METHODOLOGY OF A PROSPECTIVE STUDY OF CHANGES IN LIVER ENZYME CONCENTRATIONS FOLLOWING REPEAT ANAESTHETICS


SUMMARY

The methodology of a large prospective study on the influence of repeated anaesthetics on liver function is reported and the problems involved are discussed. The most suitable patients were those presenting for endoscopic examination of the bladder and urethra, for urethral dilatation and for cervical implantation of radium. Blood samples were taken immediately before induction of anaesthesia and on days 3–4 and 13–15 after operation, when a clinical assessment of the patient was also carried out. The concentrations of six enzymes (lactate dehydrogenase, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, serum cholinesterase and gamma glutamyl transpeptidase) were chosen specifically as indices of liver function. The eosinophil count was measured to reflect any hypersensitivity reaction. The non-Gaussian distribution of these necessitated using appropriate non-parametric tests together with parametric tests on logarithmic transformed data. In addition a quantal method was used to measure the frequency of patients showing an "abnormal" increase in enzyme concentrations.

Unexplained hepatitis in some patients following halothane anaesthesia resulted in the setting up of the National Halothane Study in the United States in 1963. This retrospective survey of more than 850,000 anaesthetics implicated halothane as a cause of massive hepatic necrosis in less than 1 in 10,000 administrations (Bunker et al., 1969). Subsequently, various workers (Muslim, Rosen and Jones, 1971; Sharpstone, Medley and Williams, 1971; Inman and Mushin, 1974) incriminated repeated exposure to halothane and reported that there was an increase in the likelihood of liver dysfunction as the interval between successive halothane anaesthetics decreased.

"Halothane hepatitis" is characterized by the appearance of fever up to 2 weeks after anaesthesia, followed by symptoms resembling viral hepatitis. Often eosinophilia is found, which is in keeping with the view that the liver damage may be the result of a delayed hypersensitivity response to halothane or one of its metabolites (Moult and Sherlock, 1975; Walton et al., 1976).

However, controversy continues about a direct relationship between halothane and liver damage after operation as it has not been shown conclusively that halothane is implicated to a greater extent than any other anaesthetic agent (Simpson, Strunin and Walton, 1973; Conn, 1974; Dykes, 1977).

In 1975 the Medical Research Council set up a working party on "The effect of repeated exposure to anaesthetics" and later expressed willingness to finance studies on the influence of repeated anaesthetics on liver function. They supported partially a large prospective study, organized in Belfast. This paper reports on the methodology of this study, which has been in progress since early 1976, and discusses the problems involved and the solutions which have been suggested. The selection of suitable patients, the choice of anaesthetic technique, the biochemical monitoring of liver function and the recording of data and its analysis are discussed. In addition, the staffing of the study and the costs involved are worthy of note. Although there is some overlap between these various points, each will be considered separately.

OBJECTIVES

The study was concerned with the total effects of the anaesthetic agents and surgical procedures on the patients as reflected by morbidity and mortality during and after operation. In addition to clinical assessment it involved biochemical monitoring to
detect any deleterious effects on hepatic function. It was hoped that the data obtained would allow a comparison between halothane and other agents, with particular reference to the problems of repeated exposure.

The study was designed to answer the following questions:

1. What is the extent of the relationship between the repeated administration of halothane and other anaesthetic agents and the subsequent development of liver dysfunction?

2. What groups of patients are especially liable to develop liver damage after halothane anaesthesia?

3. How does the safety of repeated anaesthesia with halothane compare with that of other anaesthetic agents?

In the initial programme the other agents to be considered included trichloroethylene and methoxyflurane, but the use of these has diminished markedly with the availability of enfurane which became eventually the main alternative anaesthetic agent.

CHOICE OF PATIENTS

The greatest problem was obtaining access to a distinct, easily identified group of patients who have a reasonable chance of receiving another anaesthetic within a period of less than 6 months. They must be relatively free from sepsis and not receiving therapy which would adversely affect liver function. The operation must not be major abdominal or thoracic and blood transfusion must not be necessary; one must be able to vary the form of anaesthetic safely at will. Initially it was thought that patients presenting for minor urological procedures, burns and plastic surgery and orthopaedic operations would be particularly suitable. In practice, however, the last two groups were not available in sufficient numbers and the study eventually centred on patients presenting for endoscopic examination of the bladder and urethra, for urethral dilatation and for cervical implantation of radium. These include a large proportion of outpatients which made time-consuming domiciliary visits necessary and required a greater degree of patient co-operation than with inpatients. In addition there was less opportunity for clinical assessment of the patient than had been hoped for originally.

Organization of this study required very close co-operation with surgical and other colleagues. Hospital lists had to be scrutinized for suitable patients and the necessary arrangements made. Each patient was given an explanatory letter with a contact telephone number (fig. 1), and a card setting out details of the expected home visits.

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As you know we are studying patients who are having several anaesthetics. We are grateful for your help with this. If you should need an anaesthetic, e.g. for appendix, hernia, tooth extraction etc., within the next six months please contact DR. MORPHEUS at the above telephone number.

Date: ______________________

Fig. 1. Explanatory letter to patients.
Even with this selection it was found that a high percentage of patients admitted did not have a repeat anaesthetic within 6 months. In 1977, 103 new patients were admitted to the study, of which 57 had no repeat anaesthetic within 6 months. During this period a total of 760 biochemical and clinical assessments were made. Some patients who had a further anaesthetic at another hospital, unknown to the study team, were lost from the study. Others changed address, went on holiday or had returned to work after the operation and so were unable to be seen on the required days after operation. Two patients died, although this was not associated with their operations and neither had a repeat anaesthetic.

A decision was taken not to include any new patients when the study had been fully established for a period of 2 years. Those patients presenting for repeat anaesthetics only were followed for a further 6 months. The total number of patients having one or more of the two anaesthetic agents is given in table I.

**TABLE I.** Total numbers of patients having one or more of the two principal anaesthetic agents

<table>
<thead>
<tr>
<th>Halothane administrations</th>
<th>Enflurane administrations</th>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
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<tr>
<td>2</td>
<td>2</td>
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<tr>
<td>3</td>
<td>3</td>
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<tr>
<td>4</td>
<td>4</td>
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<tr>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

### ALLOCATION OF PATIENTS

The study started initially with halothane and over the 1st year all new patients were given this as their first anaesthetic. It was proposed to follow four sequences for patients having repeat anaesthetics (H = halothane; E = enfurane):

- Sequence A (H) H H H
- Sequence B (H) H E E
- Sequence C E E E
- Sequence D E E H H

In practice, the shortage of patients having up to four anaesthetics limited the study to the first three sequences. Also included are a number of patients who had an exposure to halothane (H) in the 6 months before admission to the study. These gave the following additional groups:

1 2 3
Sequence E (H) H H H
Sequence F (H) H H E
Sequence G (H) H E E
Sequence H (H) E E E

### ANAESTHESIA

Patients presenting for anaesthesia were premedicated, at the discretion of the anaesthetist, with atropine, diazepam or pethidine, alone or in combination.

Before induction, heart rate and arterial pressure were measured. Anaesthesia, induced with sodium thiopentone 4–5 mg kg\(^{-1}\), was maintained with nitrous oxide 6 litre min\(^{-1}\) in oxygen 3 litre min\(^{-1}\) plus either halothane or enfurane as required. A semi-closed circuit with face mask was used in all patients, with a separate anaesthetic machine and tubing being used for each agent.

Following the anaesthetic, patients were taken to the recovery ward and, if outpatients, accompanied home later the same day.

### RECORDING OF DATA

Information was obtained relating to the patient's age, sex, A.S.A. grading of physical status, weight and body build. Past medical and surgical history and any recent or present drug therapy were noted also. Any complication before operation was assessed as mild, moderate or severe, depending on its effect on the system involved. The main intraoperative agent and any premedication, supplementary anaesthetic agent or neuromuscular blocking drugs used were recorded, as were details of any i.v. fluid therapy.

The site of surgery and the total anaesthetic time were noted. Any intraoperative anaesthetic complications were assessed as having a mild, moderate or severe effect on the system involved and the patient's condition on leaving the operating theatre was noted. The findings were recorded on four computer-compatible charts, one of which is shown in figure 2. The chart relating to the complications and condition of the patients after operation is shown in figure 3.

### BIOCHEMICAL MONITORING OF LIVER FUNCTION

The problems which arose here concerned which tests to carry out and the time of blood sampling in relation to anaesthesia. With regard to the former, a serum "block analysis" was available at the Department of Biochemistry, the Royal Victoria Hospital, Belfast,
Fig. 2. Computer-compatible patient chart.

Study number Card
[ ] [ ] [ ] [ ] [ ] [ ] [ ]

Postoperative complications: 1 Yes, 2 No, 9 Not Known

<table>
<thead>
<tr>
<th>4th day</th>
<th>15th day</th>
<th>4th day</th>
<th>15th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Usual sleep</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Usual appetite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Feel well</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Feel awful</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Nausea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Vomiting</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>7. Diarrhoea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Constipation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Confused/disoriented</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Unconscious</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Anaemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14. Oliguria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. Infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. Cough</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17. Spit</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Fig. 3. Chart for recording complications after operation.
Fig. 4. Summary chart of biochemical and clinical data. A.P. = alkaline phosphatase; Chol. = serum cholinesterase.

using Technicon SMAC (Sequential Multiple Analyser with Computer). This gave readings for the concentrations of sodium, potassium, chloride, bicarbonate, urea, total protein, albumin, calcium, glucose, cholesterol, urate, creatinine, iron, triglycerides and total bilirubin. In addition, the serum concentrations of the following were available: alkaline phosphatase (ALP), lactate dehydrogenase (LDH), aspartate aminotransferase (AST or SGOT), alanine aminotransferase (ALT or SGPT) and creatine kinase (CPK). It was possible also to estimate serum cholinesterase (CHS) (Michel, 1949) and gamma glutamyl transferase (GGT) (Szasz, 1969). The Department of Haematology at the Royal Victoria Hospital provided a full haematological examination which included an eosinophil count.
Six enzymes were chosen specifically as indices of liver function. Serum alkaline phosphatase (ALP) reflects both damage to liver cells and biliary obstruction. Serum lactate dehydrogenase (LDH) is widely distributed throughout the body, with high concentrations in the heart, skeletal muscle, liver, kidney, brain and erythrocytes and consequently is increased in a wide variety of disease states. Serum aspartate aminotransferase (AST or SGOT) concentrations become increased in damage to the liver as well as skeletal and cardiac muscle. Serum alanine aminotransferase (ALT or SGPT) concentrations are more specifically related to liver cell damage than those of AST. Gamma glutamyl transferase (GGT) concentrations in serum originate primarily from the hepatobiliary system and are increased in all forms of liver damage. Serum cholinesterase (CHS) reflects liver cell dysfunction and is reduced in most forms of liver disease, especially those affecting protein synthesis. The eosinophil count was measured to reflect any hypersensitivity reaction.

Changes in the concentrations of the enzymes are known to be at a maximum within 3–4 days after any hepatic insult and should have decreased to their pre-operative values within 2 weeks. Furthermore, as the clinical signs of “halothane hepatitis” are reported to be evident within 2 weeks after anaesthesia, it was decided that blood samples should be drawn before operation and on days 3–4 and 13–15 after operation. As far as possible these were taken at the same time each day, to eliminate changes which may have been attributable to diurnal variation. On each of the visits after operation a further clinical assessment of the patient’s condition was made.

When the study had been in progress for about 1 year, the paucity of positive enzymatic evidence led us to doubt that the chosen enzyme tests were sufficiently sensitive to detect changes in liver function. However, concurrent studies in this department on the effect of ketamine infusions showed a sufficiently great frequency of changes in the enzyme concentrations (Dundee et al., 1980) to re-establish our confidence in the method.

IMMUNOLOGY
Walton and others (1976) have found a greater frequency of liver, kidney, microsomal and thyroid antibodies in association with unexplained hepatitis following halothane anaesthesia. As part of the study, a portion of each serum sample was stored at $-20\,^\circ\mathrm{C}$ and detection and titration of auto-antibodies to smooth muscle, mitochondria, reticulum, gastric parietal cells, nuclei, thyroid colloid and thyroid microsomes were made using the indirect immunofluorescent technique. These were tested for in the three immunoglobulin classes IgG, IgA and IgM, at the Department of Immunology, Belfast City Hospital, and the results compared with base-line data from approximately 600 healthy persons of different ages and sexes.
ANALYSIS OF DATA
A summary chart of the biochemical and clinical data was prepared (fig. 4) and this formed the basis for the initial statistical analysis.

The distribution of enzyme concentrations in the overall population did not follow a Gaussian pattern, as is illustrated by the preoperative ALT values from the patients in the study (fig. 5). The statistical handling of the data was by two different approaches.

The first of these used a quantal method to measure the frequency of patients showing an "abnormal" increase in enzyme concentrations from the value before anaesthesia. The criterion for "abnormality" was that the value after anaesthesia would have changed by more than three standard deviations of the method of analysis from the value before anaesthesia. The standard deviation of the method of analysis was calculated from the coefficient of variation of several thousand repeat estimations on "control" sera over the period of the study and is expressed at the upper limit of the "normal" range. The "normal" enzyme ranges and corresponding standard deviations are as shown (table II). Chi-squared tests were carried out using this criterion.

The second method was an analysis of the enzyme concentrations before and after anaesthesia using appropriate parametric and non-parametric tests. Where applicable, the skew distribution of the enzyme concentrations was corrected by logarithmic transformation and analysis of variance and Student’s paired t test used. Various non-parametric tests, including the Wilcoxon T test and the Mann-Whitney U test, were used on the non-transformed data.

Initially, the repeat halothane anaesthetics and the repeat enflurane anaesthetics were dealt with separately, and any statistically significant findings noted. The clinical sub-groups in each series were then analysed to see if there was any specific group of patients more prone to enzymatic changes than the overall study population. Those groups of patients having had a particular sequence of anaesthetics as outlined above were then considered. These were suitable for within-group analysis by the various statistical methods.

STAFF
This study required the continuous services of three full-time senior registrar anaesthetists and a biochemist as well as additional assistance from the staff of the University Department of Anaesthesia. This was in addition to laboratory, computer and secretarial personnel. It was under the supervision of two consultant anaesthetists, a consultant biochemist and a consultant immunologist.

TABLE II. "Normal" enzyme concentration ranges and corresponding standard deviations of the method of estimation

<table>
<thead>
<tr>
<th>Liver function test</th>
<th>&quot;Normal&quot; range</th>
<th>SD</th>
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<tbody>
<tr>
<td>ALP</td>
<td>35-150 u. litre⁻¹</td>
<td>7 u. litre⁻¹</td>
</tr>
<tr>
<td>LDH</td>
<td>130-270 u. litre⁻¹</td>
<td>14 u. litre⁻¹</td>
</tr>
<tr>
<td>AST</td>
<td>10-40 u. litre⁻¹</td>
<td>7 u. litre⁻¹</td>
</tr>
<tr>
<td>ALT</td>
<td>10-45 u. litre⁻¹</td>
<td>7 u. litre⁻¹</td>
</tr>
<tr>
<td>GGT</td>
<td>5-34 u. litre⁻¹</td>
<td>4 u. litre⁻¹</td>
</tr>
<tr>
<td>CHS</td>
<td>50-110 Michel units</td>
<td>6 Michel units</td>
</tr>
</tbody>
</table>

REFERENCES
METHODOLOGIE D'UNE ETUDE SUR LES CHANGEMENTS SURVENANT DANS LES ENZYMES DU FOIE APRES L'ADMINISTRATION REPETEE D'AGENTS ANESTHESIANTS

RESUME

Cet article contient un rapport sur la méthodologie adoptée lors d'une importante étude sur l'influence de l'administration répétée d'agents anesthésiants sur la fonction hépatique, ainsi qu'une discussion sur les problèmes en cause. Les malades les plus appropriés sont ceux qui se présentent pour un examen endoscopique de la vessie et de l'urètre, pour une dilatation de l'urètre ou pour l'implantation cervicale de radium. On a pris des échantillons de sang immédiatement avant l'induction de l'anesthésie, les 3-4ème et 13-15ème jours après l'opération, moments où l'on a aussi procédé à une évaluation clinique du malade. On a choisi spécifiquement la mesure des concentrations de six enzymes pour servir d'indices de la fonction hépatique (lactate déshydrogénase, alcaline phosphatase, aspartate aminotransférase, alanine aminotransférase, cholinestérase du sérum et gamma glutamyl transférase). La numération des éosinophiles a été faite de manière à refléter toute réaction d'hypersensibilité. Leur répartition non conforme à la courbe de Gauss a nécessité l'usage de tests non paramétriques appropriés, et de tests paramétriques sur des données logarithmiques transformées. De plus on s'est servi d'une méthode de quantum pour mesurer la fréquence des malades accusant une augmentation "anormale" des concentrations d'enzymes.

BLAULIESTE EINER PROSPEKTIVEN STUDIE VON LEBERENZYMVERÄNDERUNGEN AUF WIEDERHOLT VERABREICHTE NARKOSEMITTEL

ZUSAMMENFASSUNG


METODOLOGIA DE UN ESTUDIO EXPLORATORIO DE LAS ALTERACIONES DE LA ENZIMA HEPATICA A RAIZ DE ADMINISTRACIONES REPETIDAS DE ANESTETICOS

SUMARIO

Se da una reseña de la metodología usada en un amplio estudio exploratorio de la influencia de repetidas administraciones de anestésicos sobre la función hepática y se discuten los problemas que plantean. Los pacientes más adecuados eran los que se sometían a un examen endoscópico de la vejiga y de la uretra, a una dilatación de la uretra y a una implantación cervical de radio. Se tomaron muestras de sangre justo antes de la inducción de la anestesia y en los días 3-4 y 13-15 después de la intervención, cuando se procedió también a una evaluación clínica del paciente. Las concentraciones de seis enzimas (dehidrogenasa de lactato, fosfatasa alcalina, aminotransferasa de aspartato, aminotransferasa de alanina, colinesterasa serosa y transferasa de glutamil gamma) fueron escogidas especialmente como índices de la función hepática. Se midió la numeración eosinófila para reflejar cualquier reacción de hipersensibilidad. La distribución no-Gaussiana de las mismas hizo necesario el uso de pruebas no-paramétricas apropiadas, junto con pruebas paramétricas en datos trasbados a logaritmos. Además, se usó un método de tanteo para medir la frecuencia de un aumento "anormal" de las concentraciones enzimáticas en los pacientes.