INITIAL EXPERIENCES WITH THE CHOLINESTERASE RESEARCH UNIT

BY

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SUMMARY

During its first eighteen months of operation, fifty-three samples have been sent to the Cholinesterase Research Unit, following an apnoea. Attempts have been made to follow up thirty-seven potentially interesting patients. Sixteen families have been successfully investigated. Some of the unusual points that have arisen are discussed.

Following a suggestion in an Editorial in the British Journal of Anaesthesia in November of 1967, we wrote to this Journal and others in the United Kingdom, offering to provide a service of cholinesterase estimations and dibucaine and fluoride numbers for anaesthetists in the United Kingdom. It was hoped that, on their part, those who sent serum would co-operate with us in elucidating any interesting families. By providing a standard methodology we hoped to prevent erroneous genotyping and to ensure that family studies were not rendered less valuable by doubts as to the exact results. This paper records our experiences during the first eighteen months of this venture.

Material received.

Fifty-three samples were received, each from a patient who had had apnoea following suxamethonium and these are broadly classified in table I. Twenty-one had a normal phenotype, and these could be divided into three broad categories. Ten samples not only had a normal phenotype but had normal levels of activity as well. A further six samples with a normal phenotype had activities between 60 and 80 units/ml, which while below the normal range for our method (80–120 u.) were at least more than half the upper limit. In both these groups the implication of abnormal metabolism of suxamethonium as a cause of apnoea is dubious, and it was not thought worthwhile to follow-up the families of these sixteen patients. Five samples showed a reduction in activity sufficient to give cause to suspect they were either samples of the silent gene or possibly possessed a low activity gene and therefore worth following up. In the remaining thirty-two samples there was evidence of at least one abnormal gene, making a total of thirty-seven samples for which family studies were indicated.

Methods used.

Plasma cholinesterase was assayed using benzoyl choline chloride as substrate in M/15 phosphate buffer, pH 7.4, at 26.5°C by the method of Kalow and Lindsay (1955). Dibucaine numbers were measured by the method of Kalow and Genest (1957) and fluoride numbers were measured by the method of Harris and Whittaker (1961). Electrophoretic patterns were obtained by a modification of the method of Harris et al. (1963).

Follow-up failures.

Of the thirty-seven families whom we thought it worthwhile to study, we have failed to obtain any samples from the family in twenty-one cases.

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The reasons for this high failure rate have been analyzed. The majority are due to two causes; in the biggest group (nine cases) the situation is dynamic in that the initial samples have been only relatively recently received and it is still possible that family samples will be obtained. In two of these we have reason to expect a favourable outcome because of previous liaison with the anaesthetist concerned, but in six, inadequate co-operation with the referring anaesthetist can already be suspected. The next largest group (eight cases) can only be ascribed to inadequate co-operation. In only one of these cases was the patient primarily responsible for the failure to obtain the specimens. In the remaining seven cases the anaesthetist who sent the initial sample has either not replied to our letters or has failed to make any arrangements, however tenuous, for obtaining the samples. Other causes of failure have been rare. In one case there were no close blood relations; in a second case all the relatives were living permanently abroad; in a third case clerical inadequacies caused us to overlook the patient; whilst in the fourth case the results were borderline and it was likely to be difficult to obtain the samples; this combination made it hardly worth the effort.

Initial method of referral.

Slightly more than half of the material (twenty-six cases) was referred to us from doctors who knew our individual interest in the problem. One sample was sent to Professor H. Harris of the MRC Biochemical Genetics Unit, who passed it on, whilst another case was reported in a letter in the British Medical Journal, and we wrote and contacted the writer. There remain, therefore, twenty-five cases in which we presume material was sent to us because of the letters published in the medical press.

Distribution of phenotypes.

It is surprising to find so many cases of apnoea (ten in all) apparently sufficiently striking to stimulate the sending of a specimen, which yet had a normal phenotype with normal enzyme activity. There must be a strong presumption that if a neuromuscular stimulator had been available and had been used, neuromuscular block would not have been found. It is particularly disturbing to find that one large teaching hospital sent no less than four of these samples.

It is of interest, however, to compare the current results with those obtained in three previous surveys. These are presented in table II together with the combined findings of the four independent investigations. The apparent constancy of the percentage of normal phenotypes in all surveys of suxamethonium-sensitive individuals is striking. Some of these "normal" phenotypes have a low enzymic activity but, as the present study confirms, a large proportion of this group have serum cholinesterase activity well within the average range for a random population. It is, of course,
possible that there are still unknown factors which determine the occurrence of apnoea and which are not revealed by current biochemical methods, but unless there is clear evidence that the apnoea is associated with neuromuscular block, it is pointless to follow up these biochemically “normal” cases.

Normal phenotypes with atypical features.

If the current concepts concerning this abnormality are correct, both the type and quantity of plasma cholinesterase activity are determined by a single pair of allelomorphic, non-dominant autosomal genes, each responsible for the elaboration of approximately half the total activity. The normal range of activities by the methods used in this laboratory is 80–120 u., and this implies that each gene is responsible for the elaboration of about 40–60 u. The so-called silent gene when present in the homozygous form is usually associated with levels of activity below about 5 u. An individual heterozygous for the normal and the silent gene might therefore be expected to have an activity a little above the 40–60 u. range, although in fact it has been our experience that the silent gene has an enhancing effect on the activity of the usual gene, and levels of 50–80 u. would not be unlikely. However, we have received several specimens well below this range, yet with family studies that make it unlikely for them to be homozygous for the silent gene. One such example is illustrated in figure 1. The father (I,) would normally be classified as heterozygous $E^u E^s$. The mother (I$^2$) and two of the children (II$^1$ and II$^2$) have a normal phenotype and activity levels which are too high for homozygous silent $E^s$, but lower than expected for the heterozygote $E^u E^s$. The other two children (II$^3$ and II$^4$) have a normal phenotype also, with activity levels, however, which imply homozygous silent $E^s$. In none of the patients was there any reason to suspect a temporary reduction of activity due, for example, to concurrent drug therapy or any known pathology which is associated with a low cholinesterase level (Thompson and Whittaker, 1965).

In this family it is tempting to postulate a low activity gene of normal phenotype in the mother which is inherited by two of the children, the other two children inheriting a silent gene from both parents. Other samples have been received in which this explanation could be postulated also. It will be of considerable interest to follow-up such patients over a period of time and to institute family studies to see whether a family inheritance of low activity exists.

Abnormal phenotypes.

The constancy of the frequency of the atypical homozygote $E^u E^u$ in all four surveys shown in table II is not surprising since the presence of the atypical phenotype is the most common explanation advanced for suxamethonium sensitivity. The varying frequencies of the fluoride-resistant phenotypes is readily explained when one recalls the low frequency of this gene and also the fact that it is the most difficult to identify (Whittaker, 1967). There is an indication that heterozygotes $E^u E^f$ showing apnoea have decreased, whereas $E^u E^u$ have increased, but the sample is too small to draw definite conclusions. The relatively large number of heterozygous propositi now being reported suggests that anaesthetists are either becoming more skilful in detecting minor abnormalities of response to suxamethonium or are more ready to investigate their suspicions.

\[\begin{array}{cccc}
I_1 & I_2 \\
\text{♂} & \text{♀} \\
61u & 31u \\
\hline
DN 79 & DN 79 \\
FN 61 & FN 63 \\
\hline
II_1 & II_2 & II_3 & II_4 \\
♀ & ♀ & ♀ & ♀ \\
31u & 31u & 4u & 5u \\
\hline
DN 79 & DN 79 & DN 79 & DN 79 \\
FN 61 & FN 61 & FN 61 & FN 60 \\
\end{array}\]

Cholinesterase activity, dibucaine and fluoride numbers in a family. The dibucaine and fluoride numbers in II$^1$ and II$^2$ were achieved by using prolonged hydrolysis times. For suggested inheritance pattern, see text.
Warning cards.

We think it desirable that warning cards should be issued to all appropriate cases, on the basis of the biochemical findings. It should be clear and authoritative, and give the name and address of a person who can substantiate the findings. Figure 2 shows the card which we issue.

CHOLINESTERASE RESEARCH UNIT

This is to certify that

specimen No. CRU XX/69 is sensitive to SUXAMETHONIUM. Further details can be obtained from the above address.

Date 1/6/69

Dr. Mary Whittaker

Suxamethonium warning card.

In two cases we have obtained samples of sera from patients who have been issued with cards warning them of their sensitivity to suxamethonium, in which we have been unable to detect any abnormality in enzyme pattern. One such patient was subsequently given suxamethonium uneventfully (Bray, 1968). We wrote to Dr Bray and obtained some of the patient’s serum, and found an activity of 106 u. with DN 80 and FN 59, i.e. normal enzyme, in normal quantities. Nevertheless, he carried a card warning anaesthetists of the dangers of the administration of suxamethonium; a card warning him of the dangers of a particular anaesthetist might have been equally appropriate.

The correct policy with regard to heterozygotes is not clear cut. Where heterozygotes have been referred to us because of apnoea, even though the enzyme activity appears to be adequate, it is difficult to deny that some incident worthy of attention occurred. In the absence of any reported abnormalities of technique one must presume that other anaesthetists might well obtain a similar result and it seems desirable to issue a warning card. However, the majority of heterozygotes are detected by family studies, and many have never been exposed to suxamethonium. Our present policy is to issue a warning card to such heterozygotes only when the total activity is less than 40 u. although we recognize that this is arbitrary. Such a level of activity is well outside the range expected of these genotypes. It is our experience that heterozygotes developing an apnoea have a lower level of plasma cholinesterase activity than other individuals with a comparable genotype (Thompson and Whittaker, 1966).

General problems.

The problems involved in setting up this sort of service on a voluntary basis have been considerable. The transfer of both of us away from London has made it more difficult to maintain good documentation between the clinical details and the biochemical investigation of the families. We are aware that delays in notifying results may result in loss of interest, and that where the referring anaesthetist is a trainee, he may be no longer in a position to provide any follow-up.

There is no doubt that it is sometimes difficult for anaesthetists to obtain the names and addresses of all relations and their doctors, particularly if the importance of this is not realized during the patient’s stay in hospital. It is then not easy to arrange for them to attend hospital and give samples of blood which must then be transmitted by post. If we are informed of these difficulties we have found that general practitioners will often assist by taking samples of blood from those relatives of apnoea cases who are on their list. We have, however, been disappointed by the number of cases which have not been adequately investigated as a result of lack of cooperation. Although only seven failures have been listed as due to this cause, it seems likely that six of the nine cases which are classified as still pending will ultimately prove to be failures from this cause also. This will mean that two-thirds of all failures to complete family studies will have been due to inadequate co-operation between us and the anaesthetists sending samples. It is perhaps worth emphasizing that without the option to perform family studies, the investigation of the patient can be a waste of time, particularly as the results may not even be entered in the patient’s notes. One sample of blood was sent to us after an apnoea during Caesarean section and an abnormal phenotype was found. It was then discovered that the patient had been investigated.
for an apnoea after a previous Caesarean section in another hospital. These two hospitals were in the same group and only 300 yards apart.

The potential benefit of applying a constant methodology has already been amply demonstrated. Several cases classified as abnormal as a result of biochemical tests performed elsewhere have been shown to be biochemically normal. Such patients would have been classified incorrectly, and their families unjustifiably bothered. Conversely, results which might have been thought of as slight aberrations, owing to unfamiliarity with the method, assume greater significance when they do not fall into one of the patterns that are expected. Electrophoretic studies of such cases have already revealed the presence of a band which is associated with an increased level of cholinesterase activity (Harris et al., 1963).

An obvious omission in this survey is the absence of any attempt to derive a correlation between the various genotypes and the duration of apnoea. This is due to the inadequacy of the clinical details usually supplied with the specimens. However, the results achieved during the first eighteen months of this venture suggest that there are still unsolved problems and inconsistencies in this field, and it is to be hoped that by bringing forward this short general report, other anaesthetists will be encouraged to send specimens which they feel may be of interest.

Specimens of serum or heparinized blood should be sent in future to Dr M. Whittaker, Dept. of Chemistry, University of Exeter, Devon.

REFERENCES


EXPERIENCE INITIALE DU CENTRE DE RECHERCHE DE LA CHOLINESTERASE

Cinquante-trois échantillons ont été envoyés, après une apnée, au centre de recherches de la cholinesterase, au cours des dix-huit premiers mois de son existence. On a essayé de suivre trente-sept patients, puis semblaient intéressants. Seize familles ont été étudiées avec succès. Certaines des observations inhabituelles sont discutées.

ERSTE ERFahrungen MIT DER CHOLINESTERASE-FORSCHUNGS-ABTEILUNG

Während der ersten achtzehn Monaten ihrer Tätigkeit ist der Cholinesterase-Forschungsabteilung nach Auftreten eines Atemstillstandes 53mal Untersuchungsmaterial zugeschickt worden. Versuche sind unternommen worden, 37 potentiell interessante Patienten weiter zu beobachten. 16 Familien sind erfolgreich untersucht worden. Einige der ungewöhnlichen Aspekte, die festgestellt worden sind, werden diskutiert.