TWO FURTHER SERUM PSEUDOCHOLINESTERASE PHENOTYPES AS CAUSES OF SUXAMETHONIUM APNOEA

BY

H. LEHMANN, M.D., Sc.D., M.R.C.P., F.R.I.C.*Reader in Chemical Pathology,
St. Bartholomew's Hospital, London***J. LIDDELL, M.D.***Senior Registrar in Clinical Biochemistry,
Radcliffe Infirmary, Oxford***B. BLACKWELL, B.A., M.B., B.Chir.***Psychiatric Registrar, Bethlem Royal Hospital, Beckenham, Kent***D. C. O'CONNOR, Ph.D., L.R.C.P.&S.***Deputy Medical Director, The Priory, London***ANN V. DAWS, M.B., B.S., D.A.***Anaesthetic Registrar, Aylesbury Hospitals Group, Bucks*

Suxamethonium, a muscle relaxant whose properties were first described by Bovet and his colleagues in 1949, has proved useful in anaesthesia because the duration of its action is short. Thus after the administration of 50–100 mg. the muscle paralysis and associated apnoea last only about two minutes. This brief action is due to the rapid destruction of the drug by serum pseudocholinesterase (Bovet-Nitti, 1949). Rare individuals exist who possess a low level of serum pseudocholinesterase, and who, after the usual dose of suxamethonium, develop a prolonged apnoea which may last several hours (Bourne, Collier, and Somers, 1952; Evans, Gray, Lehmann, and Silk, 1952). This low serum pseudocholinesterase activity and the associated suxamethonium sensitivity have been shown to be inherited (Lehmann and Ryan, 1956).

Kalow and his colleagues have shown that these individuals are homozygotes for a rare gene, and possess not a low level of the normal enzyme but an abnormal serum pseudocholinesterase. This abnormal or "atypical" enzyme differs from the usual type in two ways: it is less active in hydrolysing a large number of substrates, including suxamethonium (Davies, Marton, and Kalow, 1960), and more resistant to a majority of cholinesterase inhibitors (Kalow and Davies, 1958). Kalow and Genest (1957) have proposed the use of one such inhibitor, the local anaesthetic dibucaine (cinchocaine, "nupercaine"), as a screening test to identify these homozygotes. In this test a 10^{-5} M concentration of dibucaine inhibits the normal enzyme by about 80% and that found in suxamethonium-sensitive individuals by only 20%. The percentage inhibition is called the dibucaine number, or DN. Heterozygotes, possessing a mixture of both enzymes, have dibucaine numbers of between 50 and 69. Various population surveys have shown that the suxamethonium-sensitive homozygote occurs with a frequency of about 1 in 2,800, while 3.8% of the population are heterozygotes (Kalow and Gunn, 1958; Kattamis, Zannos-Marioleta, Franco, Liddell, Lehmann, and Davies, 1962).

Another genetic variant has been described in which the homozygote, detected because of a prolonged apnoea following suxamethonium administration, showed a complete absence of serum pseudocholinesterase activity (Liddell, Lehmann, and Silk, 1962). The gene responsible has been called the "silent" pseudocholinesterase gene.

Harris and Whittaker (1961, 1962) have described a third variant of the enzyme. They discovered that sodium fluoride, like dibucaine, inhibits the atypical enzyme less strongly than normal pseudocholinesterase.

Thus a 5×10^{-5} M concentration of sodium fluoride inhibits the normal enzyme by about 60%, the atypical enzyme by about 25%, and sera from heterozygotes by about 45%. Harris and Whittaker called this inhibition the fluoride number, or FN. When sera from large numbers of persons were examined a few individuals were found the dibucaine numbers of whose sera were slightly lower and the fluoride numbers markedly lower than expected. Harris and Whittaker suggested that this was due to the presence of another variant of pseudocholinesterase with a slightly lower dibucaine number and markedly lower fluoride number than normal. Using both the dibucaine and fluoride techniques, five types of individual can be recognized:

1. DN 80, FN 63; normal enzyme.

2. DN about 74, FN about 52; heterozygotes possessing both the normal enzyme and the fluoride-resistant enzyme described by Harris and Whittaker.

3. DN about 64, FN about 48; heterozygotes possessing both the normal and atypical enzymes.

4. DN about 54, FN about 34; heterozygotes possessing both the atypical and the fluoride-resistant enzymes.

5. DN about 20, FN about 23; homozygotes for the atypical enzyme.

Three points about this fluoride-resistant variant are still uncertain: (1) Genetic studies from only four families have been published to date—further studies are therefore required to confirm the type of inheritance; (2) there is no evidence to show which, if any, of these genotypes may show a sensitivity to suxamethonium; and (3) the homozygote for the fluoride-resistant enzyme has not been described. We have recently seen four individuals, all discovered because of suxamethonium sensitivity, the investigation of whom has provided some evidence on these three points.

Methods.—Pseudocholinesterase levels were determined by the micromanometric method of Ammon (1933). The normal range is 60 to 120 units/ml.

Dibucaine numbers were estimated by the technique of Kalow and Genest (1957) and fluoride numbers by the method described by Harris and Whittaker (1961).

Suxamethonium-sensitive Proposit

Propositus No. 1

The patient is a fit woman of 53 with no significant medical history. She is Italian by birth and has been settled in this country for 15 years. She was referred to Tindal General Hospital for six weekly electric-convulsion therapy (E.C.T.) sessions for the treatment of menopausal depression with paranoid delusions.

On the first occasion she was premedicated with atropine 1/50 gr. (1.3 mg.) and methahexital 50 mg. This was

followed by 40 mg. of suxamethonium, and the convulsive shock was given when depolarization appeared complete. Spontaneous respiration did not return for 30 minutes, and this necessitated intubation and intermittent positive-pressure respiration.

For the second treatment 25 mg. of suxamethonium was given and the resulting apnoea lasted for 13 minutes. On the third and fourth occasions 10 mg. of suxamethonium was administered and the apnoea lasted for eight minutes and 10 minutes respectively. On the fifth and sixth occasions the apnoea lasted for five minutes and two minutes after a dose of about 7 mg. of suxamethonium.

The serum pseudocholinesterase activity was 52 units/ml. and the dibucaine and fluoride numbers were 48 and 35 respectively. These results suggested that the patient was a heterozygote for the genes for the atypical and fluoride-resistant enzymes respectively.

Propositus No. 2

A girl of 14 had a nasal polypectomy in October, 1962. Following the administration of 75 mg. of suxamethonium she developed a 20-minute period of apnoea. Investigation of her plasma showed the dibucaine and fluoride numbers to be 48 and 31 respectively, and suggested that this patient was a heterozygote for the genes for the atypical and fluoride-resistant enzymes respectively. Her pseudocholinesterase level was 43 units/ml.

Propositus No. 3

A man of 49 had a reconstruction of a partial gastrectomy in October, 1962, because of the dumping syndrome. Following the administration of thiopentone and 100 mg. of suxamethonium he developed an apnoea which lasted for two hours and 40 minutes. It is of interest that during a partial gastrectomy in 1959 he was given 75 mg. of suxamethonium and no apnoea was observed.

His plasma pseudocholinesterase was 53 units/ml. and the dibucaine and fluoride numbers were 53 and 36 respectively, suggesting that he also was a heterozygote for the genes for the atypical and fluoride-resistant enzymes respectively.

Propositus No. 4

The patient, a 46-year-old Cypriot of Greek extraction, has suffered from annual recurrences of depression for over 20 years. He is married, with three children, and is the fifth of seven siblings, of whom one younger sister has twice suffered from depression. In April, 1961, he emigrated to this country and has since spent over a third of his time in mental hospitals. In June, 1961, he was given E.C.T. at the Priory Hospital, Roehampton; he was treated 16 times, and had apnoeas varying from 20 minutes after 40 mg. of suxamethonium to 10 minutes after 10 mg.

He was first admitted to the Bethlem Royal Hospital under Dr. Linford Rees in March, 1962. Because of the previous history he was given 8 E.C.T. using gallamine triethiodide as the muscle relaxant, without apnoea. In December, 1962, he was admitted with a further depressive illness. Because of the possibility of future out-patient E.C.T. and the technical difficulties involved in the use of

gallamine triethiodide it was decided to investigate further the apnoea produced by suxamethonium. During a course of seven treatments he received dosages varying from 20 to 30 mg., and on every occasion developed apnoea lasting from five to eight minutes. Anaesthesia was induced with 30 mg. of intravenous thiopentone; oxygenation and the airway were maintained without laryngeal intubation and recovery was spontaneous and uneventful. Investigation of the plasma showed a pseudocholinesterase activity of 68 units, and dibucaine and fluoride numbers of 67 and 34 respectively. Values of this type have not previously been recorded, and they suggest that the patient is a homozygote for the fluoride-resistant pseudocholinesterase gene.

It may be surprising that the FN for the supposed homozygote is the same as that of the heterozygotes just described. The important difference lies in the relatively high DN of the homozygote. To present the further evidence for the homozygous state of the fluoride-resistant enzyme gene in this propositus would be outside the scope of this article, and this will be published separately (Liddell, Lehmann, and Davies, 1963).

Family Studies

Table I shows the genotypes of the relatives of the four propiti, as determined by the fluoride and dibucaine numbers. It can be seen that the results confirm that the first three suxamethonium-sensitive patients are heterozygotes possessing both the atypical and the fluoride-resistant pseudocholinesterases. The results in the fourth family support the theory that the patient and one sibling are homozygotes for the gene for the fluoride-resistant enzyme.

Discussion

Before family counselling can be given it is important to appreciate the genetics underlying suxamethonium sensitivity. At first the situation appeared to be simple. There were two genes responsible for the "atypical" and the usual pseudocholinesterases. Thus there were three genotypes: usual homozygote, heterozygote, and atypical homozygote, and of these one, the atypical homozygote, was suxamethonium-sensitive. In our experience the heterozygote for these two genes is not sensitive to suxamethonium. For instance, during the period we have been studying the problem approximately 80,000 patients have been given suxamethonium in our hospitals. Of these patients 3.8%, or more than 3,000 individuals, must have been heterozygotes, yet we have not seen a case of suxamethonium apnoea with this genotype.

More recently the genetic mechanisms have been shown to be more complex. A suxamethonium apnoea has been seen by us in a woman without any serum pseudocholinesterase activity, and it was thought that this patient was a homozygote for a silent pseudo-

TABLE I.—Genotypes* of the Suxamethonium-sensitive Propiti and their Immediate Relatives

Propositus	Spouse	Offspring			Parents		Siblings				
		1	2	3	1	2	1	2	3	4	5
1. D-F	N-N (80, 62)	N-D (63, 50)	N-D (60, 46)	N-F (74, 49)	—	—	—	—	—	—	—
2. D-F	—	—	—	—	D-F (47, 36)	N-F (73, 47)	N-D (45, 39)	D-F (48, 31)	—	—	—
3. D-F	N-N (83, 62)	N-F (76, 51)	N-F (75, 51)	N-D (68, 45)	—	—	N-F (77, 52)	N-D (61, 55)	N-D (64, 50)	—	—
4. F-F	N-N (82, 61)	N-F (73, 55)	N-F (71, 52)	N-F (71, 53)	—	N-F (71, 53)	N-F (73, 54)	N-F (78, 64)	F-F (64, 35)	N-N (79, 63)	N-F (74, 55)

N=Normal pseudocholinesterase gene. D=Atypical pseudocholinesterase gene (dibucaine-resistant). F=Fluoride-resistant pseudocholinesterase gene. The dibucaine and fluoride numbers are shown in parentheses underneath the genotype of each individual.

* On the assumption that the genes are allelic.

cholinesterase gene. Other patients with suxamethonium sensitivity have been described who appeared to be heterozygotes for both the silent and atypical genes (Liddell *et al.*, 1962). The present findings show that both the homozygote for the gene responsible for the fluoride-resistant enzyme and the heterozygote for this gene and the atypical gene are also subject to suxamethonium apnoea of moderate severity.

It is probable that still other pseudocholinesterase genes will be discovered in patients presenting with an unexpected suxamethonium apnoea. For instance, Lehmann, Silk, Harris, and Whittaker (1961) have described the family of a suxamethonium-sensitive propositus in whom the pseudocholinesterase levels and dibucaine numbers did not fit into any of the recognized genetic patterns. Further examples of this type must be seen before the genetic mechanism can be analysed. It is obviously important that the families of all suxamethonium-sensitive individuals should be investigated. Table II gives a summary of the pseudocholinesterase genotypes which have been described up to the present.

TABLE II.—Possible Genotypes Determined by the Four Pseudocholinesterase Genes: Normal (N), Atypical or Dibucaine-resistant (D), Fluoride-resistant (F), and Silent (S)

Genotype	Suxamethonium Sensitivity	Comments
<i>Homozygotes</i>		
<i>N-N</i>	Not sensitive	The normal population Frequency = 1 : 2,800 Rare. Frequency not known " " " "
<i>D-D</i>	Markedly sensitive	
<i>S-S</i>	"	
<i>F-F</i>	Moderately "	
<i>Heterozygotes</i>		
<i>N-D</i>	Not sensitive	3.8% of the population
<i>N-S</i>	Probably not sensitive	
<i>N-F</i>	"	3 cases of apnoea described Frequency unknown Not yet described
<i>D-S</i>	Markedly sensitive	
<i>D-F</i>	Moderately "	
<i>F-S</i>	Expected to be moderately sensitive	

It is obvious that an agreed nomenclature for the pseudocholinesterase variants should soon be decided upon, and the present naming of the genes N, D, F, and S is purely temporary.

The unfolding of the genetic background of suxamethonium apnoea is remarkable in the way it follows that of sickle-cell anaemia. There also three genotypes were known at first, based on the genes for normal adult haemoglobin (A) and sickle-cell haemoglobin (S). The normal homozygote (AA) and the heterozygote, the sickle-cell trait carrier (AS), were not anaemic, but the abnormal homozygote (SS) had a severe anaemia. Other types of sickle-cell anaemia were discovered when heterozygotes for the S gene and for the thalassaemia gene were seen. Thalassaemia is a condition where haemoglobin A is suppressed and the combination of the S gene and the "silent" A gene—that is, the thalassaemia gene—resulted in sickle-cell thalassaemia. Yet other modified sickle-cell anaemias were found when heterozygotes for the S gene and another gene responsible for an abnormal haemoglobin were seen—that is, SC in sickle-cell HbC disease, SD in sickle-cell HbD disease, etc. It thus seems that we can observe the emergence of a general pattern in the inheritance of abnormal proteins and the pathological states which result.

Summary

Several pseudocholinesterase genotypes have been observed in suxamethonium apnoea. To these are now added (1) the heterozygote for the gene for the "atypical" enzyme of Kalow and his colleagues and for

that for the "fluoride-resistant" enzyme of Harris and Whittaker; and (2) the homozygote for the second gene, who is being reported for the first time.

The gradual unfolding of the varied genetical background of suxamethonium apnoea repeats the events in the history of sickle-cell anaemia and related haemoglobinopathies. It seems that there may be a general pattern for pathological conditions arising from genetically determined protein variants, whether they are chromoproteins such as the haemoglobins or enzymes such as the pseudocholinesterases.

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"Now as to the future. It is true no one can forecast the future with certainty. But having got off to a flying start we do not intend to pursue our course rudderless and uncharted. It is our intention that by 1967 our student intake will be of the order of 150 medical and 50 dental students every year. It has been said that this is too great a number to accommodate in one medical school, but we do not accept this argument. As long as we retain the ratio of teacher to student at about one to ten in each department it should be possible to train students, with the rich clinical facilities existing in Nigeria, to the highest standards expected of our profession. When one considers that the population of the Federation of Nigeria is probably over 40 million and that the ratio of doctors to the population is one to 40,000 the reason becomes obvious for our determination to step up our intake of medical students and in every way to augment the training of medical personnel generally. In planning our programmes of studies we have placed emphasis on the study of the diseases of childhood and on social medicine and public health. So much of the disease-complex in a developing country like ours is due to lack of public health facilities—lack of purified water, improper care of foodstuff, lack of control of insect pests, just to mention a few. It is our intention to train doctors who will regard public health service as an essential part of their work. All this offers a challenge which must be taken up by our School and future medical schools which we hope will soon be set up in other parts of the country. The challenge of independence is not only political and economic but also medical." (*W. Afr. med. J.*, Professor H. O. Thomas, Dean, at official opening of Lagos Medical School, October 3, 1962.)