It seems probable that other silent gene variants may occur and these may be differentiated by inhibition, electrophoretic or immunological studies. This is not surprising since the genetically determined lack of enzymic activity can be explained by quite minor changes in amino acid sequence in the ChE molecule which though quite minor in themselves can nevertheless produce a large effect at the catalytic active site of the molecule. Improved and simpler techniques for the purification of the enzyme will make possible finger-printing the amino acid sequence of not only these apparently different silent genotypes but also other variants. It appears that the silent genes are allelic with the usual and atypical genes.[142,143] Although the differences in the identity of allelic silent genes are important to the geneticist and biochemist, it is proposed in this article to discuss the silent gene as a single entity since all individuals with very low or zero activity show marked sensitivity to suxamethonium.

Fluoride resistant gene

A fourth gene controlling the biosynthesis of ChE is the fluoride resistant gene E_1^f, which was recognised by using sodium fluoride as the differential inhibitor.[18,144] It must be emphasised that the strong dependence on temperature of the fluoride inhibition adds greatly to the difficulties in the use of this inhibitor. The fluoride number (FN) is defined in a similar manner to the DN.[18]

Other variants

A family has been reported[145] in which the ChE activities and DNs cannot be explained by the known allelic genes at the E_1 locus. Other alleles functioning at the E_1 locus have been postulated from their unusual inhibition characteristics with sodium chloride[146] or with n-butanol[147] but these observations have yet to be

confirmed by family studies. Two rare genes E_1^j and E_1^k have been described. The E_1^j gene has been shown segregating in one family[148] and the findings have been supported by immunological evidence.[149] E_1^k has been found in two families as a consequence of family screening of individuals found to be sensitive to suxamethonium.[150] A family has been reported in which several members have very high ChE activity[70] and the responsible gene has been termed E Cynthiana[71] but it is not known at which locus this rare gene is functioning. An unusual cholinesterase pattern in five siblings of a suxamethonium sensitive individual with the genotype $E_1^sE_1^s$ has been reported[151] and it has been tentatively suggested that a mutation from a normal or silent gene to an abnormal gene may have occurred.

As new techniques become available the number of ChE variants will increase. The enzyme is a large molecule consisting of four polypeptide chains each of molecular weight ~80 000 daltons. Assuming that the average molecular weight of the twenty different component amino acids is ~200 then each polypeptide chain would consist of 400 amino acids and even if only one amino acid at one position is changed, many variants are possible. Such a development has occurred with haemoglobin where more than 200 variants are recognised; haemoglobin is very easily purified and the subsequent amino acid sequencing is becoming almost routine with pure proteins. An electrophoretic variant which functions at a different locus E_2[69] is not important to the anaesthetist and will not be considered in this review.

Tissue variants

The cholinesterase present in different tissues (liver, kidney, brain, ileum and skin) is controlled by the same E_1 locus responsible for the biosynthesis of the plasma enzyme.[152,153] Additional confirmatory evidence was provided by an individual anenzymic for both plasma and liver enzyme.[154] In other words the phenotype of the cholinesterase (ChE) in human tissues is the same as that found in plasma and presumably the amino acid sequence of ChE found in various tissues is identical.

The E_1 locus for ChE has provisionally been assigned to chromosome 1 and has been sandwiched between transferrin (Tf) and phosphogluconate dehydrogenase (PGD).[155]

Characteristics of genotypes

At the present time, from multiple family studies, four allelic genes are recognised: the normal gene E_1^u the atypical or dibucaine resistant gene E_1^a, the silent gene E_1^s and the fluoride resistant gene E_1^f. These give rise to ten genotypes, all of which have been recognised, whose characteristics under our conditions are given in Table 5. The phenotype of the ChE in a sample of serum or plasma can usually be

Table 5. Distribution, suxamethonium sensitivity and biochemical characteristics of the plasma cholinesterase variants in a British population

Genotype	Relative mean enzymic activity	Dibucaine number		Fluoride number		Frequency	Suxamethonium sensitivity
		Mean	Range	Mean	Range		
$E_1^uE_1^u$	100	80	77–83	61	56–56	96%	? 1 in 2500 moderately sensitive
$E_1^uE_1^s$	50	80	77–83	61	56–68	1 in 190	? 1 in 1000 moderately sensitive
$E_1^uE_1^f$	86	74	70–83	52	46–54	1 in 200	? 1 in 100 moderately sensitive
$E_1^uE_1^a$	77	62	48–69	50	44–54	1 in 25	? 1 in 500 moderately sensitive
$E_1^aE_1^f$	59	53	45–59	33	28–39	1 in 20 000	All moderately sensitive
$E_1^fE_1^f$	74	67	64–69	36	34–43	1 in 154,000	All moderately sensitive
$E_1^fE_1^s$	37	67	64–69	36	34–43	1 in 150,000	All moderately sensitive
$E_1^aE_1^a$	43	21	8–28	19	10–28	1 in 2000	All very sensitive
$E_1^aE_1^s$	22	21	8–28	19	10–28	1 in 29 000	All very sensitive
$E_1^sE_1^s$	Enzymic activity nil or too low to measure					1 in 100,000	All very sensitive

obtained by comparing the DN and FN found for it with the figures given in this table. These figures will not differentiate between a homozygote and the corresponding heterozygote containing a silent gene, e.g. $E_1^uE_1^u$ and $E_1^uE_1^s$. To convert these phenotypes into genotypes it is necessary to determine not only enzymic activity but also examine families to ascertain whether the silent gene is segregating. The DN and FN of any individual will change only after transfusion with blood from an individual with a different genotype and this should be borne in mind when taking blood samples from surgical patients who have had prolonged apnoea after suxamethonium. The data given in Table 5 lack sharp boundaries and sometimes it is difficult to assign a definite genotype. For such individuals family studies may be helpful but a repeat sample should be sought. The presence of the silent gene is readily diagnosed for the homozygote $E_1^sE_1^s$ where there is little or no enzymic activity. The detection of a heterozygote with a silent gene is much more difficult. The task is easier in family studies where it is known that the silent gene is segregating. Each parent and all children of a homozygote $E_1^sE_1^s$ must have one E_1^s gene but for other individuals resort must be made to a comparison of enzymic activities. Individuals with the common genotype $E_1^uE_1^u$ have a large range of enzymic activities and it has been found from figures quoted in the literature that heterozygotes $E_1^uE_1^s$ average almost 70% of the average $E_1^uE_1^u$ activity.[143,156] It has been found that in proven pedigrees—all heterozygotes with a silent gene—have an average activity which is appreciably greater than the expected 50% mean activity for the corresponding homozygote but there is a large range of activities for all genotypes. Attention must be focussed on the many causes of decreased ChE activity shown in Table 1.[11,17–68] Such contributory factors must be eliminated before assuming that a low enzymic activity in any individual is caused by the segregation of the silent gene. Table 2[15,45,69–79] shows some conditions associated with increased ChE and in these cases a low activity would be indicative of the probable segregation of a silent gene.

Genetically important relatives (siblings, parents and children) of individuals known to be sensitive to suxamethonium should be investigated for ChE variants. Thirteen per cent of such relatives are themselves sensitive to the drug.[157] The screening of these relatives could overburden the clinical laboratory of many hospitals and to overcome this obstacle several reference centres in Britain and one in Denmark[158] provide such a service. These centres will assist with any difficulties in phenotyping ChE but without the opportunity to perform family studies the investigation of a suxamethonium sensitive individual could be a waste of time especially when some anaesthetists fail to record the results in the patient's hospital notes. Warning cards should be issued to all appropriate patients and relatives on the basis of the biochemical findings. The card should be clear and authoritative giving the name and address of a person who can substantiate the findings. The card issued by the Exeter laboratory is shown in Fig. 4. It has

CHOLINESTERASE RESEARCH UNIT

Chemistry Department, The University, Exeter EX4 4QD
Tel. (0392) 77811.

This is to certify that

A. N. OTHER

specimen No. CRU
is sensitive to SUXAMETHONIUM. Further details can be obtained from the above address.

Date Dr. Mary Whittaker

Fig. 4. Suxamethonium warning card.

apparently taken on a new role as recently one physician wrote to enquire about membership of *The Cholinesterase Club*.

Table 6 records the distribution of the various genotypes found in surveys of individuals sensitive to suxamethonium. In all surveys the proportion of the individuals typing as $E_1^uE_1^u$ has remained fairly constant over a period of ~20 years. In a Danish survey of 225 'suxamethonium-sensitive' patients,[159] 14 of the 77 individuals typing as $E_1^uE_1^u$, had low ChE activity which could be explained by a pathological condition or by chemotherapy of an anticholinesterase (*cf.* Table 1[11,17–68]). Of the remaining 63 patients with the usual genotype it would appear that the apnoea of 34 patients could be attributed to the anaesthetist. Even if the contributions of the above factors are eliminated, about half of the 34% of apnoeic patients with the usual genotype found in the Danish survey had normal ChE activity and their sensitivity remains unexplained.

Table 6. Percentage distribution of genotypes in eight surveys of suxamethonium-sensitive individuals

Genotype	A	B	C	D	E	Total (A–E)	F	G	H	Total (A–H)
$E_1^uE_1^u$	37·4	32·1	40·0	39·5	31·3	36·1	32·2	51	28	34·6
$E_1^uE_1^f$	1·0	7·7	0·6	5·7	5·9	4·2	5·7	0	0	4·1
$E_1^uE_1^a$	10·6	12·8	7·7	1·9	4·6	7·5	19·3	21	2·6	11·2
$E_1^aE_1^f$	1·9	9·1	3·2	7·6	12·5	6·9	8·6	9	0	6·8
$E_1^aE_1^a$	48·0	38·5	45·2	45·3	43·5	44·1	31·4	17	66·8	41·3
$E_1^sE_1^s$	1·0	0·0	1·3	0·0	2·7	1·0	1·7	2	2·6	1·4
$E_1^fE_1^f$	0·0	0·0	1·9	0·0	0·0	0·4	1·1	0	0	0·5
Number investigated	104	78	155	53	220	610	348	47	116	1121

A = Kalow W. *Federation Proceedings* 1965; **24:** 1259: B = Thompson JC, Whittaker M. *Acta genetica et statistica medica* 1966; **16:** 209: C = Lehmann H, Liddell J. *British Journal of Anaesthesia* 1969; **41:** 235: D = Whittaker M, Vickers MD. *British Journal of Anaesthesia* 1970; **42:** 1016: E = Berry M, Whittaker M. *British Journal of Anaesthesia* 1975; **47:** 1195: F = Whittaker M. *Unpublished results*, 1979: G = Bauld HW *et al. British Journal of Anaesthesia* 1974; **46:** 273: H = Goedde HW *et al. Praktische Anästhesie* 1976; **11**: 329.

During the past 2–3 years the distribution of some genotypes in suxamethonium sensitive individuals appears to have changed and Group F in Table 6 shows a remarkable increase in the frequency of the heterozygote $E_1^uE_1^a$. The reason for this was perplexing—all the more so since the correct policy for heterozygotes is ambiguous. When heterozygotes have been referred because of apnoea it is difficult to deny that some incident worthy of attention has occurred and if no abnormality of technique is reported, it must be assumed that other anaesthetists would obtain a similar result and a warning card is therefore desirable. Heterozygotes found by family screening were given warning cards only when the ChE activity was less than 50% of the average found for the usual homozygote $E_1^uE_1^u$. This is contrary to the practice of the Danish Cholinesterase Research Unit. The Danes issue sensitivity cards for all rare ChE genes whether they occur as homozygotes or heterozygotes.[159] Of the total of 513 cards issued in 3 years from 1973 to 1976, 342 have been issued to individuals having genotype $E_1^uE_1^a$.[158] Analysis of the heterozygotes $E_1^uE_1^a$ in a recent survey shows a large excess of female over male patients and it was subsequently found that a large proportion of these women at the time of anaesthesia were having elective Caesarean section (Fig. 5). It appears that, because of the known decrease in ChE activity, during pregnancy heterozygotes become sensitive to suxamethonium so that it has become advisable to issue warning cards to premenopausal women having the genotype $E_1^uE_1^a$. Sensitivity cards are not issued to male heterozygotes unless they have either a low enzymic activity or a convincing history of suxamethonium sensitivity. This policy is contrary to that of the Danes. However, there is no conflict about this difference in policy and it is agreed that, until more evidence is available, except for premenopausal women, it is impossible to be dogmatic about the issue of sensitivity cards to heterozygotes $E_1^uE_1^a$.

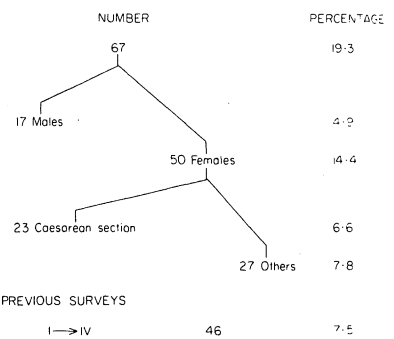

Fig. 5. Analysis of genotype $E_1^uE_1^a$ in Survey F of Table 6.

A further complication of the pregnant

heterozygotes $E_1^u E_1^a$ has emerged. The author has recently investigated the ChE of a heterozygote who was given suxamethonium prior to Caesarean section. The baby had marked respiratory failure and was shown to be homozygous for the atypical gene. Such a finding suggests that suxamethonium may have crossed the placental barrier and indeed placental transmission of the drug into the foetus of monkeys has been reported.[160] Since monkey placenta, like human, is haemochorial, these results could have clinical implications. If a sufficient quantity of suxamethonium reaches the intervillous space blood, then pharmacologically active amounts may cross the placenta. Hypoventilation in a neonate with low ChE activity has been reported[161] following suxamethonium to the mother whose ChE activity was also low—unfortunately neither mother nor infant was genotyped. It would seem worthwhile to establish firstly the proportion of 'flat' babies with an unusual ChE genotype, secondly whether the transfer of suxamethonium is dependent on the ChE genotype of the mother and thirdly whether the known decrease in ChE activity during pregnancy depends on the genotype. It has been suggested[162] that the placenta forms a relative rather than an absolute barrier to the passage of suxamethonium. Under normal conditions clinical doses of suxamethonium do not cross the placenta because of the low fat solubility and polar nature of the drug.[163] However, in atypical homozygous mothers the normal clinical dose, which is hydrolysed very slowly, becomes a relative overdose and this may cross the placenta and result in a prolonged apnoea of the newborn.

The duration of neuromuscular block following equal doses (based on surface area) of suxamethonium are similar in newborn infants and adults.[164] However, in three of the nine infants studied the effect was considerably longer than in the others, but attempts to explain the prolonged block in these infants have omitted phenotyping ChE.[165] No demonstrable amounts of suxamethonium have been detected in umbilical vein blood after maternal doses up to 300 mg but a trace of the drug has been found in cord blood with higher maternal doses.[166] None of the infants had respiratory distress. It is, however, regrettable that these studies did not include patients with any rare genotype.[167]

Cholinesterase in human placenta and amniotic fluid

Placental cholinesterase hydrolyses both acetylcholine and acetyl-β-methylcholine but not benzoylcholine indicating that AChE is present in the placenta.[168] Later workers[169] showed that butyrylcholine was also hydrolysed, albeit at a slower rate than acetylcholine, and it is now accepted that the small amount of cholinesterase found in placenta is predominantly AChE with a little ChE. The cholinesterase activity of amniotic fluid is very weak, ~40% of the activity is AChE and the remainder is ChE.[173]

ChE activity during pregnancy

There is abundant evidence that a decrease in activity of ChE occurs during pregnancy and during the immediate post-partum period.[20,21,174–177] The diminution in ChE activity is associated with pregnancy and has nothing to do with phenomena such as antibody formation since consecutive pregnancies have no repercussions on ChE levels.[174] Decreased activity appears after the tenth week of pregnancy[21] and continues for several days post-partum before returning to normal non-pregnant levels between the tenth day[175] and the sixth week post-partum.[176] The mean ChE activities found in pregnant patients are shown in Table 7[20,21,35,174,179,181] and these measurements from different laboratories show remarkable agreement. Compared with the non-pregnant the mean enzymic activity is reduced by 24% during pregnancy, 25% one-day postpartum and 33% 3-days post-partum. Such decreases would not cause an increased suxamethonium sensitivity. But as is shown by the histograms in Fig. 6, there is a wide distribution of ChE activities in any group of individuals. In the author's opinion the individuals having 50% or less of the average normal activity will probably be sensitive to suxamethonium. All individuals in this survey[174] had the usual genotype and the figures in Table 5 show that healthy individuals with rare phenotypes have lower activity on average than those with the usual genotype. A higher proportion of heterozygotes would be expected to show sensitivity to suxamethonium during pregnancy than when non-pregnant. It is not suggested, however, that suxamethonium

Table 7. Decrease in ChE activity during pregnancy

Study & reference	Controls: Non-pregnant ChE	Controls: Non-pregnant No	Pregnancy: 1st and 2nd trimester ChE	Pregnancy: 1st and 2nd trimester No	Pregnancy: 3rd trimester ChE	Pregnancy: 3rd trimester No	Pregnancy: Term ChE	Pregnancy: Term No	Post-partum: 1st day ChE	Post-partum: 1st day No	Post-partum: 2nd day ChE	Post-partum: 2nd day No	Post-partum: 3rd day ChE	Post-partum: 3rd day No	Post-partum: 4th day ChE	Post-partum: 4th day No
Levine & Hoyt[35]	100	80	76	48	74·5	38	74	52								
Hazel & Monier[174]	100	47			79	43	79	43			72	23	67	68		
Blitt *et al.*[179]	100	30					80	20			63	20			65	20
Robertson[21]	100	40			82	144										
Shnider[20]	100	10			72·3	10	84·4	13	75·3	10			67·7	10		
Redderson[181]	100	14			70·2											
Average ChE from various groups (disregarding numbers in each survey)					75·6		79·3		75·3		67·5		67·3		65	

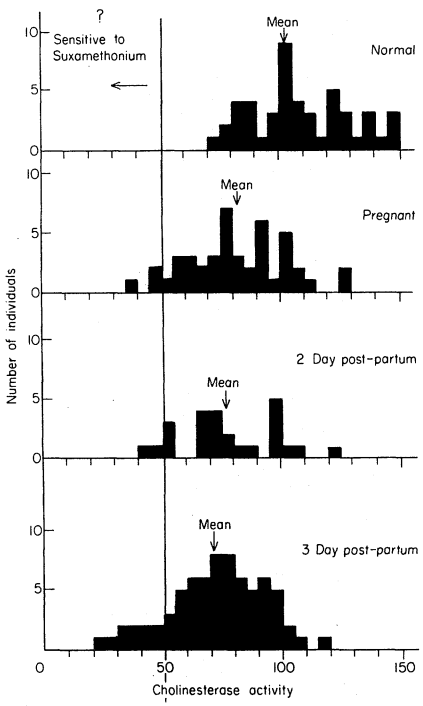

Fig. 6. Histograms of plasma cholinesterase activity in non-pregnant, pregnant, 2 days post-partum and 3 days post-partum from data of Hazel B, Monier D. *Canadian Anaesthetists' Society Journal* 1971; **18**: 272.

be abandoned in obstetric anaesthesia because of low ChE activity but merely point out that its use during labour and the immediate post-partum period may occasionally result in a prolonged apnoea. The reduced enzymic activity associated with pregnancy is not modified by toxaemia[21] but treatment of the latter with magnesium sulphate appears to reduce the amount of muscle relaxant required during anaesthesia.[177] No correlation of ChE activity and duration of action of suxamethonium in pregnant patients has been observed.[178,179]

Oral contraceptives

Oral contraceptives containing oestrogen not only decrease ChE activity[49] but modify the ChE isoenzymes[80] but whereas the decrease averages 26% in pregnant women it averages 20% in the contraceptive group.[49] The changes are readily reversible by withdrawal of the contraceptive[181] and it has been suggested that the effects are caused by a steroid-induced depression of hepatic ChE synthesis.[181]

Potentiation of suxamethonium by anticholinesterase drugs

The prolongation of the action of suxamethonium by many drugs such as procaine,[182] hexafluorenium[183] and tetrahydroaminacridine[184] has been attributed to their anticholinesterase activity and their greater affinity for ChE than for suxamethonium.[185] As, however, it is very

doubtful that this can account for the potentiation of suxamethonium by propanidid,[52] it has been proposed that the latter reacts directly with the neuro-muscular junction.[186]

It is generally accepted that anticholinesterases, e.g. neostigmine and pyridostigmine, employed in the chemotherapy of myasthenia gravis act by inhibition of the AChE at the myoneural junction but additionally inhibition of circulating ChE is readily observed.[53] A lipid-soluble fluorescent substance has been found in the urine from two myasthenic patients on pyridostigmine therapy.[187] This substance was not found in urine from individuals of genotype $E_1^uE_1^u$ unless given pyridostigmine but it was isolated from an atypical homozygote $E_1^aE_1^a$ in the absence of pyridostigmine therapy. The urine of a myasthenic patient, genotype $E_1^aE_1^u$ has not been investigated.[188] The appearance of this unusual urinary compound in homozygote $E_1^aE_1^a$ could be indicative of the physiological role of ChE.

Pancuronium bromide

Pancuronium bromide is a powerful, reversible inhibitor of ChE[55,56] in individuals with the usual phenotype $E_1^uE_1^u$. The activity of ChE is still 40% inhibited, 40 min after injection of 0·7 mg/kg of the drug and after the injection 0·2 mg/kg by ~10%, a decrease which would not produce a clinically detectable prolongation of suxamethonium action.[55] Doses of pancuronium for muscular relaxation during anaesthesia (0·1–0·8 mg/kg) produce concentrations of the drug ~10^{-7} to 10^{-6} *M* in the extracellular fluid[56] which under clinical conditions is insufficient to potentiate suxamethonium action by inhibition of ChE.[56,189,190]

Pancuronium bromide crosses the placenta after rapid intravenous infusion of the drug (0·07–0·10 mg/kg).[191] The drug has been detected in varying amounts in 11/20 neonatal urines. None of the infants showed clinical evidence of myoneural block but it is impossible to deduce from these measurements the amount of drug present in neonatal plasma and the effect on an unusual ChE genotype has not been examined.

The use of ChE in suxamethonium apnoea

ChE is a stable enzyme and even stored blood retains ~40% of its ChE activity[192] and reconstituted plasma has about the same activity. However the use of plasma carries the attendant risk of hepatitis. It is well established that raising ChE level artificially shortens the apnoea produced by subsequent doses of suxamethonium[192,193] but clinical trials to terminate a prolonged apnoea by intravenous infusion of concentrated cholinesterase were unsuccessful.[193] The enzyme preparation Cholase (a concentrated human globulin containing cholinesterase) at one time available for clinical use was effective only when given before or simultaneously with suxamethonium.[194] A highly purified concentrated preparation of the enzyme is now available.[195,196] A prolonged apnoea has been successfully terminated within 8 min after the intravenous injection of this preparation.[197] Later reports confirm the successful application of the same preparation,[90,91,198] but intravenous infusion of an enriched ChE preparation did not produce any discernible effect on phase II block of suxamethonium.[199] The apparently conflicting results could be due to the different amounts of ChE given by the various authors. The consensus of opinion is that a depolarising relaxant such as suxamethonium dissociates rapidly from the end plate receptors and diffuses back from the end plate receptors into the plasma where the infused commercial enzyme hydrolyses the drug.[90] Such action upsets the equilibrium with the result that more suxamethonium dissociates from the end-plate receptors and as the process is repeated the drug action is reduced and the apnoea is ultimately terminated. In contrast to non-depolarising relaxants rapid diffusion of depolarising relaxants, from neuromuscular end plate receptors has been demonstrated by the isolated-arm technique.[200] The purified enzyme preparation is free of Australian antigen, polyoma and Newcastle disease virus.[141]

Identification of ChE variants

When Kalow and his associates established the existence of three genetically distinct ChE variants by differential inhibition of the enzyme with dibucaine, it seemed probable that more genetic variants would be identified. Other inhibitors[201] have been shown to differentiate the variants and comparable differentiation has been obtained. In some cases it is a combination

of two differential inhibitors that give an unambiguous phenotype which can be confirmed by family studies, the fluoride resistant variants are classical examples of this.[18,144] The heterozygote $E_1^uE_1^f$ is notoriously difficult to genotype and many attempts have been made to simplify the identification. A wide variety of inhibitors have been used NaCl[202–205] urea,[206,207] NaBr[208] succinyl-dicholine[209–211] R02-0683 the dimethyl carbamate of (2-hydroxy-5-phenylbenzyl) trimethylammonium bromide[201,212,213] formaldehyde,[214] thyroxine[215] and alkyl alcohols.[147,213,216] These inhibitors have been used with either choline esters or non-choline esters as substrates.[209,213,217–223] Most groups have used M/15 phosphate buffer pH 7·4 used by Kalow, but Garry[224] has studied the effect of salts and different buffers on ChE activity and developed methods for identification of the variants using butyrylthiocholine as substrate in tris and M/20 phosphate buffers.[225,226]

Such numerous and diverse systems, not all of which are quoted above, can be daunting. It must be remembered, however, that when NaF was used in combination with dibucaine new phenotypes, previously unsuspected, were recognised. Furthermore the use of NaCl[146,149,227] and *n*-butyl alcohol[147] as inhibitors and succinyldicholine as substrate[219,220] have indicated the possibility of new phenotypes so that it may be that the introduction of new parameters in the field could unmask additional genotypes and therefore are to be encouraged.

It is essential, however, that each research group should establish its own standard range for each genotype and to have regard to the high temperature dependence of fluoride inhibition.[227] Dibucaine and NaF as differential inhibitors with benzoylcholine as substrate in phosphate buffer (M/15, pH 7·4) at 26°C are recommended[228] for phenotyping. Whenever phenotyping is difficult succinylcholine,[210] NaBr,[208] NaCl,[203] urea[206,207] and *n*-butylalcohol[147] are useful ancillary aids in the benzoyl choline system. With butyrythiocholine as substrate the two buffer system of Garry[224] or the use of *n*-butanol and R02, 0683[213] are most useful. The use of a programmable calculator in processing and interpreting the data obtained with dibucaine, fluoride, chloride and succinylcholine as differential inhibitors has been reported.[229] It is recognised[230] that the 'Acholest' test strip method for the detection of patients with low ChE activity is inadequate.

Distribution of gene frequencies in different diseases

It is very desirable that when an anaesthetist sends a blood sample to a laboratory for phenotyping he should state the diagnosis and specialised treatment of the patient. Such information enables the biochemist to spot any unusual correlations between a diseased state and altered gene frequency and allows the exploration of such a relationship to proceed quickly and efficiently with minimal frustration. A striking example of this arose when it was noticed that over 50% of the suxamethonium-sensitive individuals from one source carried the fluoride-resistant gene instead of the customary 11% found in other anaesthetists' apnoeic patients and the <1% that would be expected in a random British population.[231] The Cleveland area of the North East of England is notorious to geneticists as the location of many unusual plasma proteins and it was assumed that this or the presence of a large immigrant population had distorted the usual distribution of genotypes. Accordingly over 700 residents in the area were screened before it was realised that the anaesthetists' specimens were taken from mentally ill patients having electroconvulsive therapy (ECT). Patients having anaesthesia before ECT have a higher incidence of suxamethonium sensitivity (23/1676) than is found in general surgical patients (1/2000) and it appears from the results in Table 8 that the distribution of genotypes in these two groups differ.[160] The cause for these changes is unknown; but it is becoming apparent that drug therapy can potentiate the action of muscle relaxants. All the ECT patients were depressed and as phenelzine, a monoamine oxidase inhibitor used in the treatment of depressive illness, does potentiate suxamethonium, it may be that such an explanation could account for the increased sensitivity of mentally ill patients to suxamethonium; it does not, however, explain the changed distribution of phenotypes. This hypothesis has some support from the case reports of two patients who were given ECT during phenelzine treatment and developed 'alarming' reactions a few minutes after an apparently normal recovery from their

Table 8. Gene frequencies of ChE variants found in suxamethonium-sensitive individuals

	Number	Gene frequencies			
		E_1^u	E_1^a	E_1^s	E_1^f
General surgery*	958	0·4233	0·4916	0·0162	0·0689
Mentally ill patients†	23	0·4130	0·3261	—	0·2609

* Combined data of: Kalow W. *Federation Proceedings* 1965; **24:** 1259: Thompson JC, Whittaker M. *Acta genetica et statistica medica* 1966; **16:** 209: Lehmann H, Liddell J. *British Journal of Anaesthesia* 1969; **41:** 235: Whittaker M, Vickers MD. *British Journal of Anaesthesia* 1970; **42:** 1016: Berry M, Whittaker M. *British Journal of Anaesthesia* 1975; **47:** 1195: Whittaker M. *Unpublished results.*
† Berry M, Whittaker M. *British Journal of Anaesthesia* 1975; **47:** 1195.

'seizure'.[234] Moreover, low ChE activity has been reported in four patients on phenelzine therapy, and in one of the patients an apnoea of 1 h now following the cessation of ECT. The ChE levels of all the patients returned to normal levels after withdrawal of phenelzine but no genotyping was done.[48] The duration of apnoea after different amounts of suxamethonium (25–1000 mg) given to patients having courses of ECT has been reported.[235]

The results of a survey of the ChE variants found in hospitalised mentally ill patients in North-East England are shown in Table 9.[236] All patients were psychologically disturbed but none had Down's syndrome nor any known enzyme deficiency disease or inherited disorder except for the patients with Huntington's disease. The patients showed a greater tendency to have a rare phenotype than individuals from a random population. The distribution of the E_1^a gene of Table 10 was not significantly abnormal in different diagnostic groups confirming earlier work[101] but there is strong statistical evidence that patients in Group IV

Table 9. Distribution of the plasma cholinesterase variants in mentally ill patients and normal controls*

	Number	Phenotypes				Gene frequency		
		$E_1^uE_1^u$	$E_1^uE_1^a$	$E_1^uE_1^f$	$E_1^aE_1^f$	E_1^u	E_1^a	E_1^f
Mentally ill patients	1374	1253	53	64	4	0·9545	0·0207	0·0247
Random controls (same area)	736	700	20	16	0	0·9755	0·0136	0·0109
British students	780	752	23	3	2	0·9808	0·0160	0·0032

* After Whittaker M, Berry M. *British Journal of Psychiatry* 1977; **130**: 397.

Table 10. Distribution of rare cholinesterase phenotypes in mentally ill patients*

Diagnosis group	Total number	Gene frequency	
		E_1^a	E_1^f
I	53	0·0283	0·0094
II	5	0	0·1000
III	226	0·0177	0·0155
IV	1090	0·0211	0·0271
Total	1374	0·0207	0·0247

* After Whittaker M, Berry M. *British Journal of Psychiatry* 1977; **130:** 397.

(Psychosis) have a higher frequency of the E_1^f gene than those in other groups. In this group it was noticeable that patients with Huntington's disease had a high incidence of the rare E_1^f gene which has been substantiated by further studies.[237]

It has been shown that ChE activity in Down's syndrome (58 patients) is significantly lower than that found in either mentally retarded individuals (80 patients) or in 40 healthy controls. It is unfortunate that inhibition studies were confined to four Downs patients and four controls[238]—significance is irrelevant with such a small sample. The ChE variants in Down's syndrome have been investigated and the results of various laboratories are shown in Table 11. All cases were trisomic for the 21st chromosome. A suxamethonium sensitive individual with Down's syndrome and characteristic 21-trisomy pattern has been reported with genotype $E_1^aE_1^a$.[242] The inhibition characteristics quoted for this family are unusual and it is unfortunate that a reference laboratory was not consulted. It is most improbable that the conflicting results shown in Table 11[102,236,239–241] can be explained by ethnic differences since individuals in the three surveys were essentially Caucasian.

Lithium therapy

The potentiation of suxamethonium neuromuscular block by lithium carbonate has been reported[243] in a patient with manic depressive psychosis. An emergency Caesarean section was performed because of fetal bradycardia. The patient was given 310 mg suxamethonium and a 4 h apnoea resulted. Postoperatively her DN was 73 but FN was not measured and unwisely DN >70 was assumed to be normal genotype $E_1^uE_1^u$. A significantly higher frequency of the E_1^f gene has been observed in patients undergoing lithium therapy.[244] *In vitro* studies using lithium and sodium nitrate (the carbonate is insoluble in the buffer system) indicated that inhibition of the ChE variants was identical for the two salts, both differentiated the phenotypes equally well and neither interfered with the phenotyping. Such a result agrees with observations of the effect of lithium or sodium ions on isolated enzyme and tissue preparations when similar inhibition properties were found.[245] Although lithium, which does inhibit ChE, has been reported to be an anaesthetic risk for patients undergoing ECT,[246] Schou considered it unlikely to cause complications in ECT treatment or when used in combination with other drugs.[247]

Leprosy

Conflicting results of the distribution of ChE variants in leprosy patients when compared with healthy individuals domiciled in the same locality are shown in Table 12.[248–251] Dapsone,[252] the standard drug used in leprosy chemotherapy does not influence the phenotyping.[250] Thomas and co-workers[248,249] have measured only DNs and have made the unsatisfactory assumption that DN >70 correspond to $E_1^uE_1^u$. DNs in the range 40–70 are $E_1^uE_1^a$ and DNs < 20 are $E_1^aE_1^a$. Such assumptions will not, however, account for the differences obtained for the control group and the leper group. It does seem significant that the ethnic group lacking the E_1^a gene, e.g. Zimbabwe-Rhodesia Africans,[251] are still very susceptible to leprosy.

Table 11. Distribution of ChE genotypes in Down's syndrome, mentally retarded patients and healthy individuals

		Gene frequencies		
	Number	E_1^u	E_1^a	E_1^f
Healthy controls[102,236]	1224	0·9714	0·0233	0·0053
	1516	0·9782	0·0148	0·0069
Down's syndrome[239,240,241]	80	0·9437	0·0062	0·0500
	307	0·9544	0·0293	0·0163
	130	0·9960	0·0038	0
Mentally retarded[240,241]	206	0·9733	0·0146	0·0121
	53	0·9810	0·0189	0

Table 12. Comparative results of ChE gene frequencies found in leprosy patients

Subjects and References	Total	Gene frequency		
		E_1^u	E_1^a	E_1^f
Karigini, South India				
Normal Controls[248]	343	0·9737	0·0263	NT
Leprosy Patients[248]	390	0·6564	0·3436	NT
Normal Controls[249]	701	0·9861		NT
Leprosy Patients[249]	400	0·7566	0·2434	NT
Harari, Ethiopia[250]				
Normal Controls	150	0·9700	0·0300	NT
Leprosy Patients	206	0·9805	0·0195	NT
Salisbury Rhodesia[251]				
Normal Controls	1,034	0·9440	0	0·0560
Leprosy Patients	580	0·9699	0	0·0301

Malignant hyperthermia (MH)

It appears that in susceptible individuals[253] the clinical features of this syndrome result from an excess of calcium ions in the sarcoplasm. Enzyme studies have established that all individuals who are susceptible to MH have an underlying disease of the muscle. When the abnormal muscle cell membrane in susceptible individuals is exposed to an anaesthetic agent such as halothane or suxamethonium there is a rapid and excessive release of Ca^{++} into the muscle cell which, by a variety of biochemical processes, lead to the clinical features of metabolic hyperpyrexia, muscular rigidity and acidosis. The precise nature and site of the muscle membrane abnormality is still to be defined.

It is reported that three of the four individuals who have survived MH had the rare ChE genotype $E_1^uE_1^f$ and the E_1^f gene was segregating in two additional families who had lost one relative on account of MH.[254] Eleven British families with a history of this syndrome have been studied and in eight the E_1^f gene is segregating, in one, the propositus is $E_1^uE_1^a$ and in two families no rare ChE has been found.[255] A Norwegian who survived the syndrome is $E_1^aE_1^a$ [256] but five Danish patients had the usual phenotype $E_1^uE_1^u$.[158]

Relatives of patients who have experienced the syndrome have had muscle biopsies and were designated sensitive or insensitive from the results of the pharmacological investigations. DN and FN were also measured[257] and the result of this study is shown in Fig. 7. These data indicate that of 14 susceptible relatives, ten are $E_1^uE_1^f$ and four are usual phenotype $E_1^uE_1^u$ whereas of the 11 non-susceptible individuals, six have the usual phenotype $E_1^uE_1^u$ and five are heterozygotes (three $E_1^uE_1^f$, one $E_1^uE_1^a$, and one $E_1^aE_1^f$). It can therefore be concluded on the available evidence that there appears to be a high probability that one, at least, of the rare ChE genes occurs in families susceptible to MH. But this finding does present difficulties. By no means all individuals with rare ChE genes

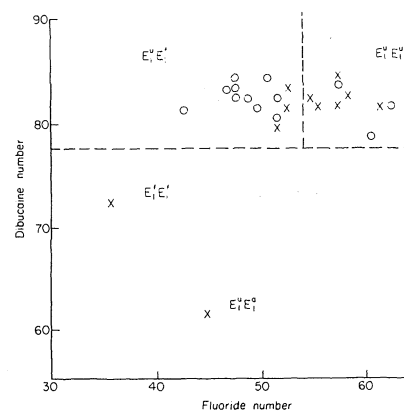

Fig. 7. Correlation between dibucaine numbers (DN) and fluoride numbers (FN) in patients susceptible (×) and non-susceptible (○) to malignant hyperthermia (after Ellis FR. *et al. British Journal of Anaesthesia* 1978; **50**: 56).

will be susceptible to MH. Moreover the inheritance of ChE variants is a recessive trait whereas the genetic information shows that MH is dominant albeit with incomplete penetrance and variable expressivity.[258] However, Denborough[259] has recently defined two classes of myopathy predisposing to MH. The commonest is believed to be subclinical and unless given an 'inappropriate' general anaesthetic, individuals with this myopathy will have little or no disability. This myopathy is inherited as a dominant Mendelian characteristic. The second predisposing myopathy occurs in young boys of normal intelligence who have a number of striking physical abnormalities, e.g. short stature, lumbar lordosis, undescended testes, thoracic kyphosis, pectus carinatum, webbing of the neck and winging of the scapulae. Their facial appearance is unusual and it seems likely that this second myopathy in young boys is inherited as a recessive characteristic. Ellis and co-workers[260] conclude from their *in vitro* muscle biopsy tests that MH is inherited as a polygenic condition.

The recognition of two classes of myopathy by Denborough does not resolve the difficulties that arise from the apparent occurrence of a rare ChE gene in individuals susceptive to MH. At this stage it seems probable that it will be a combination of two or more genetic markers, probably linked, which may supply the biochemical expression for this syndrome. The author and her colleagues have probed unsuccessfully a possible linkage between ChE genotypes and creatine phosphokinase activity and isoenzyme distribution but found no correlation. It is only with the co-operation of practising anaesthetists that biochemists can attempt to unmask the problems set not only by this syndrome but by other anaesthetic hazards, and as a bonus the physiological role of ChE may be discovered.

-6-

THE EFFECT OF TWO GENES ON ANESTHETIC RESPONSE IN THE NEMATODE CAENORHABDITIS ELEGANS

Morgan PG, Sedensky MM, Meneely PM, Cascorbi HF

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LABORATORY REPORT

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The Effect of Two Genes on Anesthetic Response in the Nematode Caenorhabditis elegans

Phil G. Morgan, M.D., Margaret M. Sedensky, M.D.,* Philip M. Meneely, Ph.D.,† Helmut F. Cascorbi, M.D., Ph.D.‡*

The authors studied the wild type strain, N2, and three mutant strains of the nematode, *Caenorhabditis elegans*, in order to measure genetically produced changes in responses to nine volatile anesthetics. They determined the anesthetic ED_{50}s of N2 for thiomethoxyflurane, methoxyflurane, chloroform, halothane, enflurane, isoflurane, fluroxene, flurothyl, and diethylether. The log-log relationship of the oil-gas partition coefficients (O/G) and the ED_{50}s of these agents for N2 yields a straight line with a slope of −.997 with a R^2 of .98 over a range of O/G (at 37° C) from 48 to 7230. When the O/Gs are corrected to 22° C, the slope is −.964 with an R^2 of .98. This relationship is similar to that found in other animals. Two mutant strains, *unc-79* and *unc-80*, show altered responses to these anesthetics. These strains are two to three times more sensitive than N2 to anesthetics with an O/G greater than that of halothane (220 at 37° C), yet they differ little from N2 in response to anesthetics with lower O/Gs. *unc-79* and *unc-80* are about 30% more sensitive than N2 to diethylether. The double mutant *unc-79;unc-80* is more sensitive to halothane, isoflurane, and fluroxene than is either mutant alone. The authors believe these data indicate an alteration at the site of action of volatile anesthetics in *unc-79* and *unc-80*. They also postulate that the interaction of *unc-79* and *unc-80* indicate these genes code for enzymes in a common pathway, and that *unc-79* precedes *unc-80* in this pathway. (Key words: Anesthetics, volatile: chloroform; diethylether; enflurane; flurothyl; fluroxene; halothane; isoflurane; methoxyflurane; thiomethoxyflurane. Nematodes: Caenorhabditis elegans. Theories of anesthesia.)

IN THE 80 YR since Meyer[1] and Overton[2] originally noted the correlation between potency of volatile anesthetics and their solubility in oil, no single explanation has gained universal acceptance for the mechanism of action of volatile anesthetics. Knowledge of the molecular composition of the site of action of volatile anesthetics should help to clarify this mechanism. Regardless of its molecular composition, the site of action of volatile anesthetics must be specified by an organism's genome. Studies in fruit flies (10^5 neurons)[3] and mice (10^8 neurons)[4] have documented genetic control of anesthetic sensitivity. However, the neuronal complexity of these animals limits them as models for understanding the molecular mechanism of anesthetic action.

We have proposed the nematode, *C. elegans*, as an animal in which to study how volatile anesthetics work.[5,6] It has been said that ". . . more is known about the genetics and development of this one millimeter roundworm than about any other multicellular creature." [7] The hermaphrodite always consists of 959 cells of which exactly 302 are neurons.[8–11] Every synapse of these 302 nerves is known,[8–11] along with all cell lineages.[12] The nematode has four neurotransmitters, acetylcholine, GABA, serotonin, and dopamine. Genetic analysis of *C. elegans* is also very extensive, and mutants are available with a variety of abnormalities in neuromuscular function. Most importantly, *C. elegans* responds to volatile anesthetics by first undergoing a period of excitation, and then by becoming immobile and unresponsive to a tap to the head.[5] Upon removal from anesthetics, they resume movement within 2–5 min and appear normal with respect to movement, feeding, and fertility. The potency of volatile agents in *C. elegans* correlates well with their oil/gas partition coefficient (O/G); the log-log plot of ED_{50}s *versus* O/G yields a slope approximating −1, from an $48 < O/G < 980$, for *C. elegans*,[5] and all other animals tested.[13]

It is possible to clone *C. elegans* genes, *i.e.*, produce *in vitro* DNA segments and use them to manufacture large amounts of their RNA and protein products. To select genes to clone, we initiated a search for mutants with abnormal responses to volatile anesthetics. The first mutation, *unc-79* (*unc* for uncoordinated in phenotype) was two to three times more sensitive to the highly lipid soluble anesthetics (halothane, chloroform, and methoxyflurane) than its normal counterpart N2, slightly resistant to two agents (enflurane and flurothyl), and unchanged in its sensitivity to fluroxene and isoflurane.[5] We postulated that it represented an animal with an altered site of action of volatile anesthetics.[5] A second mutation, *unc-80*, was also found to be hypersensi-

* Assistant Professor of Anesthesiology, Case Western Reserve University.

† Assistant Professor of Genetics, Fred Hutchinson Cancer Research Center.

‡ Professor and Chairman, Department of Anesthesiology, Case Western Reserve University.

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Address reprint requests to Dr. Morgan: Department of Anesthesiology, University Hospitals, 2074 Abington Road, Cleveland, Ohio 44106.

tive to halothane.[6] A nematode constructed by us to bear both mutations *unc-79* and *unc-80* was more sensitive to halothane than either parent.[6]

Before undertaking a molecular analysis of *unc-79* and *unc-80*, we chose to expose the mutants and N2 to nine volatile anesthetics with O/Gs ranging from 48 to 7230. This extends our data for *unc-79* and N2 by a factor of 7, and determines ED_{50}s for *unc-80* and the double mutant, *unc-79;unc-80*. We expected a deviation from the usual relationship between the log of the O/Gs and the log of the ED_{50}s, if these mutations do represent a change in the site of action of volatile anesthetics. Thus, any mutants which show such changes will be candidates for DNA cloning and subsequent analysis of the gene products controlling anesthetic response. In addition, if *unc-79* and *unc-80* use different pathways to affect the site of anesthetic action, we expected the double mutant, *unc-79;unc-80*, to be more sensitive to volatile agents than either *unc-79* or *unc-80*. We report here the results for all four strains of worms in nine anesthetic agents.

Materials and Methods

Nematodes

C. elegans var. Bristol (wild type strain = N2) and the mutant *unc-80* (el272) were obtained from the Caenorhabditis Genetics Center. We isolated *unc-79* (ecl) after exposing N2 to the chemical mutagen EMS.[5] We constructed *unc-79;unc-80* by mating the *unc-79* males (chromosome III) to *unc-80* hermaphrodites (chromosome V), bearing an easily scored homozygous marker on chromosome III.[6] Nematode cultures were kept as previously described.[5]

Anesthetics

Flurothyl (FLR) was supplied by Anaquest, Inc. Thiomethoxyflurane (TMOF) was given to us by Dr. E. I. Eger II. Chloroform (CH), methoxyflurane (MOF), halothane (H), enflurane (E), isoflurane (ISO), fluroxene (FLX), and diethylether (DE) were commercial products.

Dose-response Curves

Anesthetic response was assayed as described previously in detail.[5] Briefly, synchronized cultures of worms on agar plates were placed in a glass air-tight chamber. A liquid volume of anesthetic, calculated to give an appropriate gas concentration based on the chamber's volume, was injected with a glass syringe into the sealed chamber *via* a stopcock. The worms were observed through the chamber's lid with a dissecting microscope, and judged to be anesthetized when they assumed a straight posture and became immobile as previously described.[5] (Normal worms move in a constant sinuous motion across the plate.) They were scored after 2 h, except those exposed to thiomethoxyflurane, which required 5 h for equilibration. Anesthetic concentrations were measured with a gas chromatograph. Dose-response curves for each anesthetic were based on a minimum of 20 different concentrations, with 50 animals per concentration. ED_{50}s were defined as that concentration at which 50% of the nematodes were immobile. Observers were unaware of the strain being scored, but were aware of the anesthetic being used.

Oil-gas Partition Coefficients

We obtained O/Gs at 37° C from values published by Eger *et al.*[13–15] We used O/Gs corrected to 22° C by the method of Allott *et al.*[16] to determine a regression line for the log-log plot of O/Gs and ED_{50}s of the nine anesthetics.

Statistical Methods

Regression analysis, ED_{50}s, slope constants, and SEs were calculated using the methods described by Waud.[17] Regression curves for the log ED_{50}s and log O/G were constructed using the least-squares method. For each anesthetic, all the ED_{50}s and the slope constants of all four strains were compared using an analysis of variance to see if they satisfied the null hypothesis (*e.g.*, all means are equal).[18] If they did not satisfy the null hypothesis ($P < .05$), we compared the individual mean values of each strain. Comparison of ED_{50}s and of slope constants for the different strains was performed by Tukey's method for multiple comparisons.[18] Significance was defined as $P < .01$. The increased stringency was used to avoid Type I errors. Variances for the differences between ED_{50}s used in figure 5 were calculated by adding the variances of each ED_{50} involved.

Results

Table 1 lists the ED_{50}s ± SEs for all four strains of worms. As previously noted with N2 and *unc-79*, the ED_{50}s for *unc-80* and *unc-79;unc-80* tended to increase as the O/G of the anesthetic decreased. Unlike N2, the mutant strains went through an "excitation phase" only in the presence of diethylether and its fluorinated derivative, flurothyl. Flurothyl in low concentrations also restored the uncoordinated movement of *unc-80* to normal, as previously reported for *unc-79*.[5] We found a significant change in the slope constants between the mutant strains and N2 for halothane, methoxyflurane, and thiomethoxyflurane (table 2).

For all nine anesthetics, we noted four patterns of

TABLE 1. ED_{50}s ± SEs (v/v% at 1 atm, 22°C) for Four Strains of *C. elegans* in Nine Anesthetics

	N2	*unc-79*	*unc-80*	*unc-79; unc-80*
TMOF*	0.11 ± 0.01	0.09 ± 0.03†	0.07 ± 0.03†	0.04 ± 0.02†
MOF*	0.58 ± 0.02	0.28 ± 0.05†	0.46 ± 0.03†‡	0.25 ± 0.10†§
CH*	1.47 ± 0.02	0.50 ± 0.03†	0.80 ± 0.02†‡	0.54 ± 0.03†§
H*	3.18 ± 0.04	0.98 ± 0.02†	1.20 ± 0.02†‡	0.72 ± 0.02†‡§
E*	5.89 ± 0.08	6.24 ± 0.07†	6.06 ± 0.07†	5.82 ± 0.07‡§
ISO*	7.18 ± 0.07	6.67 ± 0.08†	6.14 ± 0.07†‡	5.84 ± 0.07†‡§
DE*	7.53 ± 0.07	5.70 ± 0.06†	5.84 ± 0.06†	5.60 ± 0.06†
FLX*	10.8 ± 0.07	10.1 ± 0.07†	10.4 ± 0.07†	9.9 ± 0.07†§
FLR*	14.3 ± 0.10	15.9 ± 0.11†	14.9 ± 0.10†‡	14.5 ± 0.08‡

TMOF = thiomethoxyflurane; MOF = methoxyflurane; CH = chloroform; H = halothane; E = enflurane; ISO = isoflurane; DE = diethylether; FLX = fluroxene; FLR = flurothyl.
* Four strains fail null hypothesis (analyzed by ANOVA) at $P < .05$ level.
† Different from N2, $P < .01$.
‡ Different from *unc-79*, $P < .01$.
§ Different from *unc-80*, $P < .01$.

TABLE 2. Slope Constants + SE for Four Strains of *C. elegans* in Nine Anesthetics

	N2	*unc-79*	*unc-80*	*unc-79; unc-80*
TMOF*	14.1 ± 5.6	3.0 ± 1.3†	4.2 ± 1.7†	3.3 ± 1.7†
MOF*	8.4 ± 3.0	2.1 ± 1.0†	1.8 ± 0.9†	0.5 ± 0.4†
CH	6.8 ± 3.8	3.2 ± 1.5	3.3 ± 1.4	4.1 ± 1.9
H*	11.6 ± 4.6	3.7 ± 1.4†	4.0 ± 1.6†	2.8 ± 1.1†
E	17.8 ± 9.6	22.4 ± 10.4	32.6 ± 14.0	30.6 ± 13.3
ISO	25.2 ± 11.5	10.8 ± 5.4	11.4 ± 5.7	16.1 ± 8.5
DE	14.3 ± 6.1	13.2 ± 6.0	9.3 ± 4.2	14.8 ± 6.1
FLX	10.7 ± 4.1	10.9 ± 4.0	12.2 ± 4.4	11.6 ± 4.2
FLR	11.7 ± 5.3	14.0 ± 6.9	8.9 ± 4.1	14.9 ± 5.6

See table 1 for abbreviations.
* Four strains fail null hypothesis (ANOVA) at $P < .05$ level.
† Different from N2, $P < .01$.

dose-response curves, which are represented in figures 1–4. Figure 1, the response of N2, *unc-79*, and *unc-80* to thiomethoxyflurane, is representative of their behavior in the four anesthetics with the highest O/G (*i.e.*, also methoxyflurane, chloroform, and halothane). The

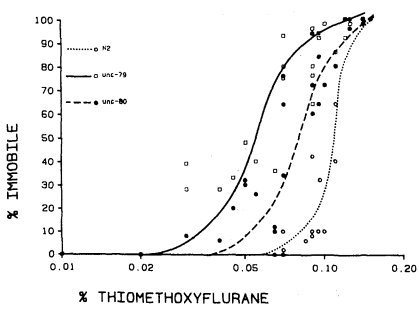

FIG. 1. Percent *C. elegans* immobile *versus* percent (v/v%) thiomethoxyflurane in air. Each point represents 50 organisms observed for 10 s each. All experiments were performed at 20–22° C and 1 atmosphere for this figure, as well as figures 2–4.

ED_{50}s of *unc-79* and *unc-80* are much less than those of N2, with the ED_{50}s of *unc-79* less than those of *unc-80*. The double mutant, *unc-79; unc-80*, has ED_{50}s not statistically different from *unc-79*. An exception exists with halothane, to which *unc-79; unc-80* is more sensitive than *unc-79*, as noted before.[6]

In contrast to the above, *unc-79* and *unc-80* are resistant to flurothyl compared to N2 ($P < .01$ for *unc-79*, $P < .01$ for *unc-80*) (fig. 2). This pattern is characteristic for both flurothyl and enflurane, two agents with convulsant activity in mammals. The ED_{50} of the double mutant is indistinguishable from those of N2 in these two agents.

In fluroxene, the mutant strains have ED_{50}s about 5–10% less than that of N2 ($P < .01$) (fig. 3). This was also true for isoflurane. The double mutant, *unc-79; unc-80*, has an ED_{50} indistinguishable from *unc-79* in fluroxene, but its ED_{50} is less than that of *unc-79* in isoflurane ($P < .01$).

With diethylether, both mutants have an ED_{50} approximately 30% less than that of N2 ($P < .01$); the double mutant resembles *unc-79* (fig. 4).

Figure 5 summarizes the data as percent change of ED_{50} for all mutant strains compared to N2. The differences in ED_{50}s for all anesthetics with an O/G

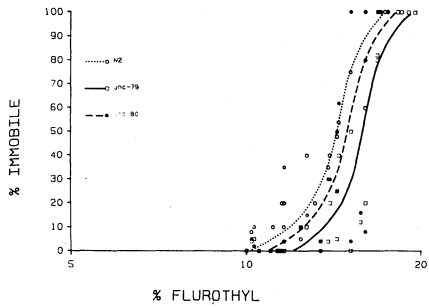

FIG. 2. Percent *C. elegans* immobile *versus* percent (v/v%) flurothyl in air.

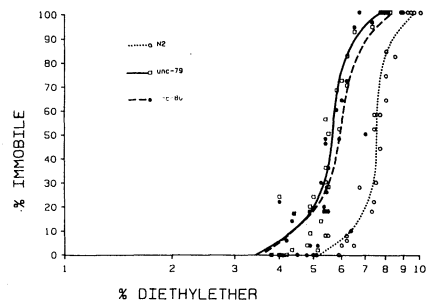

FIG. 4. Percent *C. elegans* immobile *versus* percent (v/v%) diethylether in air.

greater than or equal to that of halothane are seen to the left of the histogram; also shown are the similarities of ED_{50}s for the double mutant and *unc-79* in these agents.

The log-log relationship of O/G *versus* ED_{50}s for N2 is a straight line, with a slope of −.997 and an R^2 of .98 (fig. 6). The values for these O/G were determined at 37° C [O/G (37°)]. However, our experiments were performed at 22° C. We used a standard temperature correction described by Allott *et al.*[16] for O/Gs (37°) from 0.1 to 980 to determine O/Gs at 22° [O/G (22°)]. Since this method has only been shown to be applicable to an O/G (37° C) as high as 980 (MOF), we did not use TMOF [O/G (37° C) = 7230] to calculate the slope of the regression line. When we applied this temperature correction, we obtained a slope of −.964 with an R^2 of .98. Plotting the same data for *unc-79* or *unc-80* does not yield a straight line at either temperature. Figure 6 presents these relationships at 22° C. Each mutant generates a set of points that deviates from those of N2 for O/Gs greater than or equal to that of halothane (H, CH, MOF, TMOF).

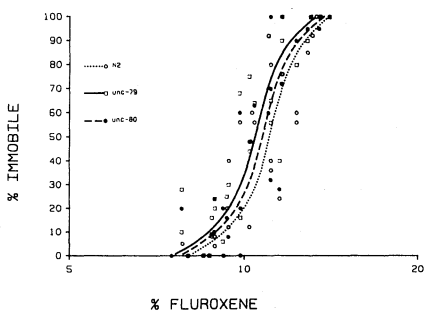

FIG. 3. Percent *C. elegans* immobile *versus* percent (v/v%) fluroxene in air.

Discussion

Our experiments show genetic control of anesthetic response in *C. elegans*, an animal unfamiliar to most anesthesiologists. Introduction of this unfamiliar model warrants discussion of limitations of our methods in scoring animals for immobility. The accuracy of scoring a plate was approximately ±5% between different scorers, which is about the same as the reproducibility of counting for any individual scorer. The animals' characteristic phenotypes were usually obvious to anyone scoring plates, making true "blinding" as to strain

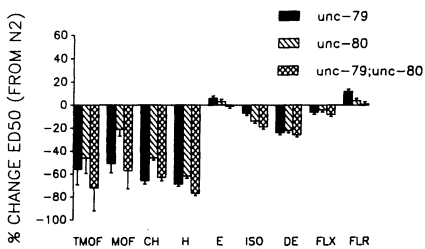

FIG. 5. Percent change in ED_{50}s for three mutant strains compared to the wild type strain (N2). Calculated as ED_{50} (strain) − ED_{50} (N_2)/ ED_{50} (N2).

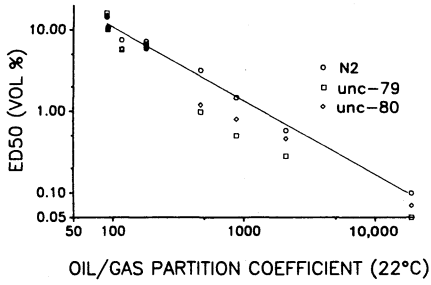

FIG. 6. Log dose required to immobilize 50% of nematodes (ED_{50}) *versus* log oil/gas partition coefficient (O/G) for nine anesthetics. O/G values determined at 37° C[15–15] are corrected to 22° C, as described by Allott *et al.*[16]

of worm intrinsically impossible. Although anesthetic agent and approximate concentration were known to the scorer, the exact concentration of gas was not known until after all worms were counted. However, we think that true double blinding of these dose-response curves is probably unnecessary. To score worms for immobility, one records an objective behavioral endpoint that is a quantal response; there is no gradation of behavior that requires evaluation by an observer. A major advantage of dose-response curves in *C. elegans* are the large numbers (relative to mammals) of animals in each curve. We have scored at least 1500–2000 animals for each dose-response curve; this, in turn, leads to small standard errors of each ED_{50}.

We do not know why 5 h of exposure were necessary for maximum effect of thiomethoxyflurane. *C. elegans* is always enveloped in a thin film of water as it moves across an agar plate; we thought that thiomethoxyflurane may be less water soluble than the other anesthetics tested. However, the water/gas partition coefficient of thiomethoxyflurane is no lower than anesthetics that required only 2 h for maximum effect.[15]

To use *C. elegans* as a model, it was necessary to establish that the non-mutated animal, N2, responds to anesthetics like other animals. We found that the log/log plot of ED_{50}s *versus* O/Gs for N2 gives a straight line with a slope very close to −1 for nine volatile anesthetics. Like all other animals tested to date, the response of N2 adheres closely to the Meyer-Overton correlation; the responses of the two mutant strains, *unc-79* and *unc-80*, do not. These two strains represent the first animals with a documented deviation from this correlation; this may have profound implications. As stated by Koblin and Eger, "The amazing closeness of this correlation (the Meyer-Overton rule) implies a *unitary molecular site of action* (the italics are ours) and suggests that anesthesia results when a specific number of anesthetic molecules occupy a crucial hydrophobic region within the CNS." [19] We agree with their conclusion and postulate that our mutants represent a genetic manipulation of the otherwise "unitary molecular site of action." Whatever the precise nature of the change in these two mutants, their deviation from the Meyer-Overton rule is consistent with a change in the "crucial hydrophobic region" or the "specific number of anesthetic molecules" necessary to achieve the anesthetic state.

The significant differences in slope constants for the mutant strains (table 2) may indicate a change in the type of molecular interaction at the site of anesthetic action for thiomethoxyflurane, methoxyflurane, and halothane. If the change in ED_{50}s for these agents were merely due to a decreased dissociation coefficient at the site of action, the slope constants should remain unaffected. Thus, something other than mere increased affinity for these anesthetics seems to be responsible for the change in ED_{50}s.

Nothing is known of the neuroanatomy or molecular defects of *unc-79* or *unc-80*. However, based on the behavior of the double mutant, *unc-79;unc-80*, we can attempt to explain the role of these two genes. *unc-79* and *unc-80* are recessive mutations with respect to halothane sensitivity.[6] This implies that both genes code for enzymatic products, as opposed to structural proteins required in stoichiometric amounts. If *unc-79* and *unc-80* affect the same enzymatic pathway, then the ED_{50}s of *unc-79;unc-80* should be the same as the ED_{50}s of either *unc-79* or *unc-80*. If the two genes affect separate pathways, the ED_{50}s of the double mutant should be less than those of either single mutant. Thus, we can speculate that *unc-79* and *unc-80* may act *via* a common enzymatic pathway in causing increased sensitivity to the more lipid-soluble anesthetics. In addition, the ED_{50}s of the double mutant are closest to those of *unc-79* in all of the more lipid soluble anesthetics, leading us to conclude that the product of the normal *unc-79* gene (*unc-79*$^+$) precedes that of *unc-80* in this pathway (fig. 7). Figure 7 shows compound A converted into B by the product of the *unc-79*$^+$ gene, and B into C by the product of the normal *unc-80* gene (*unc-80*$^+$). Accumulation of C leads to normal anesthetic sensitivity.

This model is particularly interesting in light of recent data presented by Evers *et al.*[20] These investigators showed that, by altering fatty acid composition of rat brains, they were able to change sensitivity to certain anesthetics. The fatty acid compositions were altered by controlling the diet of the animals. Genetically produced enzymatic changes may affect the same or similar systems.

We have previously described a mutation in another gene, *unc-9*, which causes *unc-79* and *unc-80* to respond

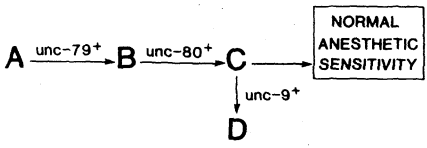

FIG. 7. A postulated metabolic pathway leading to normal anesthetic sensitivity in *C. elegans*. Compound A is converted into B by the product of the normal gene *unc-79*$^+$. B is converted, in turn, to C by the product of the normal gene *unc-80*$^+$. Adequate levels of C cause normal anesthetic sensitivity. The product of *unc-9*$^+$ normally converts C into D and, thus, levels of C may rise if the *unc-9*$^+$ gene is inactivated.

like N2 to halothane.[6] In figure 7, the *unc-9*$^+$ product degrades C to D; a mutated *unc-9* allows C to accumulate, leading to normal response in halothane by the mutant. If our model is correct, *unc-9* will be a suppressor in other volatile anesthetics.

In addition to suppressor studies, we are now screening for mutants with increased sensitivity to flurothyl and enflurane, and mutants resistant to halothane. To date, we have screened 90 of the 110 known uncoordinated mutants in *C. elegans* for alterations in anesthetic sensitivity, and have found none other than the two reported here. However, we cannot say that *unc-79* and *unc-80* are the only genes affecting anesthetic response in *C. elegans*. We are also beginning to clone both the *unc-79* and *unc-80* genes, in order to identify their gene products.

In summary, we have identified specific genes that alter anesthetic sensitivity in *C. elegans*, and proposed a model for their interaction. These genetic studies may lead to understanding the molecular determinants of anesthetic response in *C. elegans*.

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PHARMACOGENOMICS: TRANSLATING FUNCTIONAL GENOMICS INTO RATIONAL THERAPEUTICS

William E. Evans and Mary V. Relling

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GENOME
REVIEW

Pharmacogenomics: Translating Functional Genomics into Rational Therapeutics

William E. Evans* and Mary V. Relling

Genetic polymorphisms in drug-metabolizing enzymes, transporters, receptors, and other drug targets have been linked to interindividual differences in the efficacy and toxicity of many medications. Pharmacogenomic studies are rapidly elucidating the inherited nature of these differences in drug disposition and effects, thereby enhancing drug discovery and providing a stronger scientific basis for optimizing drug therapy on the basis of each patient's genetic constitution.

There is great heterogeneity in the way individuals respond to medications, in terms of both host toxicity and treatment efficacy. Potential causes for such variability in drug effects include the pathogenesis and severity of the disease being treated; drug interactions; and the individual's age, nutritional status, renal and liver function, and concomitant illnesses. Despite the potential importance of these clinical variables in determining drug effects, it is now recognized that inherited differences in the metabolism and disposition of drugs, and genetic polymorphisms in the targets of drug therapy (such as receptors), can have an even greater influence on the efficacy and toxicity of medications. Clinical observations of such inherited differences in drug effects were first documented in the 1950s, exemplified by the relation between prolonged muscle relaxation after succinylcholine and an inherited deficiency of plasma cholinesterase (*1*), hemolysis after antimalarial therapy and the inherited level of erythrocyte glucose 6-phosphate dehydrogenase activity (*2*), and peripheral neuropathy of isoniazid and inherited differences in acetylation of this medication (*3*). Such observations gave rise to the field of "pharmacogenetics," which focuses largely on genetic polymorphisms in drug-metabolizing enzymes and how this translates into inherited differences in drug effects [reviewed in (*4*)].

The molecular genetic basis for these inherited traits began to be elucidated in the late 1980s, with the initial cloning and characterization of a polymorphic human gene encoding the drug-metabolizing enzyme debrisoquin hydroxylase (*CYP2D6*) (*5*). Genes are considered functionally "polymorphic" when allelic variants exist stably in the population, one or more of which alters the activity of the encoded protein in relation to the wild-type sequence. In many cases, the genetic polymorphism is associated with reduced activity of the encoded protein, but there are also examples where the allelic variant encodes proteins with enhanced activity. Since the cloning and characterization of *CYP2D6*, human genes involved in many such pharmacogenetic traits have been isolated, their molecular mechanisms have been elucidated, and their clinical importance has been more clearly defined. Inherited differences in drug-metabolizing capacity are generally monogenic traits, and their influence on the pharmacokinetics and pharmacologic effects of medications is determined by their importance for the activation or inactivation of drug substrates. The effects can be profound toxicity for medications that have a narrow therapeutic index and are inactivated by a polymorphic enzyme (for example, mercaptopurine, azathioprine, thioguanine, and fluorouracil) (*6*) or reduced efficacy of medications that require activation by an enzyme exhibiting genetic polymorphism (such as codeine) (*7*).

However, the overall pharmacologic effects of medications are typically not monogenic traits; rather, they are determined by the interplay of several genes encoding proteins involved in multiple pathways of drug metabolism, disposition, and effects. The potential polygenic nature of drug response is illustrated in Fig. 1, which depicts the hypothetical effects of two polymorphic genes: one that determines the extent of drug inactivation and

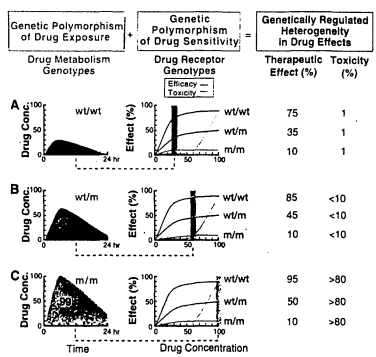

Fig. 1. Polygenic determinants of drug effects. The potential consequences of administering the same dose of a medication to individuals with different drug-metabolism genotypes and different drug-receptor genotypes is illustrated. Active drug concentrations in systemic circulation are determined by the individual's drug-metabolism genotype (green lettering), with (**A**) homozygous wild type (wt/wt) patients converting 70% of a dose to the inactive metabolite, leaving 30% to exert an effect on the target receptor. (**B**) For the patient with heterozygous (wt/m) drug-metabolism genotype, 35% is inactivated, whereas (**C**) the patient with homozygous mutant (m/m) drug metabolism inactivates only 1% of the dose by the polymorphic pathway, yielding the three drug concentration-time curves. Pharmacological effects are further influenced by different genotypes of the drug receptor (blue lettering), which have different sensitivity to the medication, as depicted by the curves of drug concentration versus effects (middle). Patients with a wt/wt receptor genotype exhibit a greater effect at any given drug concentration in comparison to those with a wt/m receptor genotype, whereas those with m/m receptor genotypes are relatively refractory to drug effects at any plasma drug concentration. These two genetic polymorphisms (in drug metabolism and drug receptors) yield nine different theoretical patterns of drug effects (right). The therapeutic ratio (efficacy: toxicity) ranges from a favorable 75 in the patient with wt/wt genotypes for drug metabolism and drug receptors to <0.13 in the patient with m/m genotypes for drug metabolism and drug receptors.

Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, TN 38105, USA, and Colleges of Pharmacy and Medicine, University of Tennessee, Memphis, TN 38105, USA.

*To whom correspondence should be addressed. E-mail: william.evans@stjude.org

another that determines the sensitivity of the drug receptor. The polymorphic drug-metabolizing enzyme, which exhibits codominant inheritance (that is, three phenotypes), determines the plasma concentrations to which each individual is exposed, whereas the polymorphic receptor determines the nature of response at any given drug concentration. This example assumes that drug toxicity (Fig. 1, red lines) is determined by nonspecific effects or through receptors that do not exhibit functionally important genetic polymorphisms, although clearly toxicity can also be determined by genetic polymorphisms in drug receptors. Thus, the individual with homozygous wild-type drug-metabolizing enzymes and drug receptors (Fig. 1A) would have a high probability of therapeutic efficacy and a low probability of toxicity, in contrast to an individual with homozygous mutant genotypes for the drug-metabolizing enzyme and the drug receptor, in which the likelihood of efficacy is low and that of toxicity is high (Fig. 1C).

Such polygenic traits are more difficult to elucidate in clinical studies, especially when a medication's metabolic fate and mechanisms of action are poorly defined. However, biomedical research is rapidly defining the molecular mechanisms of pharmacologic effects, genetic determinants of disease pathogenesis, and functionally important polymorphisms in genes that govern drug metabolism and disposition. Moreover, the Human Genome Project, coupled with functional genomics and high-throughput screening methods, is providing powerful new tools for elucidating polygenic components of human health and disease. This has spawned the field of "pharmacogenomics", which aims to capitalize on these insights to discover new therapeutic targets and interventions and to elucidate the constellation of genes that determine the efficacy and toxicity of specific medications. In this context, pharmacogenomics refers to the entire spectrum of genes that determine drug behavior and sensitivity, whereas pharmacogenetics is often used to define the more narrow spectrum of inherited differences in drug metabolism and disposition, although this distinction is arbitrary and the two terms are now commonly used interchangeably. Ultimately, knowledge of the genetic basis for drug disposition and response should make it possible to select many medications and their dosages on the basis of each patient's inherited ability to metabolize, eliminate, and respond to specific drugs. Herein, we provide examples that illustrate the current status of such pharmacogenomic research and discuss the prospects for near-term advances in this field.

Genetic Polymorphisms in Drug Metabolism and Disposition

Until recently, clinically important genetic polymorphisms in drug metabolism and disposition were typically discovered on the basis of phenotypic differences among individuals in the population (*8*), but the framework for discovery of pharmacogenetic traits is rapidly changing. With recent advances in molecular sequencing technology, gene polymorphisms [such as single-nucleotide polymorphisms (SNPs), and especially SNPs that occur in gene regulatory or coding regions (cSNPs)] may be the initiating discoveries, followed by biochemical and, ultimately, clinical studies to assess whether these genomic polymorphisms have phenotypic consequences in patients. This latter framework may permit the elucidation of polymorphisms in drug-metabolizing enzymes that have more subtle, yet clinically important consequences for interindividual variability in drug response. Such polymorphisms may or may not have clear clinical importance for affected medications, depending on the molecular basis of the polymorphism, the expression of other drug-metabolizing enzymes in the patient, the presence of concurrent medications or illnesses, and other polygenic clinical features that impact upon drug response. In Fig. 2, we have highlighted those drug-metabolizing enzymes known to exhibit genetic polymorphisms with incontrovertible clinical consequences; however, almost every gene involved in drug metabolism is subject to common genetic polymorphisms that may contribute to interindividual variability in drug response. Table 1 provides examples of how these genetic polymorphisms can translate into clinically relevant inherited differences in drug disposition and effects, a comprehensive summary of which is available at www.sciencemag.org/feature/data/1044449.shl.

All pharmacogenetic polymorphisms studied to date differ in frequency among ethnic and racial groups. In fact, the slow acetylator phenotype was originally suspected to be genetically determined because of the difference in frequency of isoniazid-induced neuropathies observed in Japan versus those observed in the United States (*9*). The marked racial and ethnic diversity in the frequency of functional polymorphisms in drug- and xenobiotic-metabolizing enzymes dictates that race be considered in studies aimed at discovering whether specific genotypes or phenotypes are associated with disease risk or drug toxicity.

It is now well recognized that adverse drug reactions may be caused by specific drug-metabolizer phenotypes. This is illustrated by the severe and potentially fatal hematopoietic toxicity that occurs when thiopurine methyltransferase–deficient patients are treated with standard does of azathioprine or mercaptopurine (*6*). Another example is the slow acetylator phenotype that has been associated with hydralazine-induced lupus, isoniazid-induced neuropathies, dye-associated bladder cancer, and sulfonamide-induced hypersensitivity reactions (*9, 10*); in all cases, acetylation of a parent drug or an active metabolite is an inactivating pathway. *N*-Acetyltransferase is an enzyme that conju-

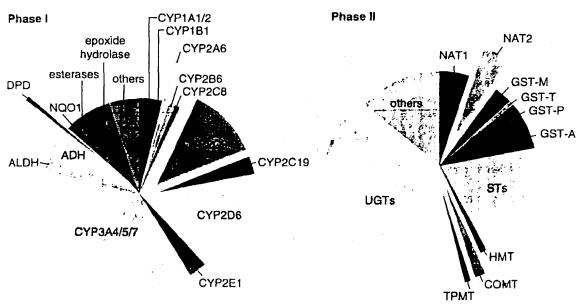

Fig. 2. Most drug-metabolizing enzymes exhibit clinically relevant genetic polymorphisms. Essentially all of the major human enzymes responsible for modification of functional groups [classified as phase I reactions (left)] or conjugation with endogenous substituents [classified as phase II reactions (right)] exhibit common polymorphisms at the genomic level; those enzyme polymorphisms that have already been associated with changes in drug effects are separated from the corresponding pie charts. The percentage of phase I and phase II metabolism of drugs that each enzyme contributes is estimated by the relative size of each section of the corresponding chart. ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP, cytochrome P450; DPD, dihydropyrimidine dehydrogenase; NQO1, NADPH:quinone oxidoreductase or DT diaphorase; COMT, catechol *O*-methyltransferase; GST, glutathione *S*-transferase; HMT, histamine methyltransferase; NAT, *N*-acetyltransferase; STs, sulfotransferases; TPMT, thiopurine methyltransferase; UGTs, uridine 5′-triphosphate glucuronosyltransferases.

gates substrates with a more water-soluble small molecular moiety. Such conjugation reactions are frequently, but not always, detoxifying, in that they often "mask" a more reactive functional group and usually enhance urinary or biliary excretion of substrates. There are many examples in which the combination of a genetic defect in a conjugation pathway (Fig. 2, right), coupled with a wild-type phenotype for an oxidation pathway (Fig. 2, left), many of which can make substrates more reactive through the insertion of oxygen or other chemical modifications, results in a phenotype particularly predisposed to adverse effects from a medication or environmental substance. Alternatively, increased CYP1A activity (an enzyme catalyzing a phase I oxidation reaction), coupled with slow acetylation (a phase II conjugation reaction), resulted in less myelosuppression from the active metabolites of the anticancer agent amonafide (*11*). Because every individual represents a combination of drug-metabolizer phenotypes, given the large number of enzymes involved in drug metabolism, it is apparent that some individuals are destined to have unusual reactions to drugs or to combinations of drugs due to the coincident occurrence of multiple genetic defects in drug-metabolizing enzymes. Such an alignment of genotypes, particularly when coupled with polymorphisms in drug receptors, is likely to constitute part of the mechanism for so-called "idiosyncratic" drug reactions.

In addition to detoxifying and eliminating drugs and metabolites, drug-metabolizing enzymes are often required for activation of prodrugs. Many opioid analgesics are activated by CYP2D6 (*7*), rendering the 2 to 10% of the population who are homozygous for nonfunctional *CYP2D6* mutant alleles relatively resistant to opioid analgesic effects. It is thus not surprising that there is remarkable interindividual variability in the adequacy of pain relief when uniform doses of codeine are widely prescribed.

For many genetic polymorphisms of drug-metabolizing enzymes, there is no evident phenotype in the absence of a drug challenge, perhaps because these enzymes are not critical for metabolism of endogenous compounds in physiologically essential pathways. However, some drug-metabolism genotypes may result in a phenotype in the absence of drug; for example, it has been postulated that CYP2D6-poor metabolizers are less pain tolerant than extensive metabolizers because of a defect in synthesizing endogenous morphine (*12*) and that certain forms of dihydropyrimidine dehydrogenase deficiency are associated with mental retardation (*13*). Moreover, the risk of some cancers has been linked to polymorphisms in drug-metabolizing enzymes, which may be due to an impaired ability to inactivate exogenous or endogenous mutagenic molecules.

As depicted in Fig. 2, CYP3A4 is the human enzyme known to be involved in the metabolism of the largest number of medications. Thus far, no completely inactivating mutations have been discovered in the human *CYP3A4* gene, although a common polymorphism in the *CYP3A4* promoter has been recently described (*14*). For enzymes that apparently do not have critical endogenous substrates (for example, *CYP2C19*, *CYP2D6*, and *TPMT*), the molecular mechanisms of inactivation include splice site mutations resulting in exon skipping (for example, *CYP2C19*), microsatellite nucleotide repeats (for example, *CYP2D6*), gene duplication (for example, CYP2D6), point mutations resulting in early stop codons (for example, *CYP2D6*), amino acid substitutions that alter protein stability or catalytic activity (for example, *TPMT*, *NAT2*, *CYP2D6*, *CYP2C19*, and *CYP2C9*), or complete gene deletions (for example, *GSTM1* and *CYP2D6*). It is remarkable that even for rare phenotypes such as thiopurine methyltransferase deficiency (which occurs in only 1 in 300 individuals), a small number of recurring mutations have been shown to account for most of the mutant alleles in humans (*6*). For this and other drug-metabolizing genes, the frequency of SNPs and other genetic defects appears to be more common than the frequency of "1 per 1000 base pairs" that is cited for the human genome. Perhaps it is because some "drug"-metabolizing enzymes are dispensable or redundant with other enzymes (such as CYP2D6 and CYP2C19) that genetic polymorphisms of drug-metabolizing enzymes are so common.

Genetic Polymorphisms in Drug Transporters

Although passive diffusion accounts for cellular uptake of some drugs and metabolites, increased emphasis (*15*) is being placed on the role of membrane transporters in absorption of oral medications across the gastrointestinal tract; excretion into the bile and urine; distribution into "therapeutic sanctuaries," such as the brain and testes; and transport into sites of action, such as cardiovascular tissue, tumor cells, and infectious microorganisms. It has been proposed that some of these transporters, such as *P*-glycoprotein, may not be essential for viability, because

Table 1. Examples of clinically relevant genetic polymorphisms influencing drug metabolism and effects. A comprehensive listing is available at www.sciencemag.org/feature/data/1044449.shl.

Gene	Medications	Drug effect linked to polymorphism	References
	Drug-metabolizing enzymes		
CYP2C9	Tolbutamide, warfarin, phenytoin, nonsteroidal anti-inflammatories	Anticoagulant effect of warfarin	(*32*)
CYP2D6	Beta blockers, antidepressants, antipsychotics, codeine, debrisoquin, dextromethorphan, encainide, flecainide, guanoxan, methoxyamphetamine, *N*-propylajmaline, perhexiline, phenacetin, phenformin, propafenone, sparteine	Tardive dyskinesia from antipsychotics; narcotic side effects, efficacy, and dependence; imipramine dose requirement; beta-blocker effect	(*6, 12, 33*)
Dihydropyrimidine dehydrogenase	Fluorouracil	Fluorouracil neurotoxicity	(*13*)
Thiopurine methyltransferase	Mercaptopurine, thioguanine, azathioprine	Thiopurine toxicity and efficacy; risk of second cancers	(*6, 34*)
	Drug targets		
ACE	Enalapril, lisinopril, captopril	Renoprotective effects, cardiac indices, blood pressure, immunoglobulin A nephropathy	(*18, 21*)
Potassium channels			
HERG	Quinidine	Drug-induced long QT syndrome	(*35*)
	Cisapride	Drug-induced torsade de pointes	
KvLQT1	Terfenadine, disopyramide, meflaquine	Drug-induced long QT syndrome	
hKCNE2	Clarithromycin	Drug-induced arrhythmia	(*26*)

knockout mice appear normal until challenged with xenobiotics. However, other transporters are likely to play critical roles in transport of endogenous substances. Although polymorphisms in *P*-glycoprotein have been reported (*16*), and such variation may have functional importance for drug absorption and elimination, the clinical relevance of polymorphisms in drug transporters has not yet been fully elucidated.

Genetic Polymorphisms in Drug Targets

Most drugs interact with specific target proteins to exert their pharmacological effects, such as receptors, enzymes, or proteins involved in signal transduction, cell cycle control, or many other cellular events. Molecular studies have revealed that many of the genes encoding these drug targets exhibit genetic polymorphism, which in many cases alters their sensitivity to specific medications. Such examples include polymorphisms in β-adrenergic receptors and their sensitivity to β-agonists in asthmatics (*17*), angiotensin converting enzyme (ACE) and its sensitivity to ACE inhibitors (*18*), angiotensin II T1 receptor and vascular reactivity to phenylephrine (*19*) or response to ACE inhibitors (*20*), sulfonylurea receptor and responsiveness to sulfonylurea hypoglycemic agents (*21*), and 5-hydroxtryptamine receptor and response to neuroleptics such as clozapine (*22*). In addition, genetic polymorphisms that underlie disease pathogenesis can also be major determinants of drug efficacy, such as mutations in the apolipoprotein E gene and responsiveness of patients with Alzheimer's disease to tacrine therapy (*23*) or cholesteryl ester transfer protein polymorphisms and efficacy of pravastatin therapy in patients with coronary atherosclerosis (*24*). Finally, the risk of adverse drug effects has been linked to genetic polymorphisms that predispose to toxicity, such as dopamine D3 receptor polymorphism and the risk of drug-induced tardive dyskinesia (*25*), potassium channel mutations and drug-induced dysrhythmias (*26*), and polymorphism in the ryanodine receptor and anesthesia-induced malignant hyperthermia (*27*). Polymorphisms in genes of pathogenic agents (human immunodeficiency virus, bacteria, tuberculosis, and others) are another important source of genetic variation in drug sensitivity, but this review focuses only on polymorphisms in human genes that determine an individual's response to specific medications.

Table 1 provides examples of genetic polymorphisms in drug targets that have been linked to altered drug sensitivity. It is anticipated that ongoing studies will rapidly expand the number of such pharmacogenomic relations. Furthermore, these examples represent monogenic determinants of drug effects, which are the easiest to recognize in population studies. It is likely, however, that drug response is often a polygenic trait, in which case more comprehensive studies will be required to define pharmacogenomic traits that are determined by multiple polymorphic genes. It should also be recognized that not all studies have reached the same conclusions about the effects of genetic polymorphisms on drug response [for example, not all studies of ACE polymorphisms have found a relation with response to ACE inhibitors (*18*)]. Such discordant results may be due to a number of factors, including the use of different end points in assessing response, the heterogeneous nature of diseases studied, and the polygenic nature of many drug effects. The rapidly expanding knowledge of the human genome, coupled with automated methods for detecting gene polymorphisms, provides the tools needed to elucidate these polygenic determinants of drug effects, thus fueling the burgeoning field of pharmacogenomics.

Relevance to Drug Discovery and Clinical Therapeutics

Substantial investments are being made within the pharmaceutical and biotechnology industries to use genomic strategies for the discovery of novel therapeutic targets (*28*). It is anticipated that, over the next decade, the Human Genome Project, coupled with DNA array technology, high-throughput screening systems, and advanced bioinformatics, will permit rapid elucidation of complex genetic components of human health and disease. Common polymorphisms in drug targets dictate that DNA sequence variations be taken into account in the genomic screening processes aimed at new drug development. This will provide new insights for the development of medications that target critical pathways in disease pathogenesis and medications that can be used to prevent diseases in individuals who are genetically predisposed to them.

Such pharmacogenomic studies should also permit the development of therapeutic agents targeted for specific, but genetically identifiable, subgroups of the population. This represents a migration from the traditional strategy of trying to develop medications that are safe and effective for every member of the population, a strategy that aims to provide a marketing bonanza but one that is a pharmacological long shot because of highly potent medications, genetically diverse patients, and diseases that have heterogeneous subtypes. Although debate about the wisdom of developing medications for only a subset of the population remains within the pharmaceutical industry (*28*), it is clear that science and technology will soon make it feasible to use molecular diagnostics to more precisely select medications and dosages that are optimal for individual patients (*29*). In this regard, automated systems are being developed to determine an individual's genotype for polymorphic genes that are known to be involved in the pathogenesis of their dis-

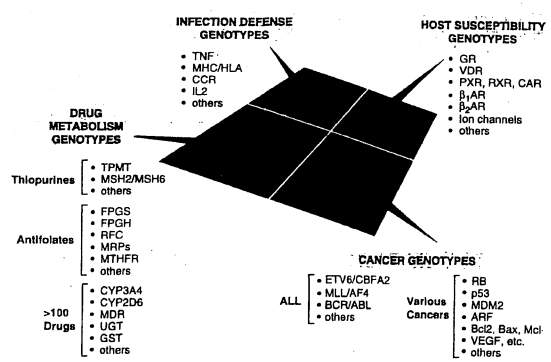

Fig. 3. Molecular diagnostics of pharmacogenomic traits. DNA arrays are being made for automated, high-throughput detection of functionally important mutations in genes that are important determinants of drug effects, such as drug-metabolizing enzymes, drug targets (receptors), disease pathogenesis, and other polymorphic genes that influence an individual's susceptibility to drug toxicities or environmental exposures (such as pathogens, carcinogens, and others). This figure exemplifies components of a potential diagnostic DNA array for genes that could influence a patient's response to chemotherapy for acute lymphoblastic leukemia, including genes that determine drug metabolism, disease sensitivity, and the risk of adverse effects of treatment (cardiovascular or endocrine toxicities, infections, and so forth).

ease, in the metabolism and disposition of medications, and in the targets of drug therapy. Such diagnostics, which need be performed only once for each battery of genes tested, can then become the blueprint for individualizing drug therapy. This is illustrated in Fig. 3, which depicts various genes that could be genotyped to guide the selection and dosing of chemotherapy for a patient with acute lymphoblastic leukemia (ALL). It is already known that genetic polymorphisms in drug-metabolizing enzymes can have a profound effect on toxicity and efficacy of medications used to treat ALL (*6*) and that individualizing drug dosages can improve clinical outcome (*30*). It has also been established that the genotype of leukemic lymphoblasts is an important prognostic variable that can be used to guide the intensity of treatment (*31*). Furthermore, genetic polymorphisms are also known to exist for cytokines and other determinants of host susceptibility to pathogens, and polymorphisms in cardiovascular, endocrine, and other receptors may be important determinants of an individual's susceptibility to drug toxicity. Putting all of these molecular diagnostics on an "ALL chip" would provide the basis for rapidly and objectively selecting therapy for each patient. These examples represent our current, relatively poor, understanding of genetic determinants of leukemia therapy and host sensitivity to treatment; ongoing studies will provide important insights that should substantially enhance the utility of such pharmacogenomic strategies for ALL and many other human illnesses.

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