GLUTATHIONE S-TRANSFERASE MEASUREMENT AS AN INDEX OF LIVER DYSFUNCTION AFTER GENERAL ANAESTHESIA WITH A VOLATILE AGENT IN MAN

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CONTENTS

DECLARATION

This thesis has been composed by myself. The first two chapters are based on personal surveys of the literature, and the rest of the thesis is composed of clinical studies performed with a group of colleagues in the Departments of Anaesthetics and Clinical Chemistry, Royal Infirmary of Edinburgh.

I was the principal investigator in the studies of the effect of nicardipine and cimetidine on plasma GST concentration; in the remaining two studies I shared the responsibility and duties of the principal investigator with Dr Louise Aldridge who has kindly allowed me to include the results of those studies in this thesis.

ABSTRACT

On rare occasions, halothane anaesthesia is associated with liver damage. Two forms of toxicity are now recognised - a mild form manifest by increased serum liver enzymes, and a rarer, fulminant form which may be fatal. The historical background, clinical features, morphology and epidemiology of halothane hepatitis are presented, and animal models of halothane hepatotoxicity are described; current perspectives on its aetiology are reviewed.

The application of serum enzymes to the recognition of acute liver injury is reviewed, and the limitations of those in current use are described. The measurement of glutathione S-transferase (GST) in plasma by specific radioimmunoassay offers potential for the early detection of drug-induced, hepatocellular damage. The structure, classification, distribution and techniques for measurement of GST are presented, and experience with their use in various liver disorders is explored. The influence of general anaesthesia on several liver enzymes is examined, and the advantages of GST over conventional liver enzymes are discussed.

The effects of halothane, enflurane and isoflurane anaesthesia on plasma GST concentration after operation are reported. GST concentration increased 3-6 hours after anaesthesia with halothane and enflurane, and more

marked increases were observed at 24 hours in some of the patients who received these agents: GST did not increase at any time in patients who received isoflurane. The incidence of abnormal GST concentrations after anaesthesia with halothane, enflurane and isoflurane directly correlated with the incidence of reported clinical hepatic dysfunction for these drugs.

Three of the proposed aetiologies of halothane hepatitis, namely altered hepatocellular calcium balance, toxic products of biotransformation and hepatic hypoxia, were investigated subsequently in more detail. Perioperative administration of the calcium channel blocker nicardipine did not influence GST release after halothane anaesthesia. Cimetidine, which reduces halothane metabolism, did not influence GST release either. In patients undergoing artificial ventilation, a greater increase in GST concentration occurred at 1 hour after anaesthesia compared with the change that occurred in patients who breathed spontaneously.

These studies suggest that a different mechanism is responsible for the two phases of toxicity recognised in the 24 hours after anaesthesia: an early phase up to 6 hours may result from hepatic hypoxia or altered calcium balance, while toxic products of metabolism may produce a secondary phase at 24 hours. This supports the proposal that the aetiology of the mild form of halothane-induced liver damage is multifactorial.

Finally, recommendations for the safe administration of halothane are proposed.

CHAPTER 1

HALOTHANE HEPATITIS - A REVIEW

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HISTORICAL BACKGROUND

Liver dysfunction following exposure to volatile halogenated anaesthetics has long been established. The first recorded case of unexplained jaundice following chloroform anaesthesia occurred in 1848 [Defalque 1968], only one year after its introduction into clinical practice by Simpson. Halothane was introduced for clinical use in 1956 and was hailed immediately as a great advance in anaesthesia [Johnstone 1956]. Its advantages of potency, lack of flammability and general smoothness of administration led rapidly to widespread use. Initial animal studies in rats [Krantz et al 1958], dogs [Raventós 1958; Virtue et al 1958] and monkeys [Stephen et al 1958] indicated minimal or no hepatotoxicity, and in particular no hepatic necrosis was observed after halothane compared to chloroform or divinyl ether [Jones, Margolis & Stephen 1958].

Early Reports

The early clinical accounts on the use of halothane in man supported the animal studies; no liver necrosis was recorded [Robson & Sheridan 1957; Little, Barbour & Given 1958]. Reports of postoperative liver necrosis after the use of halothane began to appear in 1958 [Virtue & Payne 1958; Burnap, Galla & Vandam 1958] and further

anecdotal reports followed [Temple, Cote & Gorens 1962; Bunker & Blumenfeld 1963; Lindenbaum & Leifer 1963; Brody & Sweet 1963]. By 1963, five years after the widespread international use of halothane, at least 350 cases of "halothane hepatitis" had been recorded [Brown & Gandolfi 1987].

Retrospective Studies in Man

The general concern precipitated a number of retrospective surveys of hepatic dysfunction following general anaesthesia [Slater et al 1964; Henderson & Gordon 1964; Mushin et al 1964; Allen & Metcalf 1964; Dykes et al 1965; Trey et al 1968; Klion, Schaffner & Popper 1968; Peters et al 1969]. These studies were conducted in different geographical locations and reviewed approximately 230,000 general anaesthetics of which about 50% had included halothane. When compared to other general anaesthetic agents there was no evidence that halothane had caused an increased incidence of postoperative liver damage. These reports led to the National Halothane Study which reviewed cases of fatal hepatic necrosis occurring within 6 weeks of anaesthesia in 34 hospital centres in the United States of America [National Halothane Study 1969]. Over the 4 year period of the study, 82 cases of fatal hepatic necrosis were collected from some 856,000 administrations of general anaesthesia, of which about 255,000 involved halothane. In all but nine of these cases the necrosis could be assigned to a recognisable clinical factor. Seven of

these patients with unexplained liver necrosis had received halothane, and four of the seven had received halothane on more than one occasion within the previous 6 weeks. Thus the incidence of fatal hepatic necrosis following halothane anaesthesia in this study was approximately 1 in 35,000.

The result of this massive study published in 1969 was unfortunately totally inconclusive. The intention had been to compare the incidence of fatal, massive hepatic necrosis within six weeks of halothane anaesthesia with that of other general anaesthetics. In this respect halothane compared favourably; cyclopropane was associated with the greatest overall estimated necrosis rate (1.7 per 10,000 anaesthetics compared with 1.02 per 10,000 anaesthetics for halothane). Furthermore, no patient who developed jaundice after halothane and died after a second administration, was found at post mortem to have suffered massive or intermediate hepatic necrosis. Weaknesses in the study design may have prejudiced the results: the study was retrospective, although the original intention had been to establish a prospective survey, and reporting bias might have occurred, as some of the institutions involved had already published anecdotal case reports of liver dysfunction associated with halothane. In addition, there was no information available on post mortem examination of the abdominal viscera in 40% of the deaths considered in the study. It is also noteworthy that

differences in death rates between institutions (lowest 0.27%, highest 6.41%) were greater than differences between anaesthetics, even after adjusting the rates for differences in operating patterns, and the age and physical status of the institutional cohorts. The main conclusions which emerged from this study were that massive hepatic necrosis after anaesthesia was a rare occurrence (approximately 1 in 10,000), although an association with halothane could neither be confirmed nor excluded, and that halothane had a good overall record of safety, including use in operations involving the gall bladder and biliary tract.

Other retrospective studies confirmed that halothane was associated with severe liver dysfunction, with an incidence ranging from 1 in 6,000 to 1 in 20,000 [Inman & Mushin 1974; Böttiger, Dalén & Hallén 1976]. In addition, it became apparent that there was a greater problem with repeated exposure to halothane, especially at short intervals [Moult & Sherlock 1975; Inman & Mushin 1974; Inman & Mushin 1978; Böttiger, Dalén & Hallén 1976]. In a comprehensive review, Walton and his colleagues (1976) described 203 patients with jaundice following anaesthesia in the United Kingdom. Full clinical and biochemical details of these cases were reviewed blindly by a panel of hepatologists, who considered that the hepatitis could not be attributed to an identifiable cause in 76 patients who had received halothane. Of these, 95% had received multiple

halothane anaesthetics; in 55% of cases, the repeat exposure was within 4 weeks. Also noted was an increased incidence in women, middle-aged and obese patients, and a high incidence of thyroid antibodies was found in patients whose jaundice was not related to any identifiable cause. However, as it was not possible to distinguish the clinical or biochemical course of hepatitis in these patients from that in viral hepatitis, the authors stressed that they did not attribute all of these 76 cases to halothane hepatotoxicity.

Prospective Studies in Man

Three small, but well-controlled, prospective studies of changes in liver biochemical tests after anaesthesia and minor surgery have been reported from Britain [Wright et al 1975; Trowell, Peto & Smith 1975; Fee et al 1979]. The former showed that increased serum activities of aspartate aminotransferase (AST) occurred in 15 of 76 patients who received multiple halothane anaesthetics compared with 4 of 76 patients who received trichloroethylene. Trowell, Peto and Smith (1975) found the serum activity of alanine aminotransferase (ALT) to be greater than 100 U l^{-1} in 4 out of 18 patients receiving halothane, two of whom displayed hepatocellular necrosis on liver biopsy. None of the 21 patients who received repeated non-halothane anaesthesia showed similar increases. The choice of anaesthetic technique, however, did not influence the number of patients who developed serum activities of ALT which exceeded the

upper limit of the reference range (9/18 halothane, 6/21 non-halothane).

Fee and colleagues (1979) analysed data from 129 patients who underwent minor urological operations, 63 of whom received two or more administrations of halothane, and the remainder two or more enflurane anaesthetics. Although no patient in this study showed overt signs of liver dysfunction, more liver enzyme abnormalities occurred after multiple exposures to halothane than after repeated enflurane anaesthesia. This difference between the groups disappeared when obese patients or those exposed to anaesthesia in the preceding 6 weeks were excluded. In spite of its detailed and expensive plan, this study suffered from two fundamental limitations; the number of patients available was too small to draw firm conclusions, and the interval between anaesthetics was very variable and often more than 4 weeks. This meant that no conclusion could be drawn on the validity or otherwise of the recommended minimum interval of 28 days between halothane anaesthetics which pertained at that time.

To add to the confusion, other workers had published reports which disagreed about the effect of multiple halothane anaesthetics on biochemical liver tests. McEwan (1976) found a smaller frequency of disturbance of ALT and AST in patients after repeated halothane anaesthesia than in patients receiving non-halothane

anaesthesia after previous halothane, while Allen and Downing (1977) who investigated the effect of multiple anaesthetics with either halothane or enflurane in South African black women, found only minimal increases in serum aminotransferases, although the gap between re-exposures was considerably longer than 6 weeks.

In a more recent Danish study of postoperative complications, Zaric and colleagues (1986) encountered four cases of presumed halothane hepatitis in 2609 consecutive surgical patients, of whom 1166 were anaesthetised with halothane; no case of hepatitis was reported among the other patients who had received enflurane, regional or intravenous anaesthesia. All four implicated patients were obese, middle-aged females who underwent non-abdominal surgery, and three of the four had received halothane previously.

Most of these prospective studies, however, have described only altered liver biochemical tests and not the full clinical syndrome of halothane hepatitis.

EPIDEMIOLOGY

Several possible risk factors for the development of halothane hepatitis have become apparent on the basis of reported cases (Table 1.1).

Table 1.1

Possible risk factors for the development of halothane hepatitis

Multiple exposure
Middle age
Obesity
Female gender
Genetic predisposition
Enzyme induction

Multiple exposure

The risk of halothane hepatitis is increased greatly when repeated halothane anaesthetics are given over a short period [Inman & Mushin 1974; Inman & Mushin 1978; Moult & Sherlock 1975; Böttiger, Dalén & Hallén 1976]. More than 80% of patients who develop hepatic necrosis after halothane have been exposed previously to the agent [Touloukian & Kaplowitz 1981; Walton et al 1976], and up to two-thirds of patients who develop fulminant hepatic failure after halothane may have had a documented adverse reaction to halothane, such as postoperative pyrexia, nausea and jaundice [Neuberger & Williams 1984]. Inman and Mushin (1978) reviewed 251 cases of alleged halothane hepatotoxicity reported to the Committee on Safety of Medicines. Of these, 82% involved multiple exposure to halothane, and 75% of these patients had been exposed

more than once within 28 days. Irrespective of the interval, multiple exposure was associated with a higher mortality (51%) than was single exposure (35%). Although the incidence of dysfunction appeared to be maximal when the penultimate exposure was within 28 days, cases have been reported when the penultimate exposure was several years previously [Inman & Mushin 1974; Neuberger & Williams 1984]. The shorter the interval between exposures, the more severe is the hepatic dysfunction and the shorter is the latency before presentation [Moult & Sherlock 1975; Inman & Mushin 1978; Neuberger & Williams 1984].

Middle age

Middle-aged patients have a greater propensity to develop liver damage than the young or elderly [Carney & Van Dyke 1972; Neuberger & Williams 1984; Cousins, Plummer & Hall 1989]; 70% of cases occur in patients older than 40 years, and the peak incidence occurs between 50 and 60 years. In comparison with the age distribution of patients undergoing anaesthesia, patients with halothane-induced liver disease are slightly older [Böttiger, Dalén & Hallén 1976; Carney & Van Dyke 1972; Neuberger & Williams 1984]. Severe hepatic dysfunction after halothane anaesthesia in children was not considered previously to be a hazard, but an increasing number of reports indicate that it can occur [Wark 1983; Warner et al 1984; Whitburn & Sumner 1986; Wark, O'Halloran & Overton 1986; Kenna et al 1987]. The

incidence in children is, however, considerably smaller than in adults (between 1 in 82,000 [Wark 1983] and 1 in 200,000 [Warner et al 1984]). Despite its occurrence, halothane is still particularly indicated for, even repeated, anaesthesia in children [Walton 1986; Weis & Engelhardt 1989].

Obesity

Hepatic dysfunction is more common in obese than in non-obese patients [Peters et al 1969; Carney & Van Dyke 1972; Walton et al 1976]. As halothane accumulates in adipose tisue, this could delay its excretion and, theoretically, prolong exposure to potentially reactive halothane metabolites, resulting in increased risk in obese patients. In addition, obese patients metabolise halothane more extensively than do non-obese patients [Bentley et al 1982], which might further predispose them to liver injury.

Female gender

Although there is no difference in exposure to halothane between the two sexes [Carney & Van Dyke 1972], females are at approximately double the risk than males of developing halothane hepatitis [Neuberger & Williams 1984; Benjamin et al 1985], although male patients apparently have a worse prognosis [Moult & Sherlock 1975; Walton et al 1976; Böttiger, Dalén & Hallén 1976]. In a rat model of halothane hepatotoxicity, however, halothane is less hepatotoxic in females than in males [Plummer et

al 1985; Jee et al 1980]. Recently, Jenner, Plummer and Cousins (1990) have shown that reductive biotransformation of halothane was more extensive in females than in males undergoing routine anaesthesia, which might explain the association between female gender and halothane hepatotoxicity.

Genetics and ethnic origin

Susceptibility to halothane hepatitis may, to a certain extent, be heritable. Hoft and colleagues (1981) have reported cases of halothane hepatitis in three pairs of closely related women of Mexican-Indian or Mexican-Spanish descent. In addition, Farrell and coworkers (1985) have suggested that a familial susceptibility factor may exist. Furthermore, patients with unexplained hepatic failure after halothane are reported to have different human lymphocyte antigen (HLA) frequencies to healthy controls [Otsuka et al 1985], providing additional evidence of genetic predisposition.

Enzyme induction

The association between hepatotoxicity and repeated exposure to halothane might be explained if halothane anaesthesia itself induced drug metabolising enzymes. Using antipyrine half-life or clearance as an index of drug metabolising capacity, halothane has been shown to cause microsomal enzyme induction in both adults [Nimmo, Thompson & Prescott 1981] and children [Haxholdt et al 1986]. Further support for this hypothesis is provided

in a retrospective analysis of 279 patients who underwent brain surgery during halothane anaesthesia [Nomura et al 1986]. The incidence of halothane-induced liver injury was greater in subjects treated with phenobarbitone before surgery (7/100) than in those not taking enzyme-inducing medication (1/179).

Other factors such as type of surgery and the presence of pre-existing liver disease might also be expected to influence the development of liver necrosis after anaesthesia. However, no correlation between hepatotoxicity and duration, site or severity of surgery (including hepatobiliary procedures) has been shown in man [Benjamin et al 1985], and in fact halothane hepatitis may follow apparently uneventful anaesthesia for minor surgery [Neuberger & Williams 1984]. Similarly, there is no convincing evidence that patients with pre-existing, compensated liver disease are at any greater risk of developing halothane hepatotoxicity than healthy patients [Brown 1985; Cousins et al 1989; Stock & Strunin 1985]. Radiation therapy in association with repeated exposure to halothane was considered previously to increase risk of hepatic damage [Hughes & Powell 1970] but subsequent studies have not confirmed this [Allen & Downing 1977; Wark, Clifton & Bookallil 1979].

Halothane hepatotoxicity is not restricted to patients undergoing anaesthesia. Liver damage following occupational exposure to halothane has been reported in

medical personnel including anaesthetists, surgeons and laboratory workers [Belfrage, Ahlgren & Axelson 1966; Klatskin & Kimberg 1969; Johnston & Mendelsohn 1971; Lund, Skulberg & Helle 1974; Neuberger et al 1981b; Lings 1988]. In two surgeons specific "halothane antibody" was present, and three of these individuals developed hepatitis following deliberate re-exposure to halothane, providing compelling evidence for the existence of halothane hepatotoxicity [Dykes 1977].

CLINICAL FEATURES

Halothane hepatitis is characterised by several consistent clinical features (Table 1.2). Many patients complain initially of malaise, anorexia and nonspecific gastrointestinal symptoms such as nausea and upper abdominal discomfort [Klion, Schaffner & Popper 1969; Moult & Sherlock 1975]. The onset of fever is characteristically the first sign of illness. Klion, Schaffner and Popper (1969) found pyrexia in 37 of 42 patients with presumed halothane hepatotoxicity, but only persistent or recurrent fever was related to the onset of hepatitis. Moult and Sherlock (1975) described a pattern of delayed pyrexia after exposure to halothane in eight patients, and 75% of patients in a series from King's College Hospital, London, developed unexplained fever after operation [Neuberger & Williams 1984]. The mean interval between operation and onset of fever was 7

days after a first exposure, whereas multiple exposures were associated with a reduced latent period of 4 days [Böttiger, Dalén & Hallén 1976]. Not all patients become pyrexial, however [Walton et al 1976], and the value of unexplained postoperative fever as an indicator of sensitisation to halothane has been questioned [Dykes 1971].

Table 1.2

Clinical features of halothane hepatitis

Nonspecific GI upset
Delayed pyrexia
Jaundice
Eosinophilia
Serum autoantibodies
Nonspecific rash
Arthralgia

Jaundice occurs almost invariably. Many studies have shown that its appearance is delayed until the 5th or 6th day after exposure [Moult & Sherlock 1975; Inman & Mushin 1978; Neuberger & Williams 1984], although rarely it can be delayed by up to 4 weeks [Klion, Schaffner & Popper 1969]. As with fever, multiple exposures to halothane are associated with a shorter delay in

appearance of jaundice than that after a single exposure. In a series of 251 cases reported to the Committee on Safety of Medicines, patients became icteric after a mean delay of 11 days following a single exposure and about 4 to 6 days in instances of multiple exposures [Inman & Mushin 1978].

Eosinophilia is occasionally present, its incidence ranging from 8% to 32% [Klion, Schaffner & Popper 1969; Moult & Sherlock 1975; Walton et al 1976; Böttiger, Dalén & Hallén 1976]. Serum autoantibodies are often found during the course of liver failure. Walton and colleagues (1976) found liver kidney microsomal antibodies in 19 of 76 patients with unexplained jaundice after halothane, with a similarly high incidence of thyroid antibodies. Neuberger and Williams (1984) identified serum autoantibodies (antiliver kidney microsomal, antismooth muscle, and antinuclear) in 44% of their patients. A small number of patients may develop a nonspecific rash, or arthralgia [Walton et al 1976].

Biochemical tests of liver function generally reflect changes which are typical of hepatocellular damage. Serum aminotransferase activities are frequently grossly increased (500 to 2000 U l^{-1}) [Klion, Schaffner & Popper; Moult & Sherlock 1975; Böttiger, Dalén & Hallén 1976], although the degree of increase can vary widely [Walton et al 1976]. Alkaline phosphatase activity, although increased, is generally less than twice the upper limit

of normal [Walton et al 1976]. Clinically insignificant disturbances in liver biochemical tests are common following halothane, and, to a lesser extent, after enflurane. These alterations are not related to acute necrosis in an obvious spectrum, and have not been shown to have important sequelae [Fee et al 1979].

PATHOLOGY

Light microscopy

The most notable histological feature of halothane hepatitis is centrilobular necrosis [Slater et al 1964; Blackburn, Ngai & Lindenbaum 1964; Klion, Schaffner & Popper 1969; Schatzki, Kay & McGavic 1973; Wills & Walton 1978; Uzunalimoglu, Yardley & Boitnott 1970; Benjamin et al 1985]. A spectrum of severity exists from panlobular and multifocal spotty necrosis through to massive necrosis. There is associated ballooning degeneration of hepatocytes with subsequent inflammatory infiltrate, collapse and stromal fibrosis. Fatty infiltration has been noted also by several investigators [Lindenbaum & Leifer 1963; Slater et al 1964; Blackburn, Ngai & Lindenbaum 1964; Klion, Schaffner & Popper 1969; Benjamin et al 1985]. Granulomatous aggregates are occasionally seen [Benjamin et al 1985]. As many of these features occur in viral hepatitis, it can be difficult to distinguish between the two conditions on light microscopy, although a number of authors consider that

such distinction can be made [Peters et al 1969; Klion, Schaffner & Popper 1969; Uzunalimoglu, Yardley & Boitnott 1970; Benjamin et al 1985].

Electron microscopy

Klion, Schaffner and Popper (1969) described mitochondrial membrane abnormalities as the most prominent feature seen on electron microscopy in halothane hepatitis. Although these findings were confirmed by Uzunalimoglu, Yardley and Boitnott (1970), other workers have noted only very mild or less frequent changes [Wills & Walton 1978; Schatzki, Kay & McGavic 1973]. More recently, Goldfarb and colleagues (1989) used electron microscopy to examine liver biopsy specimens taken during anaesthesia with halothane, isoflurane or droperidol. No mitochondrial abnormalities were seen, but significantly more lysosomes were found in the hepatocytes of patients receiving halothane than in those receiving isoflurane or droperidol. It appears that halothane can induce ultrastructural abnormalities very early after the beginning of its administration, while under the same conditions, isoflurane does not. However, as none of these patients subsequently developed clinically obvious liver damage, the relation of these ultrastructural changes to those seen in halothane hepatitis is not known.

AETIOLOGY

There are classically four major theories proposed to account for halothane hepatotoxicity: toxic products of metabolism, hypersensitivity, regional hepatic hypoxia and genetic predisposition. To these has been added more recently a fifth theory: that of altered hepatocellular calcium homeostasis.

Biotransformation

Inhalation anaesthetic agents have been thought classically to be inert and not to undergo biotransformation in the body. This concept was not challenged until 1964 when Van Dyke, Chenoweth and Van Poznak (1964) demonstrated the metabolism of several volatile anaesthetic agents including halothane. The same group found that halothane metabolism occurred in the microsomal fraction of the liver [Van Dyke & Chenoweth 1965], and was increased by pretreatment with phenobarbitone [Van Dyke 1966]. Halothane biotransformation in man was confirmed by Stier and colleagues (1964), and the extent of this metabolism was found to be approximately 20% [Rehder et al 1967; Cascorbi, Blake & Helrich 1970]. Two main pathways of metabolism have been identified (Figure 1.1). Oxidative metabolism is greater in the presence of high oxygen tensions, while reductive metabolism is favoured by hypoxic conditions and phenobarbitone pretreatment. Both pathways exist in man, and reductive metabolism is

known to occur in patients undergoing routine halothane anaesthesia [Cousins et al 1987a]. Metabolism begins soon after commencing the administration of halothane and continues into the period immediately after anaesthesia [Atallah & Geddes 1973; Gourlay et al 1980].

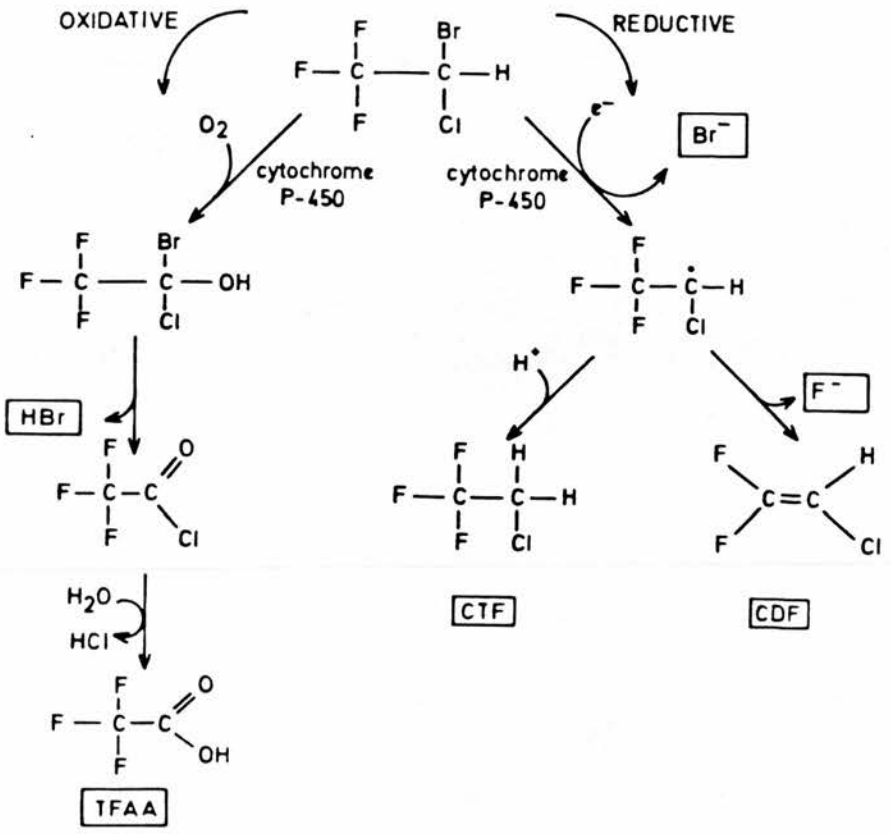

Figure 1.1 Oxidative and reductive pathways of halothane metabolism. Major products of metabolism are enclosed in boxes.

Metabolic products recovered initially in urine were predominantly bromide ion and trifluoroacetic acid (TFAA) [Stier et al 1964; Rehder et al 1967], although later

Cohen and colleagues (1975) demonstrated, in addition, the formation of N-trifluoroacetyl-2-aminoethanol and N-acetyl-S-(2-bromo-2-chloro-1,1-difluoroethyl)-L-cysteine. All but the last of these are considered to be products of oxidative metabolism. In 1977, Mukai and coworkers (1977) identified two new volatile products of reductive metabolism, 2-chloro-1,1,1-trifluoroethane (CTF) and 2-chloro-1,1-difluoroethylene (CDF) in the expired breath of rabbits exposed to halothane. The formation of these products has been confirmed subsequently in humans [Sharp, Trudell & Cohen 1979; Gourlay et al 1980]. Van Dyke and Gandolfi (1976) had found that fluoride ion was cleared from the halothane molecule under reductive biotransformation; serum fluoride, therefore, became a simple measure of reductive metabolism of halothane.

Although none of the end products of either metabolic pathway has been shown to be toxic per se, intermediate products of biotransformation may be responsible for direct hepatic injury; it has been proposed that liver damage is mediated via reactive, free radical intermediates, as occurs with the classical hepatotoxins carbon tetrachloride (CCl_4) and chloroform ($CHCl_3$). Although free radicals are produced during administration of halothane [Plummer et al 1982], there is no direct evidence that they are responsible for halothane hepatotoxicity. Moreover, Brown, Sipes and Sagalyn (1974) found that halothane hepatotoxicity does not

proceed by the same sequence of events as that caused by chloroform, and Cohen and Chance (1990) have demonstrated significant differences in hepatic pathology associated with the two agents. Trifluoroethanol, a precursor of TFAA, has been postulated as a metabolite of halothane [Cohen 1971], although it has not been identified in humans: there is conflicting evidence for its ability to produce hepatic necrosis [Rosenberg & Wahlström 1971; Blake et al 1969].

Using low-temperature autoradiography with labelled halothane [Cohen & Hood 1969], Cohen (1969) demonstrated that non-volatile metabolites were bound covalently to liver macromolecules in mice for at least 2 weeks after halothane exposure, although this could not be correlated with hepatic necrosis as none was seen. Binding occurs in the microsomal fraction of the liver [Van Dyke & Wood 1973] and depends on the transformation of volatile agents by the mixed function oxidase-cytochrome P-450 system [Karashima et al 1977]. The extent of binding is enhanced by inducers of cytochrome P-450, for example phenobarbitone [Van Dyke 1973; Brown & Sipes 1977], and is reduced by enzyme inhibitors, such as metyrapone [Gandolfi et al 1980] and SKF 525A [Van Dyke & Wood 1973]. Covalent binding of metabolites increases under hypoxic conditions [Uehleke, Hellmer & Taberelli-Poplawski 1973], although oxygen is required initially to convert halothane to the intermediate metabolite which is bound [Van Dyke 1973]. Products

from reductive metabolism are therefore thought to have more hepatotoxic potential than oxidative metabolites [Widger, Gandolfi & Van Dyke 1976; Gandolfi et al 1980]. It has been hypothesised that $CF_3C{\cdot}HCl$ free radicals are formed by cytochrome P-450, and that some of these radicals then initiate lipid peroxidation in the endoplasmic reticulum; the damage produced by this process might be responsible ultimately for cell death [de Groot & Noll 1983].

Enflurane and isoflurane undergo considerably less metabolism than halothane during routine anaesthesia (2.4% [Chase et al 1971] and 0.2% [Holaday et al 1975], respectively). Neither drug is subject to reductive metabolism, and neither produces free radicals [Plummer et al 1982]. Because the extent of metabolism of halothane, enflurane and isoflurane is directly related to the incidence of reported clinical hepatic dysfunction for these drugs, it is proposed that products of metabolism play an integral role in the production of halothane-induced liver injury. Furthermore, the increased covalent binding of metabolites under hypoxic conditions, the potential for producing free radicals as a reactive intermediate, and the absence of effect of deuterium substitution of halothane (which reduces oxidative but not reductive metabolism) on the degree of hepatotoxicity in the phenobarbitone-hypoxic rat model [Sipes et al 1980], has focused much attention on reductive halothane metabolism mediating hepatotoxicity.

Immune-mediated

Several features of halothane-induced hepatotoxicity point to hypersensitivity as a mechanism [Editorial 1986; Sherlock 1986]. These include the frequent association with multiple exposures [Inman & Mushin 1974; Inman & Mushin 1978; Moult & Sherlock 1975; Böttiger, Dalén & Hallén 1976], the common history of drug allergy or atopy [Carney & Van Dyke 1972; Walton et al 1976; Zaric et al 1986], the association with fever and eosinophilia [Klion, Schaffner & Popper 1968; Benjamin et al 1985; Touloukian & Kaplowitz 1981], a history of previous adverse reaction to halothane [Neuberger & Williams 1984], provocation of hepatitis by deliberate challenge in previously affected individuals [Belfrage, Ahlgren & Axelson 1966; Klatskin & Kimberg 1969], and the demonstration of circulating autoantibodies [Walton et al 1976; Rodriguez et al 1969; Neuberger & Williams 1984; Sharpstone, Medley & Williams 1971]. Apart from the positive challenge tests, however, none of these features can be considered unique to immune involvement.

Initial studies to detect evidence of hypersensitivity responses in patients with halothane hepatitis produced conflicting results. Paronetto and Popper (1970) demonstrated a positive lymphocyte transformation test in patients with suspected halothane-induced liver disease, but other studies failed to confirm this [Moult et al 1975; Walton et al 1973]. Following the demonstration by Uehleke, Hellmer and Tabarelli-Poplawski (1973) that

halothane metabolites could bind covalently to liver macromolecules, it was postulated that a reactive metabolite of halothane acted as a hapten which generated an immune response. However, Reves and McCracken (1976) could not induce hepatic necrosis in guinea pigs sensitised to a halothane metabolite and challenged subsequently with halothane.

In 1978, in vitro sensitisation of leucocytes to liver homogenates prepared from rabbits exposed previously to halothane was demonstrated in patients with halothane hepatitis [Vergani et al 1978]. Circulating antibodies that bound to the surface membrane of halothane-altered rabbit hepatocytes were detected subsequently in about 75% of patients in whom fulminant hepatic failure occurred after halothane anaesthesia [Kenna, Neuberger & Williams 1984; Vergani et al 1980; Neuberger et al 1983]. These antibodies could not be detected in the serum of normal healthy controls, patients with other types of liver disease, or patients exposed to halothane with no liver damage. The antibodies were of the IgG subclass and not of the IgM subclass, suggesting that sensitisation to halothane-altered liver cell components had occurred following an earlier exposure [Neuberger et al 1983]. The same antibodies were detected to a similar extent (16 of 23 patients) in patients with less severe hepatitis after halothane anaesthesia [Kenna, Neuberger & Williams 1987]. These antibodies have been identified as being cross-reactive with

trifluoroacetyl-halide $CF_3CBrClOH$ (TFA-halide), bound covalently to liver protein (anti-TFA antibody). Five of 6 patients with halothane hepatitis had a high titre of circulating anti-TFA antibody [Hubbard et al 1988b]. This antibody was present as early as 4 days after exposure in one patient, and remained elevated for 3 months after halothane in another patient.

The presence of these antibodies shown by an enzyme-linked immunosorbent assay [Kenna, Neuberger & Williams 1984] has been proposed as a diagnostic marker of halothane-induced liver damage. However, in some patients the antibodies did not appear until 8 weeks after exposure to halothane and it is not known if the antibodies are a cause, or merely arise as a consequence, of hepatic damage [Dienstag 1980]. Furthermore, several problems with the assay have been discovered that were not recognised previously [Martin, Kenna & Pohl 1990], and Spence (1987b) has warned of the limited value of the antibody test in diagnosing halothane hepatitis. A more recent tiered system of laboratory methods may, however, offer greater potential for an accurate diagnosis [Hastings et al 1989].

The theories of biotransformation and immune-mediation are not mutually exclusive. Halothane hepatitis may be initiated by a reactive metabolite that alters the surface structure of hepatocytes; in susceptible individuals these changes may induce an immune response

and subsequent cytotoxicity. In contrast with the biotransformation theory which emphasises reductive halothane metabolism, metabolism via the oxidative route is necessary for the expression of halothane-altered membrane antigen [Neuberger et al 1981a]. Oxidative metabolism of halothane proceeds via trifluoroacetyl halide [Karashima et al 1977], a reactive metabolite which can bind covalently to cellular constituents [Gandolfi et al 1980; Kenna et al 1988b]. Endogenous liver protein is, in effect, changed from "self" to "non-self", thus becoming immunogenic. Antibodies are elicited against this "non-self" protein, creating an immune response. Using immunohistochemical staining with an antibody specific for the hapten, Satoh and colleagues (1985a) detected trifluoroacetyl-conjugated macromolecules on hepatocytes isolated from rats exposed to halothane. They concluded that TFA-halide reacted directly with constituents of the plasma membranes, or with other cellular components which became incorporated into the plasma membranes. In fact, the microsomal fraction was found to contain the greatest concentration of trifluoroacetyled adducts, the major component of which was identified immunochemically as a phenobarbitone-inducible form of cytochrome P-450. The results suggested that TFA-halide was so reactive that it bound predominantly to the cytochrome P-450 which produced it [Satoh et al 1985b]. Subsequent studies, however, have established that this is not the dominant protein acetylated [Hubbard, Gandolfi & Brown 1988a].

Kenna and coworkers (1988b) have investigated the mechanism of neoantigen generation by testing liver microsomes from rats exposed to halothane for reactivity with patients' sera and also with an antiserum specific for the covalently bound TFA-halide. They characterised the drug metabolite-tissue protein neoantigens and found that the patients' antibodies recognised epitopes consisting of the TFA group plus associated structural features of the protein carriers, and not the TFA hapten alone. In the same year, results from studies in man indicated that humans exposed to halothane express liver neoantigens which are analogous to the halothane metabolite-protein neoantigens characterised previously in halothane exposed animals [Kenna, Neuberger & Williams 1988a].

Hepatic hypoxia

There is much evidence implicating the metabolism of halothane, especially via the reductive pathway, as the factor principally responsible for halothane-induced hepatotoxicity. However the following observations do not support the metabolic theory:

(a) Enflurane and isoflurane which undergo considerably less metabolism than halothane, and have no reductive pathway, can cause hepatic necrosis in phenobarbitone-pretreated, hypoxic rats [Van Dyke 1982; Shingu et al 1983].

(b) Although the products of reductive halothane metabolism have been identified, none has been

shown to be hepatotoxic.

(c) Hepatic necrosis occurs in rats pretreated with triiodothyronine and then exposed to halothane without hypoxia [Wood et al 1980; Smith et al 1983].

(d) Shingu and colleagues (1982b) found that higher doses with shorter exposures to halothane produced more toxicity than exposure to lower concentrations for prolonged periods. Higher doses might produce liver hypoxia by cardiorespiratory depression, whereas a lengthy exposure would increase the amount of halothane metabolic intermediates.

Considerable evidence now exists implicating a reduction in hepatic oxygen supply, or more accurately, an imbalance between oxygen supply and demand, in the development of halothane-induced liver damage. Hepatic oxygen deprivation may arise as a result of one or more of the following: hypoxia, reduced cardiac output, reduced hepatic blood flow, and increased hepatic oxygen demand. Hypoxia in the absence of anaesthesia can cause centrilobular necrosis in phenobarbitone-treated rats similar to that seen after exposure to halothane [Shingu, Eger & Johnson 1982a]. The region around the central vein has the lowest oxygen tension as it is the last site in the liver lobule to receive oxygen, and therefore might be most influenced by hypoxia. Hypoxaemia rarely occurs or persists during clinical anaesthesia and is therefore unlikely to be a determinant of liver damage in

anaesthetised humans. However, local liver hypoxia resulting from an imbalance between oxygen supply and demand may be relevant: factors known to increase oxygen consumption such as barbiturate treatment and hyperthyroidism are associated with increased halothane hepatotoxicity in the rat [McLain, Sipes & Brown 1979; Ross, Daggy & Cardell 1979; Wood et al 1980; Smith et al 1983].

Hepatocellular necrosis can result also from a decrease in oxygen supply secondary to reduced hepatic perfusion. Harper and colleagues (1982) measured hepatic injury in phenobarbitone-induced rats following ligation of the hepatic artery performed under thiamylal, halothane, enflurane or isoflurane anaesthesia in oxygen. They found that interrupting arterial flow resulted in liver injury only in rats anaesthetised with halothane, and that the injury was comparable to that found in the phenobarbitone-hypoxia rat model. In addition, injury was more severe following upper abdominal surgery than after lower abdominal or peripheral surgery. Gelman (1976) had shown previously that total hepatic blood flow was reduced to a greater extent in patients undergoing partial gastrectomy or cholecystectomy than in those undergoing peripheral procedures such as herniorrhaphy. These findings suggest that interference with hepatic blood flow with consequent hepatocellular hypoxia may be important in the production of liver injury.

In man, total liver blood flow is derived from two sources, the hepatic artery which supplies roughly one-third of the total flow but 50% of the oxygen supply, and the portal vein [Strunin & Davies 1983]. Normally there exists reciprocity between hepatic arterial and portal venous flows, so that total liver blood flow tends to remain fairly constant [Richardson & Withrington 1981]. Specific volatile anaesthetic agents have different effects on this reciprocity of flow. Halothane has been shown to decrease both portal blood flow and hepatic arterial blood flow in dogs [Hughes, Campbell & Fitch 1980; Gelman, Fowler & Smith 1984a], rats [Gelman et al 1984] and guinea pigs [Hursh, Gelman & Bradley 1987], although the reduction in hepatic arterial blood flow is somewhat inconsistent [Gelman 1987]; the main determinant of portal blood flow, and to a lesser extent, hepatic arterial blood flow during halothane anaesthesia is cardiac output [Gelman, Fowler & Smith 1984a].

Enflurane decreases portal blood flow and, when administered in sufficient concentration to cause a 50% reduction in cardiac output, also impairs hepatic arterial blood flow [Hughes, Campbell & Fitch 1980]. It does, however, preserve liver blood flow and oxygen supply better than halothane when used in equipotent doses. In contrast, although isoflurane decreases portal blood flow, hepatic arterial blood flow increases, resulting in little change in total blood flow [Gelman,

Fowler & Smith 1984a, 1984b]. Despite the apparent preservation of liver blood flow, concentrations of isoflurane sufficient to decrease mean arterial pressure by 30% or more significantly reduced total liver blood flow, resulting in impaired hepatic oxygen delivery, increased hepatic oxygen consumption and a deterioration in tissue oxygenation [Hobbhahn et al 1986]; hepatic oxygen supply appeared to be adequate with lower concentrations of isoflurane [Gelman, Dillard & Bradley 1987]. It would appear, therefore, that although all three volatile agents decrease portal blood flow, isoflurane and to a lesser extent enflurane facilitate oxygen delivery to the liver much better than halothane, mainly by a more effective preservation of hepatic arterial blood flow.

Pharmacogenetics

Evidence of a genetic basis for susceptibility to halothane hepatitis has been provided by several studies. The extent to which an individual metabolises halothane, particularly via the reductive pathway, may be determined genetically. Cascorbi and colleagues (1971) demonstrated less variation in halothane metabolism between identical twins than between fraternal twins. Differences in the reductive metabolism of halothane and the susceptibility to liver damage have been reported among three different strains of rats [Gourlay et al 1981], and there is a strong suggestion that genetic predisposition is an important determinant in the

aetiology of halothane-induced hepatotoxicity in the guinea pig [Lunam, Cousins & Hall 1986].

Further evidence of possible genetic determination was provided by a report of halothane hepatitis occurring in three pairs of closely related women of common ethnic origin [Hoft et al 1981]. Farrell and coworkers (1985) reported increased susceptibility of lymphocytes to electrophilic attack in eleven patients with halothane hepatitis and in ten close relatives, and proposed the existence of a predisposing, familial, constitutional susceptibility factor. In addition, HLA frequencies were found to be different in 38 patients with halothane hepatitis compared with healthy controls [Otsuka et al 1985]. Available evidence seems, however, to indicate that halothane-associated liver damage is more likely to be of multifactorial origin, of which pharmacogenetics constitutes, at most, one variable [Brown 1981, 1985].

Altered calcium homeostasis

A calciogenic hypothesis to account for hepatic damage induced by volatile anaesthetic agents has been postulated by Gelman and Van Dyke (1988). They proposed that toxicity occurs secondary to disruption of mechanisms which maintain cellular calcium homeostasis.

Free, ionised, intracellular calcium concentration is in the range of 100 - 200 nmol l^{-1}, but that in extracellular fluid is upwards of 1 mmol l^{-1} [Rasmussen

1986; Thomas & Reed 1989]. Maintenance of this large chemical gradient is vital to cell viability; thus it is conceivable that processes leading to cellular injury may include alterations in calcium homeostasis. The gradient is maintained by a low natural permeability of the plasma membrane to calcium, the binding of cellular calcium to intracellular components, and the activity of at least two ATP-dependent transport mechanisms in the plasma membrane [Cheung et al 1986; Landers, Becker & Wong 1989]. In addition, both endoplasmic reticulum and mitochondria can actively sequester calcium from the cytosol [Becker, Fiskum & Lehninger 1980], protecting the cell against calcium overload in times of excessive calcium transfer into the cell [Rasmussen 1986].

Calcium ions are thought to play a major role in mediating or propagating hepatocellular injury [Farber 1982; Thomas & Reed 1989]; it has been suggested that increased concentrations of free intracellular calcium constitute the final common pathway of cell injury [Cheung et al 1986]. Several known hepatotoxins increase cytosolic calcium [Moore et al 1985; Brattin et al 1984; Moore 1980]. Halogenated hydrocarbons may increase liver calcium by inhibition of a microsomal calcium pump [Moore 1980] or by impairing the activity of endoplasmic reticulum [Brattin et al 1984; Long & Moore 1986]. Specifically, halothane can inactivate irreversibly calcium transport in hepatic endoplasmic reticulum, provoking an immediate release of calcium

[Zucker, Diamond & Berman 1982].

Animal studies have provided evidence supporting a role for altered calcium fluxes in the mechanism of halothane-induced liver injury. In guinea pigs, hepatic calcium content was increased significantly, 24 hours after exposure to halothane [Farrell et al 1988]. Subsequent changes in liver calcium were proportional to the severity of liver necrosis, as determined morphologically. The increase in liver calcium occurred before hepatocellular necrosis was observed histologically. This antecedent relationship of altered cellular calcium homeostasis to liver necrosis is consistent with a pathogenetic role in the production of liver injury. Lind and coworkers (1984) administered lipopolysaccharides to phenobarbitone-pretreated hypoxic rats immediately after halothane anaesthesia, and found a marked potentiation of hepatic necrosis which was related to a significant increase in liver calcium content. Administration of the antiendotoxin agent lactulose reduced the hepatic damage, and prevented the observed increase in liver calcium. More recently, it was shown that halothane, enflurane and isoflurane each stimulated a significant, dose-dependent release of radio-labelled calcium from internal calcium stores in isolated rat hepatocytes [Iaizzo et al 1990]. Halothane produced the greatest calcium release, and isoflurane produced the least - a pattern which is consistent with the known ability of these anaesthetics to induce hepatic necrosis.

Further evidence in support of the calciogenic hypothesis of cell injury is offered by studies in which the administration of a calcium channel blocker reduced the extent of hepatic necrosis in animals exposed to hepatotoxic agents, including halothane [Landon, Naukam & Sastry 1986; Garay, Annesley & Burnette 1986; Goto et al 1990]. Calcium channel blockers most probably act by interfering with the influx of calcium through the ionic channel of the hepatocyte membrane, and preventing the increase in cytosolic calcium concentration. Clearly, calcium plays a major role in chemically induced cell injury, although the consequences of increased cytosolic calcium remain incompletely understood.

Current perspectives

It is now recognised that two types of halothane-induced hepatic dysfunction exist [Brown & Gandolfi 1987; Touloukian & Kaplowitz 1981; Pohl & Gillette 1982; Neuberger & Williams 1984]. A mild subclinical form manifest by abnormal biochemical indices of liver function may occur in up to 20% of patients exposed to halothane. This could be caused by toxic products of halothane metabolism, possibly determined by genetic factors, or by hepatic hypoxia resulting from an imbalance between hepatic oxygen supply and demand; altered hepatocellular calcium balance cannot be excluded as a possible mechanism. A much rarer fulminant form may occur with severe necrosis which may prove fatal. It is probable that this form results from an immune

reaction: an oxidative metabolite binds covalently to liver proteins producing a hapten, which, in turn, provokes an immune reaction and the formation of a circulating antibody.

Hepatotoxicity following all volatile halogenated anaesthetics may be linked by a common mechanism. Christ and colleagues (1988b) demonstrated that enflurane and isoflurane are converted to reactive metabolites that form covalently bound, acylated protein adducts which are recognised by specific anti-TFA antibodies. Halothane formed much greater amounts of immunoreactive protein adducts than enflurane which, in turn, formed much greater amounts than isoflurane. Thus the degree of anti-TFA immunoreactive adduct formation correlated directly with the extents of metabolism and incidence of reported clinical hepatic dysfunction for these drugs (ie, halothane > enflurane > isoflurane). Immunoblotting techniques used by the same group showed that antibodies in the sera of six patients with halothane hepatitis recognised liver microsomal antigens formed in rats treated with enflurane or halothane [Christ et al 1988a]. This suggested that a halide metabolite of enflurane bound covalently to liver proteins and could similarly provoke an immune response. This mechanism could account also for the apparent cross-senstisation between halothane and enflurane. Isoflurane did not produce detectable liver antigen, although the principal reason for this may have been

related to the level of biotransformation in their particular animal preparation. However, isoflurane, enflurane and halothane all have the potential for producing acetylating intermediates that can alter liver proteins, rendering them immunogenic. Thus a common mechanism for producing liver damage may exist between the volatile halogenated anaesthetics.

ANIMAL STUDIES

Early attempts to produce an animal model of halothane hepatitis proved disappointing. Since 1976, however, five animal models have been developed, in each of which massive hepatic necrosis was produced.

Polychlorinated biphenyl rat model

Sipes and Brown (1976) described a rat model in which hepatic necrosis was induced by a single exposure to 1% halothane in 99% oxygen following pretreatment with Aroclor 1254, a polychlorinated biphenyl (PCB). The lesion produced was centrilobular and multifocal, and morphologically similar to that described in man. This damage was apparent within 2 hours of the end of anaesthesia [Reynolds & Moslen 1977]. PCBs are potent inducers of a wide variety of biotransformation pathways, but unfortunately can themselves cause liver damage [Brown & Sipes 1977], so this model is probably not appropriate.

Phenobarbitone-hypoxia rat model

Certain strains of male rats develop hepatic necrosis when exposed to halothane under hypoxic conditions after phenobarbitone pretreatment [McLain, Sipes & Brown 1979; Ross, Daggy & Cardell 1979; Cousins et al 1979]. Both hypoxia and phenobarbitone are required to cause necrosis. As the severity of damage correlated with the amount of inorganic fluoride produced, it was proposed that the liver damage was mediated through reductive metabolism of halothane [Cousins et al 1987b]. This hypothesis was supported by Jee and colleagues (1980), who found that the administration of the biotransformation inhibitors metyrapone and SKF 525A inhibited hepatic necrosis even when administered up to 4 hours after anaesthesia. Later, Wilhelm and others (1987) postulated that glutathione was responsible for the detoxification of reactive intermediate products of reductive metabolism, and found that liver damage in this model was significantly aggravated by glutathione depletion.

The validity of this particular model has, however, been questioned. The reproducibility of liver damage depends on species, sex, age, temperature and even season variations [Gelman 1986]. Severe hypoxia (F_IO_2 0.06 - 0.08) alone can cause necrosis in phenobarbitone pretreated rats [Shingu, Eger & Johnson 1982a]. Both isoflurane and enflurane, neither of which undergo reductive metabolism, can cause necrosis in starved rats

pretreated with phenobarbitone under conditions of profound hypoxia (F_IO_2 0.08) [Van Dyke 1982], but not under moderate hypoxia (F_IO_2 0.14) [Lind et al 1985]. Fed rats exposed to enflurane or isoflurane did not develop liver necrosis. The effect of fasting is unclear, although starvation is known to increase the solubility of volatile anaesthetics in rat liver enhancing hepatic uptake of anaesthetic [Fassoulaki & Eger 1986].

In the phenobarbitone-hypoxia rat model, it is unclear whether halothane initiates liver injury by reducing hepatic blood flow thereby inducing hypoxia, or whether products of halothane metabolism are responsible. These variables are impossible to separate during in vivo halothane exposure, as reductive metabolism requires hypoxia. However, Schlieble and colleagues (1988) have attempted to separate these effects of halothane in an in vitro experimental system, using monolayer cultures of rat hepatocytes to eliminate the effect of halothane on hepatic perfusion. They found that three essential components were necessary for the development of necrosis in this model, namely hypoxia, phenobarbitone induction and exposure to halothane.

Triiodothyronine rat model

Hepatic necrosis occurs in rats pretreated with triiodothyronine (T_3) and then exposed to halothane in non-hypoxic environments [Wood et al 1980; Smith et al

1983]. Because the necrosis is caused in the absence of hypoxia, reductive metabolism is not implicated. Both enflurane and isoflurane can also cause necrosis in this model [Berman et al 1983]. In contrast to the phenobarbitone-hypoxia model, it appears that a mechanism other than reduction of halothane may be involved in the T_3 model [Uetrecht et al 1983]. It is proposed that hypoxic damage to the hypermetabolic centrilobular cells resulting from depression of splanchnic blood flow is responsible for the liver cell necrosis [Berman et al 1983].

Isoniazid rat model

Rice and colleagues (1987) treated Fischer 344 rats with isoniazid or saline for 7 days, and then exposed them to either halothane in air, or air alone. Hepatocellular necrosis occurred only in rats administered halothane; the most severe lesions were observed in the isoniazid treated animals, mirroring increases in serum aminotransferase activities. Hypoxia was not required for lesion development, and as isoniazid treatment enhanced oxidative but not reductive halothane biotransformation, it was suggested that oxidative metabolism was responsible for hepatotoxicity in this model.

Guinea pig model

Recently, a model has been described in which halothane produced hepatic damage in an outbred strain of guinea

pig [Lunam, Cousins & Hall 1985]. Neither enzyme induction nor hypoxia was required for this effect. Subsequently, liver necrosis was described in certain other strains of guinea pig [Lind et al 1987]. Unlike other animal models in which all animals developed severe centrilobular necrosis within hours of exposure, only 30% of guinea pigs show a similar degree of severity. In addition, the development of necrosis is delayed for 48-72 hours, and both sexes are affected. Although both reductive and oxidative metabolism of halothane occur in the guinea pig, it is the oxidative pathway which has been implicated as a mechanism of hepatic injury [Lind, Gandolfi & Hall 1989]. Other workers have suggested, however, that it is the marked reduction in both portal and hepatic arterial blood flows during halothane anaesthesia which is responsible for hepatic damage in the guinea pig [Hursh, Gelman & Bradley 1987]. The guinea pig has also been used to study the potential contribution to liver damage of a halothane-induced immune response [Siadat-Pajouh et al 1987].

Other workers have attempted to produce an immune-mediated model of halothane hepatotoxicity. Neuberger, Kenna and Williams (1987) immunised rabbits with halothane antigen-bearing hepatocytes isolated from litter mates, to induce circulating levels of "halothane antibodies". The rabbits were then exposed to halothane; none of these rabbits showed any evidence of liver cell necrosis. More successfully, Callis and

coworkers (1987) developed a rabbit model to study the production of humoral immunity towards a biotransformation intermediate of halothane. In this model, rabbits exposed many times to halothane in 75% oxygen produced an antibody in response to an intermediate product of oxidative metabolism. Although the evidence presented is convincing, this model has yet to be validated by other workers.

How relevant these animal studies are to the problem of human hepatotoxicity is not known. The lesion in man is rare and unpredictable, and animal models have not been in keeping with this clinical picture [Cousins 1980]. All three rat models require the presence of demanding conditional factors which would not be expected to pertain to man. These rat models display considerable sex, age and strain differences regarding susceptibility to hepatic necrosis. In addition, the phenobarbitone-hypoxia rat model shows a dose-response relationship [Jee et al 1980] which is not the case in man, and the incidence or severity of hepatic dysfunction does not increase with multiple exposures [Reynolds & Moslen 1974]. In contrast, the guinea pig model more closely resembles clinical observations in humans with regard to halothane biotransformation and lesion morphology [Lind et al 1987], possible genetic predisposition [Lunam, Cousins & Hall 1986], and induction of an immune response following multiple halothane exposures [Siadat-Pajouh et al 1987].

Therefore, although the animal models have enabled an understanding of some of the issues involved, they have not greatly advanced the quest for the cause of halothane hepatotoxicity in man.

OTHER INHALATION ANAESTHETIC AGENTS

Enflurane

There is not the same concern regarding hepatotoxicity after anaesthesia with enflurane as there is with halothane. Nonetheless, a few case reports of alleged enflurane hepatitis have been published [Denlinger, Lecky & Nahrwold 1974; Danilewitz et al 1980; Kline 1980; Ona, Patanella & Ayub 1980; Paull & Fortune 1987]. In several of these reports, however, other causes of liver damage, such as viral hepatitis, could not be excluded. In a review of 58 cases of suspected enflurane hepatitis, Lewis and colleagues (1983) considered that 24 could be explained only as a result of the administration of enflurane. Analysis of these cases demonstrated that the clinical, biochemical and histological features were similar to those seen with halothane-induced hepatitis. However, the vague and non-specific criteria for ascribing hepatotoxicity in that review have been criticised [Dykes 1984]. Furthermore, Eger and coworkers (1986) reviewed 88 cases of hepatic injury attributed to enflurane, including the material reviewed by Lewis, and could demonstrate neither a causal

relationship between enflurane anaesthesia and subsequent liver injury, nor consistent histological changes in liver specimens.

It cannot be denied that unexplained severe liver damage follows enflurane anaesthesia in the rare patient: the known incidence is of the order of 1 in 800,000, which is less than the spontaneous attack rate of viral hepatitis [Brown & Gandolfi 1987]. This incidence is too low to suggest a causal association and, in addition, a molecular mechanism by which enflurane might produce hepatotoxicity analogous to that produced by halothane is difficult to propose and support experimentally [Eger et al 1986]. There are few, if any, parallels between the metabolism of halothane and enflurane, and the pattern of enflurane hepatitis does not tend to support an immune-mediated mechanism [Eger et al 1986]. However, Christ and colleagues (1988b) have suggested a possible hypersensitivity basis for enflurane hepatitis, involving the covalent binding of an oxidative metabolite to liver microsomal adducts. The case for enflurane hepatitis currently remains not proven, but further studies such as that undertaken by Christ may add weight to the argument for its existence.

Isoflurane

Two case reports have been published attributing hepatic injury to isoflurane, but in neither report did the results show a definitive causal relationship between

isoflurane and liver dysfunction [Grégoire & Smiley 1986; Carrigan & Straughen 1987]. Stoelting and colleagues (1987) reviewed 45 cases of hepatic dysfunction after isoflurane anaesthesia reported to the Food and Drug Administration in the United States of America. They concluded that causes other than isoflurane could account for the postoperative liver damage in 29 patients, while in the remaining 16, isoflurane might have been one of several possible causes of injury. McLaughlin and Eger (1984) described a patient given repeated isoflurane anaesthetics in the presence of abnormal liver biochemistry; isoflurane was not implicated in the aetiology of the hepatic injury and, in fact, liver function improved during the course of the anaesthetics. Current evidence suggests that it is highly unlikely that isoflurane is even rarely responsible for postoperative hepatotoxicity [Stoelting 1987].

Sevoflurane

First synthesised in the early 1970's, sevoflurane is a halogenated methyl isopropyl ether, which at present is marketed for clinical use only in Japan [Wallin et al 1975]. The extent of biotransformation of sevoflurane is similar to that of enflurane, but its low solubility and rapid elimination confine this to the period of exposure [Holaday & Smith 1981]. Findings from studies of its effect on liver blood flow are not consistent, demonstrating an increase [Manohar & Parks 1984], decrease [Wouters et al 1989], or no effect [Pilato et al

1989], although different concentrations of sevoflurane were used in each of these studies. Original animal studies suggested that sevoflurane was not hepatotoxic [Cook et al 1975], but subsequent investigations do not support this; it may cause hepatic injury of a degree similar to isoflurane in enzyme-induced hypoxic rats [Strum et al 1987], and its administration to guinea pigs may also result in liver damage, probably by altering liver blood flow [Lind et al 1989].

Desflurane (I-653)

Desflurane is a new volatile anaesthetic agent structurally very similar to isoflurane, which is currently being evaluated for clinical use [Jones, Cashman & Mant 1990; Jones et al 1990a]. Although it undergoes some oxidative biotransformation [Sutton et al 1990], it resists metabolic degradation more than other volatile agents [Koblin et al 1988; Koblin et al 1989; Jones et al 1990b]. Desflurane has not been reported to cause hepatic injury in hypoxic, enzyme-induced rats [Eger et al 1987], rats anaesthetised repeatedly [Eger, Johnson & Ferrell 1987], or pigs [Holmes et al 1990], or to influence biochemical tests of liver function in human volunteers [Jones et al 1990b].

Nitrous oxide

In conventional anaesthetic practice nitrous oxide is commonly administered in combination with volatile anaesthetics. Might nitrous oxide be hepatotoxic and

contribute to the hepatic damage associated with other anaesthetic agents? Ross and coworkers (1984) demonstrated in phenobarbitone-pretreated rats exposed to 14% oxygen, that nitrous oxide produced no effect in the absence of halothane, but potentiated the hepatotoxicity of 0.75% halothane. In a more profoundly hypoxic rat model (F_IO_2 0.075), however, nitrous oxide has been shown to cause centrilobular necrosis [Fassoulaki et al 1984]. Indeed nitrous oxide was found to be more hepatotoxic than enflurane or isoflurane, which did not produce hepatic necrosis under identical conditions. This toxicity is unlikely to result from biotransformation of nitrous oxide as there is no evidence of metabolism of nitrous oxide by the liver [Ross, Monk & Duffy 1984]. Fassoulaki and colleagues (1984) have suggested that nitrous oxide, like other anaesthetics, causes a disruption in hepatocellular calcium homeostasis, resulting in damage.

There is one human report of jaundice following anaesthesia in which the only inhalation agent used was nitrous oxide [Hart & Fitzgerald 1975]. The clinical and laboratory features were very similar to those attributed to halothane anaesthesia. Although halothane was not administered to the patient, the anaesthetic gases were delivered from an anaesthetic machine fitted with a halothane vaporiser which was switched off. It is more likely that halothane was responsible for the jaundice, resulting from inapparent circuit contamination

[Varma, Whitesell & Iskandarani 1985].

SUMMARY

The historical background, clinical features, morphology, epidemiology and aetiology of halothane hepatitis have been presented. Animal models of halothane hepatotoxicity have been described, although their application to humans is of doubtful significance. Two, probably distinct, forms of liver damage associated with halothane have been identified. The much more common mild form may result from reductive biotransformation of halothane, possibly influenced by genetic factors, or reduced liver oxygenation, whereas the rare fulminant form is most likely to be immune-mediated. The role of altered calcium homeostasis has not yet been established. In addition, a common mechanism for liver dysfunction associated with the halogenated volatile anaesthetic agents has been proposed. The hepatotoxicity of enflurane cannot be excluded; while hepatic dysfunction after isoflurane and nitrous oxide is considered unlikely, further attention is necessary. It is too soon to comment on the hepatotoxic potential of sevoflurane and desflurane.

CHAPTER 2

SERUM ENZYMES IN ACUTE LIVER INJURY

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Many biochemical tests have been introduced which are said to assess liver "function" - an inaccurate term. Some liver tests can detect cell injury or the response to injury, while others indicate impairment of a particular aspect of liver function. A good function test must satisfy the following criteria: specificity, sensitivity, reproducibility, and ease of performance. Furthermore, to infer hepatotoxicity by a suspected agent, it is important that the biochemical test correlates well with histological abnormalities [Zimmerman 1978]. Assessment of chemical hepatic injury in early studies was based on an impaired ability of the liver to synthetise urea, cholesterol and its esters, and plasma proteins including clotting factors, and to maintain normal blood levels of glucose and amino acids. These measures, however, are too insensitive, nonspecific or cumbersome to be useful in monitoring the severity of damage, comparing the relative toxicity of different agents, or assessing the potentiation or inhibition of toxic phenomena by various treatments. Much more useful is the measurement of serum enzymes which has been the standard biochemical approach to the assessment of hepatic injury during the past 30 years.

More than 50 enzymes have been identified in serum or plasma. For most of them, abnormal values have been

found in patients with hepatic disease. Their application to the diagnosis of hepatic injury requires analysis of their specificity for, and sensitivity to, liver damage. In this regard, enzymes identified in serum fall into four groups (Table 2.1). In one group of enzymes, increased serum levels reflect cholestasis more sensitively than parenchymal injury. A second group contains enzymes that are more sensitive to parenchymal injury than reduced biliary flow, while a third group consists of enzymes that are insensitive for hepatic injury but serum levels of which may be increased in disease of other tissues. In a fourth group are enzymes whose serum levels are characteristically depressed in hepatic injury. It is the enzymes in the first and second groups that are most useful for the investigation of liver disease in general, and those in the second group that are used most widely in the study of toxic hepatic injury.

As with all other methods for assessing liver function, serum enzyme tests have some limitations. Some of the enzymes that are considered to reflect liver injury may actually derive from other organs; that is, they may not be specific for hepatic disease. A further problem common to measurement of all serum enzymes is that increased serum levels may not reflect tissue damage. Rather they may result from:

(a) an increase in the tissue source of enzymes because of an increased rate of production per

cell or an increase in the number of cells, or

(b) impaired disposition or excretion, or

(c) leakage from cells with altered membrane permeability.

In spite of these limitations, measurement of serum enzymes remains the cornerstone in the investigation of chemical hepatic injury. Some of these enzymes warrant further consideration.

Table 2.1

Examples of serum enzymes in liver disease

Enzymes that detect hepatocellular necrosis	
Standard tests:	Aspartate aminotransferase Alanine aminotransferase Lactate dehydrogenase
Specific tests:	Isocitrate dehydrogenase Sorbitol dehydrogenase Ornithine carbamyl transferase Argininosuccinate lyase
Enzymes that detect cholestasis	
Standard tests:	Alkaline phosphatase Gamma-glutamyl transferase
Specific tests:	5'-Nucleotidase Leucine aminopeptidase
Enzymes increased in disease of other tissues	
	Creatine phosphokinase
Enzymes with reduced serum levels in liver disease	
	Cholinesterase

ENZYMES THAT DETECT HEPATOCELLULAR NECROSIS

Aminotransferases

Alanine aminotransferase (ALT; serum glutamic-pyruvic transaminase; SGPT) and aspartate aminotransferase (AST; serum glutamic-oxaloacetic transaminase; SGOT) are the most frequently measured indicators of liver disease. These enzymes catalyse the transfer of the α-amino group of alanine and aspartate respectively to the α-keto group of ketoglutaric acid, resulting in the formation of pyruvic acid and oxaloacetic acid [Kaplan 1987]. The increase in serum ALT and AST activities is related to damage to, or destruction of, tissue rich in aminotransferases, or to changes in cell membrane permeability that allow ALT and AST to leak into the circulation.

Neither ALT nor AST is specific to the liver. AST is present in large quantities in liver, cardiac and skeletal muscle, and kidney, and to a lesser extent in brain, pancreas, lung, leucocytes and erythrocytes [Strunin 1977]. Although ALT is present in greatest concentration in liver, it is also distributed widely in the body [Zimmermann & Seeff 1970]. AST is present in both mitochondria and cytosol of the liver, but ALT is limited to cytosol. The isoenzymes of AST from mitochondria and cytosol are immunochemically distinct and may be assayed individually. Eighty per cent of AST in liver biopsy specimens from normal adults is of

mitochondrial origin; in contrast, AST in serum is primarily cytosolic enzyme, possibly because of the greater lability of mitochondrial AST in serum. However, large increases in mitochondrial AST are found early in diseases associated with massive tissue necrosis, perhaps reflecting a sudden release of intracellular contents into the circulation, and for this reason, specific assay of mitochondrial AST may be useful in predicting the extent of liver necrosis [Stolz & Kaplowitz 1990].

Although serum aminotransferase activities are increased in almost all forms of liver disease, they are not specific for hepatobiliary disorders [Kools & Bloomer 1987; McIntyre 1983]. Increased activities may be found also in patients with cardiac and skeletal muscle damage [Wróblewski 1959], although grossly increased activities (>1000 U l^{-1}) usually indicate hepatic origin. It is therefore not surprising that increased serum aminotransferase activity correlates poorly with abnormal liver histology [James et al 1975; Kaplan 1987; Galambos & Wills 1978]. A further complication is the finding in healthy populations that a significant percentage of those tested have abnormally increased activities of serum aminotransferases. Kahn and colleagues (1982) found 5.5% of 10,034 asymptomatic blood donors had abnormal AST activity, while Friedman and coworkers (1987) could find no cause for increased ALT activity (>100 U l^{-1}) in 22 of 92 healthy blood donors.

One further note of caution is that aminotransferase activities may be falsely diminished or inhibited under certain circumstances. For instance, serum levels of AST are diminished in uraemia but increase after dialysis, suggesting that a dialysable substance in the blood of uraemic patients inhibits AST [Cohen et al 1976]. In addition, serum AST activity diminishes after 24 hours when stored at room temperature, although the enzyme is stable for 28 days when serum is refrigerated or frozen [Stolz & Kaplowitz 1990].

Lactate dehydrogenase

Lactate dehydrogenase (LDH) is distributed widely in the body, particularly in skeletal and cardiac muscle, liver and erythrocytes. Although LDH is a sensitive indicator of cell damage, it is not specific for liver disease. Increased serum activities of this enzyme have been found in such disparate disorders as myocardial infarction, carcinomatosis, haemolytic anaemia, leukaemia, infectious mononucleosis and progressive muscular dystrophy, as well as in intrinsic hepatic disease [Zimmerman & Seeff 1970]. Five isoenzymes can be detected electrophoretically but fractionation yields no further useful information in the diagnosis of hepatobiliary disease [Hargreaves 1986]. Consequently, measurement of LDH activity has limited application in the diagnosis of hepatic disease.

Because these standard enzyme tests show poor specificity

and variable sensitivity in detecting hepatocellular damage, a number of other serum enzyme tests which might offer greater organ specificity have been developed. These include isocitrate dehydrogenase (ICDH), sorbitol dehydrogenase (SDH), ornithine carbamyl transferase (OCT) and argininosuccinate lyase (ASAL).

Isocitrate dehydrogenase

ICDH is a cytoplasmic enzyme which is relatively specific for liver disease, but is less sensitive than the aminotransferases [Rosalki 1976]. It has been shown to be particularly sensitive in detecting minor episodes of hepatic damage caused by drugs and alcohol [Ellis et al 1978]. However, disseminated malignancy without detectable hepatic involvement can lead to increased ICDH activity [West et al 1964]. As it does not provide the specific reflection of hepatic disease initially believed, it offers no diagnostic advantage over aminotransferase measurements.

Sorbitol dehydrogenase

Virtually all sorbitol dehydrogenase is found in the liver; only relatively low concentrations are present in the prostate and kidney [Wilkinson 1976]. Its serum activity becomes rapidly and distinctly increased in acute hepatic parenchymal damage, and it would seem to provide a useful biochemical test in the early detection of acute liver disease [Zimmerman & Seeff 1970]. However, aminotransferase measurements have greater

sensitivity, and values of SDH activity may be normal in cirrhosis and other chronic liver disorders [Asada & Galambos 1963]. Its instability in serum further limits its diagnostic usefulness [Wilkinson 1976].

Ornithine carbamyl transferase

OCT, an enzyme involved intimately in the synthesis of urea, is found almost exclusively in the liver; there is virtually no activity in other tissues [Reichard 1961]. Serum activity is very low in normal individuals but is markedly increased in patients with hepatic necrosis [Reichard 1961]. Tegeris and colleagues (1969) induced selective tissue damage in dogs and swine, either chemically by the nephrotoxic agent uranyl nitrate or the hepatotoxin CCl_4, or surgically by bile duct or coronary artery ligation. They measured the activities in serum of several liver enzymes including AST, ALT and OCT, and demonstrated that OCT became increased only when the hepatic parenchyma was damaged. This increase occurred just as early and was much greater than that of the other enzymes assayed. Furthermore, in rats administered chemical hepatotoxins, OCT activity was found to be as sensitive as histopathology for detecting liver damage [Drotman & Lawhorn 1978]. Despite the apparent advantages of OCT over aminotransferase measurements, however, it has not replaced them in clinical use.

Argininosuccinate lyase

Argininosuccinate lyase mediates the degradation of

argininosuccinic acid to arginine and fumaric acid. The reaction is reversible. ASAL occurs in liver, kidney and erythrocytes [Takahara & Natelson 1967]. In the liver, the enzyme is involved in the urea cycle and tends to act as a lyase. In the kidney, however, the equilibrium is reversed so that the enzyme acts as a synthetising enzyme to form argininosuccinic acid [Campanini et al 1970]. Serum concentrations of ASAL have been determined in patients with parenchymal liver disease. Its measurement has been found to be more specific and sensitive for hepatocellular integrity than the more commonly applied biochemical liver tests [Campanini et al 1970; Sims & Rautanen 1975]. Like OCT, however, ASAL has not proved to be more useful in practice than the aminotransferases, and is not widely used.

ENZYMES THAT DETECT CHOLESTASIS

Alkaline phosphatase

Alkaline phosphatase is the name given to a group of enzymes which catalyse the hydrolysis of a large number of organic phosphate esters at an optimum alkaline pH. They are found mainly in bone osteoblasts and the canalicular membranes of hepatocytes, but are also present in small intestine, kidney, white blood cells and placenta [Kaplan 1987]. Two distinct types of alkaline phosphatase are present in the liver, although their precise roles are unknown [Stolz & Kaplowitz 1990].

Serum electrophoresis allows the separation of the isoenzymes produced in these various locations [Hargreaves 1986]; in most clinical situations this amounts to separating liver from bone alkaline phosphatase. Serum alkaline phosphatase activity may be increased in bone, kidney and intestinal disorders as well as in hepatobiliary disease, and increased activity is observed also as a normal physiological response in children (due to influx of enzyme from osteoid tissue during bone growth) and in late normal pregnancy (due to release of placental enzyme). The major value of serum alkaline phosphatase in the diagnosis of liver disorders is in the recognition of cholestasis. Increased serum activity of liver alkaline phophatase does not necessarily, however, indicate the presence of liver disease. Brensilver and Kaplan (1975) studied 317 patients who had increased serum alkaline phosphatase activity. Although the source of the increase was liver isoenzyme in 253 patients, there was no evidence of liver disease in almost one-third of these patients. Measurement of alkaline phosphatase activity, therefore, has little application in the detection of acute hepatocellular damage.

Gamma-glutamyl transferase

Gamma-glutamyl transferase (GGT) is present in cell membranes in many tissues, including kidney, pancreas, liver, spleen, heart, brain and seminal vesicles [Goldberg 1980]. Increased serum activity of this

enzyme is found predominantly in diseases of the liver, biliary tract and pancreas. There are two main clinical values of GGT. It can be used to confer organ specificity to an increased serum activity of alkaline phosphatase, as GGT activity is not increased in disorders of bone [Kaplan 1987]; secondly, isolated increased GGT activity with normal other biochemical indices of hepatocellular function may be an indicator of alcohol abuse or alcoholic liver disease. Increased serum GGT activity, however, is not always associated with hepatobiliary disease. In addition, hyperbilirubinaemia can interfere with measured activity of GGT in vitro, impairing the diagnostic usefulness of this enzyme test [Combes et al 1977]. In a large prospective study, 68% of hospital inpatients who had abnormal levels of GGT activity had diseases which did not involve the liver [Burrows, Feldman & McBride 1975]. Although GGT provides a sensitive index of hepatocellular dysfunction, it has poor specificity and offers no advantage over measurement of aminotransferases and alkaline phosphatase in the detection of acute liver disease.

5'-Nucleotidase

Despite its presence in several other organs, increased serum activity of 5'-nucleotidase is purported to be of hepatobiliary origin only [Kaplan 1987]; in clinical hepatic disease, activity of 5'-nucleotidase correlates with serum alkaline phosphatase activity. Its major

advantage over alkaline phosphatase is enhancement of specificity. It is used mainly to confirm that increased alkaline phosphatase activity is of hepatic origin, although measurement of alkaline phosphatase isoenzymes and gamma-glutamyl transferase activity have generally replaced this use. It may be useful in the diagnosis of cholestasis during pregnancy and childhood as its activity is not increased physiologically in these states, unlike that of alkaline phosphatase [Stolz & Kaplowitz 1990].

Leucine aminopeptidase

This enzyme, which is probably involved in the regulation of protein metabolism and turnover, is found in virtually all human tissues [Kaplan 1987]. Increased activity is found only in pregnancy and hepatobiliary disorders. Its activity is increased in most types of liver disease but greatest increases are seen in biliary obstruction, and thus its major application would seem to be in the recognition of cholestasis. Clinically, its primary use is to specify the origin of increased serum alkaline phosphatase activity; in this respect it appears similar to 5'-nucleotidase and GGT [Stolz & Kaplowitz 1990]. As these latter tests are more convenient to perform, and offer broadly equal sensitivity and specificity for hepatobiliary disorders, leucine aminopeptidase is now rarely measured.

ENZYMES WITH REDUCED SERUM LEVELS IN HEPATIC DISEASE

Pseudocholinesterase

Serum pseudocholinesterase concentration is characteristically depressed in patients with hepatic disease. Low concentrations are observed also in patients with a low serum concentration of albumin. It is believed that the low pseudocholinesterase concentration reflects impaired hepatic protein synthesis. However, serum pseudocholinesterase concentration cannot be used as an index of acute hepatic dysfunction as its half-life is some 15 days [Strunin 1977]. Furthermore, the test is not sufficiently consistent to be useful in the differential diagnosis of liver disease, and adds little to the more commonly used laboratory measurements.

Enzymes commonly measured in routine clinical practice include AST, ALT, alkaline phosphatase and gamma-glutamyl transferase. These tests are generally simple to perform, are relatively quantitative and inexpensive, and often are included in the output of multiple channel automatic analysers. Furthermore, there is enormous experience with their serum levels in a wide variety of liver disorders. Along with measurement of serum concentrations of bilirubin and albumin, they are useful general tests in the initial investigation of liver disease. Their poor hepatospecificity and variable

sensitivity, however, limit their specific application. Individual tests are generally unreliable indicators of impaired liver function, and therefore a battery of liver tests is usually performed. By measuring several serum enzymes, sensitivity and specificity are enhanced, and the predictive accuracy for the presence of liver disease is improved also if more than one test is abnormal. The measurement of more hepatospecific enzymes such as SDH, OCT and ASAL provides little additional information in the routine investigation of liver disease. These more specific tests are often less sensitive for liver disease than the aminotransferases, and, in addition, they may be more complicated to perform, and cost more in terms of laboratory time and materials used. For these reasons, coupled with the relative lack of experience with their serum activities in liver disease, they have not replaced the conventional liver enzyme tests in widespread use. They have greater application, however, in the study of acute, toxic liver injury, when measurement of enzymes that are sensitive and specific indicators of hepatocellular necrosis allows earlier, less invasive, easier and possibly more accurate detection of hepatotoxicity than by histological methods [Zimmerman 1978].

None of the enzyme tests examined here in detail meets all the requirements (ie, sensitivity, hepatospecificity, ease of measurement and reproducibility) necessary to allow accurate diagnosis and monitoring of minor,

drug-induced hepatocellular damage. More recently, attention has focused on glutathione S-transferase as an alternative test of hepatocellular damage which, in theory, should be highly sensitive and hepatospecific.

GLUTATHIONE S-TRANSFERASE

The glutathione S-transferases (GST) are multifunctional proteins which serve as intracellular binding proteins and as catalysts for the reaction of electrophilic compounds with glutathione [Mannervik 1985; Jakoby 1985; Wolkoff 1980]. This conjugation reaction is the first step in the formation of the mercapturic acids, a pathway that is a major excretory route for the elimination of foreign compounds [Chasseaud 1979; Mannervik 1985]. In addition, GST bind a number of compounds that they do not metabolise (non-susbtrate ligands), and they have been suggested to act as intracellular transport proteins for compounds that have limited solubility in water [Boyer 1989]. As many GST substrates are environmental pollutants, drugs or carcinogens, the primary role of GST is thought to be one of detoxification [Beckett & Hayes 1987]: indeed, evidence exists that GST protect against chemical carcinogenesis [Chasseaud 1979; Smith, Ohl & Litwack 1977; Sparnins, Venegas & Wattenberg 1982].

STRUCTURE, NOMENCLATURE AND CLASSIFICATION

GST activity is widely distributed throughout the animal

kingdom and in all species multiple forms of the enzyme are a prominent feature [Mannervik 1985; Jakoby 1985]. These can be purified using chromatographic and electrophoretic techniques [Beckett & Hayes 1987; Mannervik 1985]. Early workers assumed that separate enzymes were responsible for the metabolism of different substrates, and the enzymes were variously named aryl, alkyl, aralkyl and alkene glutathione S-transferases. However, it became apparent that the GST displayed overlapping substrate specificities. Consequently, the original nomenclature was replaced by designations based on the physical or structural properties of the proteins rather than on their enzymatic properties. The various hepatic rat GST isoenzymes isolated were named E, D, C, B, A and AA in order of their elution from a carboxymethyl cellulose column [Jakoby 1978]. This system of nomenclature also proved inadequate, as rat liver was soon discovered to contain many more forms of GST than had been identified originally.

Each form of GST comprises two subunits of similar size, with a total molecular mass (Mr) of 45,000 - 55,000. Both homodimers and heterodimers have been described [Mannervik 1985]. The different forms of GST are best described by their subunit composition, as the individual subunits function independently. Rat GST have been studied extensively. At least seven subunits exist, designated according to their mobilities during sodium dodecyl sulphate - polyacrylamide gel electrophoresis as

Ya, Yb (b_1 and b_2), Yc, Ye, Yf (or Yp), Yk and Yn (or Yt) [Beckett & Hayes 1987; Boyer 1989]. The large number of GST isoenzymes in the rat has caused considerable confusion in the past when different means of naming them were used. In an attempt to avoid confusion, a more recent system of nomenclature has been adopted, in which each distinct GST subunit is denoted by an Arabic numeral, and in which subunits are numbered sequentially in the order that they are discovered and characterised [Jakoby, Ketterer & Mannervik 1984].

On the basis of immunochemical analysis, rat GST subunits can be divided into at least three groups or families. Group I comprises subunits Ya, Yc and Yk; group II contains Yb_1, Yb_2 and Yn; and group III is composed of Ye and Yf subunits. Subunit hybridisation can occur in the rat, but only between subunits in the same family. At least 12 GST have been described in rat liver as a consequence of such hybridisation [Hayes 1984, 1986]. Although GST are present in many tissues in the rat, a marked tissue-specific distribution of GST subunits exists [Hayes & Mantle 1986a]. For example, Ya is found only in liver, kidney and small intestine, whereas Yb is present in high concentration in most organs except kidney. Yf is distributed widely in extrahepatic tissues, and although not observed normally in rat liver, its concentration is increased markedly during the development of experimental, chemically-induced, hepatic carcinoma. For this reason, much attention has focused

on its potential to serve as a preneoplastic marker for hepatocarcinogenesis [Satoh et al 1985c].

Table 2.2

Rat cytosolic glutathione S-transferase isoenzymes

Source of GST	Class [a]	Adopted Name [b]	Enzyme [c]	Family [d]	Subunit Mr	pI
Liver	Alpha	1-1	YaYa	I	25,500	10.0
Liver	Alpha	1-2	YaYc			9.9
Liver	Alpha	2-2	YcYc	I	27,500	9.8
Liver	Mu	3-3	Yb_1Yb_1	II	26,300	8.4
	Mu	3-4	Yb_1Yb_2			8.0
Liver	Mu	4-4	Yb_2Yb_2	II	26,300	6.8
	[e]	5-5			26,500	7.3
Testis	Mu	6-6	YnYn	II	26,000	5.8
Kidney	Pi	7-7	YfYf	III	24,000	6.9
Liver	Alpha	8-8	YkYk	I	25,000	6.1

[a] Mannervik et al 1985; Mannervik & Danielson 1988

[b] Jakoby, Ketterer & Mannervik 1984

[c] Bass et al 1977

[d] Hayes & Mantle 1986a

[e] not yet classified

Human GST

In man, the glutathione S-transferases can be divided on the basis of their isoelectric points (pI) into three groups - basic, neutral and acidic [Mannervik 1985; Beckett & Hayes 1987; Warholm, Guthenberg & Mannervik 1983]. Within each group there is a range of pI values; the isoelectric points range from 9.0-7.3 for the basic GST, 6.5-5.5 for neutral, and 5.3-4.5 for the acidic group. Immunochemically, the basic, neutral and acidic GST correspond to the rat GST groups I, II and III respectively [Hayes & Mantle 1986a, 1986b]; physicochemically they most closely resemble rat Ya, Yb_2 and Yf subunits respectively [Hayes & Mantle 1986a, 1986b; Stockman, Beckett & Hayes 1985; Hayes et al 1983].

Table 2.3

Human glutathione S-transferase isoenzymes

Source of GST	Class	Enzyme	Group	Subunit Mr	pI
Liver	Alpha	B_1B_1	Basic	25,900	8.9
Liver	Alpha	B_1B_2	Basic	25,900	8.75
Liver	Alpha	B_2B_2	Basic	25,900	8.4
Liver	Mu	μ	Neutral	26,500	6.1
Liver	Mu	ψ	Neutral	26,500	5.5
Lung	Pi	λ	Acidic	24,800	4.8
Erythrocyte	Pi	ρ	Acidic	24,800	4.7
Placenta	Pi	π	Acidic	25,200	4.8

The nomenclature used to describe human basic GST was based initially on the purification scheme devised by Kamisaka and colleagues (1975). These workers isolated and characterised five basic transferases from human liver, designated α, β, gamma, δ and ϵ on the basis of increasing isoelectric points. More recently, other workers have isolated a neutral form (transferase μ) [Warholm, Guthenberg & Mannervik 1983] and three acidic forms (transferases lambda [Dao et al 1984], π [Mannervik & Guthenberg 1981] and rho [Marcus, Habig & Jakoby 1978]) from human tissue. There is evidence to suggest that transferase π is identical to transferase rho [Guthenberg & Mannervik 1981], although Vander Jagt and coworkers (1985) have shown that small size differences exist between the subunits of these transferases.

The subunit composition of the GST isoenzymes in man has been identified for only three of the basic GST; that of the neutral and acidic forms remains unknown. At least two distinct basic subunits exist, B_1 and B_2, both with Mr 25,900. These can hybridise to form B_1B_1, B_1B_2 and B_2B_2 subunit combinations [Stockman, Beckett & Hayes 1985], known formerly as "basic", N/A1 and N/A2b respectively [Hayes et al 1983]. These forms appear to correspond to transferases ϵ, δ and gamma respectively, described by Kamisaka and coworkers (1975). The term, ligandin, used in earlier literature [Bass et al 1978; Sherman et al 1983a; Tsuru et al 1978], appears to refer to both B_1 and B_2 subunits (Table 2.4). There is

controversy as to whether basic GST can hybridise with a subunit of an acidic form. Although Singh and colleagues (1985) demonstrated that this may be possible, most other workers agree that such combination cannot occur [Stockman, Beckett & Hayes 1985; Soma, Satoh & Sato 1986; Hussey et al 1986b]. Much, therefore, remains to be learned about the quaternary structure of the GST isoenzymes in man.

Table 2.4

Equivalent nomenclature for human basic GST

Reference	[a]	[b]	[c]	[d]
	B_1B_1	basic	ϵ	ligandin
	B_1B_2	N/A1	δ	ligandin
	B_2B_2	N/A2b	gamma	ligandin

[a] Stockman, Beckett & Hayes 1985

[b] Hayes et al 1983

[c] Kamisaka et al 1975

[d] Bass et al 1978; Sherman et al 1983a; Tsuru et al 1978

Until recently it was considered that major species differences existed in the occurrence of the multiple forms of GST. Further characterisation and comparisons of GST from three mammalian species (rat, mouse and man)

has, however, suggested that mammalian GST should be divided into three classes, based on enzymatic, immunological and physicochemical properties. Further support for this classification has been obtained by elucidating the amino-terminal amino acid sequences of the various GST and identifying the similarities between species in each class. The three classes now recognised are alpha, mu and pi [Mannervik et al 1985]. These would appear to correspond to rat GST families I, II and III, and human basic, neutral and acidic GST respectively (Tables 2.2 and 2.3).

TISSUE DISTRIBUTION

GST are cytosolic proteins, with the exception of a microsomal form that has been identified in rodents [Morgenstern, DePierre & Jörnvall 1985]. Several isoenzymes of GST have been demonstrated in each of the animal species investigated. However, when different organs in the same animal are examined, it is generally found that not all of the multiple forms of enzyme are present in every tissue. In the rat, the organ studied most intensively is the liver; at least 12 different isoenzymes occur in the cytosolic fraction. GST have been identified also in other rat organs including testis, seminiferous tubules, epididymis, kidney, lung, heart and gastrointestinal tract, although definitive characterisation has not been possible for all sources [Mannervik 1985].

Accurate description of the tissue distribution of human GST isoenzymes is hampered by the possibility of heterogeneity in each of the three groups characterised, ie acidic, neutral and basic. GST are found in greatest concentration in liver, kidney, duodenum, testis and adrenal [Sherman, Titmuss & Kirsch 1983b], although they have been demonstrated also in other tissues, most notably placenta [Mannervik & Guthenberg 1981] and erythrocytes [Marcus, Habig & Jakoby 1978]. Human liver contains predominantly basic GST; neutral GST are found in the liver in approximately 60% of individuals [Sherman, Titmuss & Kirsch 1983b; Hussey et al 1986b; Warholm, Guthenberg & Mannervik 1983]; acidic GST are found only in low amounts in normal liver [Awasthi, Dao & Saneto 1980], but their concentration increases greatly in the presence of hepatic tumour [Soma, Satoh & Sato 1986]. Basic GST have been isolated also from human kidney [Sherman, Titmuss & Kirsch 1983b], whereas the GST present in lung, placenta and erythrocytes are acidic in nature [Beckett & Hayes 1987]. Using immunohistochemical staining, Redick, Jakoby and Baron (1982) demonstrated that GST were not distributed uniformly throughout the liver lobule, but that they were present in greatest concentration in the centrilobular region, with much lower concentrations in the midzonal and periportal regions; this contrasts with the mainly periportal distribution of the aminotransferases.

Human GST are subject to marked polymorphism [Hussey et

al 1986b]; genetic variation has been proposed to account for this. Using starch-gel electrophoresis and histochemical staining, three gene loci, GST-1 (mu class, neutral), GST-2 (alpha class, basic) and GST-3 (pi class, acidic), have been described [Board 1981]. More recently, two products from the GST-1 locus have been discovered, termed GST-1 type 1 and GST-1 type 2 [Suzuki et al 1987], and there are also at least two products from the GST-2 locus [Boyer 1989]. Therefore, human GST are thought to be the products of at least three, and possibly five, separate gene families. The presence of three genetically distinct types of human GST has been confirmed by Warholm and colleagues (1983).

MEASUREMENT OF PLASMA GST

Intracellular enzymes released into the peripheral circulation following tissue damage can be measured in two main ways. The much commoner method is to measure their catalytic activity, but an alternative is to measure their concentration using immunological methods such as radioimmunoassay (RIA). There are advantages and disadvantages to both techniques. Enzymic activity measurement is generally rapid and easy to perform, but is susceptible to sources of error in the presence of circulating inhibitors. It can also be difficult to distinguish among different isoenzyme forms. Immunoassay methods are not affected by either of these limitations, but they are often slow and tedious to perform, and the availability of the necessary antisera

may be limited. Plasma levels of GST have been measured using both techniques.

Enzymic activity

GST activity was first demonstrated in rats, using bromsulphthalein [Combes & Stakelum 1961] and 3,4-dichloronitrobenzene [Booth, Boyland & Sims 1961] as electrophilic substrates. Both these substrates are specific for the rat Yb_1 subunit, and display virtually no activity with other subunits, including human GST [Beckett & Hayes 1987]. Another substrate, 1-chloro-2,4-dinitrobenzene (CDNB), which is active with most forms of GST, was introduced some 10 years later [Clark, Smith & Speir 1973]. Unfortunately, the specific activity of different isoenzymes with CDNB varies significantly, making it difficult to obtain sufficient sensitivity to allow precise measurement of plasma GST activity. Two further sources of serious error apply to GST activity measurement. Firstly, many non-substrate ligands including bilirubin and bile salts, the plasma concentration of which may be increased in liver disease, can bind to GST thus inhibiting their activity [Kamisaka et al 1975]. Secondly, acidic forms of GST from lung, placenta, erythrocytes and other tissues also conjugate CDNB with glutathione, resulting in poor organ specificity. In addition, haemolysis can interfere with activity measurement due to the release of erythrocyte GST.

Radioimmunoassay

With the introduction of immunoassay methods, specific forms of GST can be measured in plasma, with greater sensitivity than enzymic activity methods. The first RIA technique, which measured rat ligandin (YaYa), was developed by Bass and coworkers (1977) to investigate the tissue distribution of ligandin. Later these workers demonstrated that measurement of plasma GST YaYa concentration was a more sensitive index of experimental hepatocellular necrosis than the measurement of serum aminotransferase activity [Bass et al 1978]. Species specificity however, precludes the use of the rat RIA in man.

Tsuru and colleagues (1978) first described a RIA for human ligandin (alpha class; basic GST), and showed increased ligandin concentrations in a variety of liver disorders. However, there was considerable overlap between the concentrations measured in controls and in those with liver disease. Unfortunately, the poor sensitivity of this method was such that the lower limit of the reference range could not be defined; rather the plasma ligandin concentration in control patients did not exceed 5.3 ng ml^{-1}. Sherman and coworkers (1983a) have also described a RIA method for human ligandin, in which serum ligandin concentrations correlated significantly with histological severity of disease. Again though, this particular RIA was not sufficiently sensitive to define the lower limit of the reference range, and the

minimum detection limit of 1 ng is rather high.

In the same year a RIA was developed in man for the detection of GST B_2B_2. This method had sufficient precision and sensitivity to define both an upper and lower limit of a reference range (0.5 - 2.6 µg l^{-1}); it had a detection limit of 12 pg [Hayes et al 1983]. Subsequently, a RIA method for measuring GST B_1B_1 was described which had similar sensitivity [Beckett & Hayes 1984]. The range of GST B_1B_1 concentrations in healthy volunteers who had no clinical or biochemical evidence of liver disease was found to be 1.2 - 4.1 µg l^{-1}. Both B_1B_1 and B_2B_2 GST are equally effective as immunogens. The antisera produced to these immunogens are highly specific (Table 2.5).

Table 2.5

Specificities of antisera used in the radioimmunoassay of B_1B_1 and B_2B_2 GST (modified from Beckett et al 1985b)

	Relative cross-reactivity (%)	
GST	Anti-B_2B_2 GST	Anti-B_1B_1 GST
B_1B_1	0.6	100
B_1B_2	58	55
B_2B_2	100	0.2
Erythrocyte	<0.1	<0.1

Antisera raised to B_1B_1 GST immunogens show little cross-reactivity with GST B_2B_2, while the converse is true for antisera raised to a B_2B_2 immunogen. However, considerable cross-reactivity of GST B_1B_2 occurs with antisera raised to either B_1B_1 or B_2B_2 GST, indicating that GST B_1B_2 is a hybrid enzyme comprising a monomer from each of B_1B_1 and B_2B_2 GST. Erythrocyte GST shows no cross-reactivity with either of the antisera raised to hepatic GST, allowing specific measurement of hepatic GST even in haemolysed plasma [Beckett et al 1985b].

The radioimmunoassay methods developed for the measurement of GST B_1B_1 and B_2B_2 in plasma are much more sensitive than those described by other workers. Both assays have good organ specificity; there is little or no cross-reactivity with the neutral or acidic GST, and even gross haemolysis produces no change in GST concentrations when measured by RIA [Hayes et al 1983]. GST B_1B_1 is now measured in preference to B_2B_2 as it provides greater sensitivity, and measurement of both types offers no additional useful information. As with other immunoassay techniques, the chief limitation of these methods is the long incubation period required (66 hours) which contrasts with the few minutes it takes to perform measurement of serum aminotransferase activities. Clinical studies have been performed to assess the usefulness of plasma GST measurement compared with standard biochemical liver tests.

GST AND LIVER DISEASE

Adachi and coworkers (1980) measured serum GST activity in a variety of liver diseases including acute, fulminant and chronic hepatitis, using CDNB as substrate. GST activity increased significantly in cases of acute hepatitis, primary hepatoma and metastatic liver disease, with extremely high activity occurring in some cases of fulminant hepatitis. Serum GST and aminotransferase activities correlated well in acute and fulminant hepatitis, and primary liver carcinoma, while in chronic hepatitis and cirrhosis the correlation was poor. Serum GST activity decreased much more rapidly than did that of ALT or AST in cases of acute and fulminant hepatitis. The authors concluded that serum GST measurements would provide valuable diagnostic information in acute liver disease. Interpretation of their data is difficult, however, not only because of the poor sensitivity of the enzymic method of GST measurement, but also because many patients had an increased serum concentration of bilirubin which can inhibit serum GST activity. Although there existed a reasonable correlation between GST activity and serum ligandin concentration measured by radioimmunoassay, the numbers involved were very small and the correlation itself was not particularly strong. Therefore, their assumption that measurements of GST activity and ligandin concentration in serum have almost the same clinical significance is not well founded.

Early animal and human studies in which plasma ligandin

concentrations were measured by radioimmunoassay had shown that GST provides a more sensitive index of acute hepatocellular necrosis than does serum aminotransferase activity [Bass et al 1978; Sherman et al 1983a]. Furthermore, in patients with chronic active hepatitis, plasma GST concentration correlated strongly with the histological severity of disease, unlike ALT activity [Sherman et al 1983a]. More recently, plasma GST concentrations have been measured in patients with acute and chronic forms of liver disease.

Beckett and colleagues (1985a, 1985b) measured GST B_1B_1 and B_2B_2 concentrations by specific radioimmunoassays in patients with paracetamol poisoning, to investigate the time course of hepatocellular damage, and to monitor the response to treatment with N-acetyl cysteine. Seventeen of the 18 patients studied had abnormal GST concentrations; in contrast, aminotransferase activity was increased in only seven patients. The high incidence of abnormal GST concentrations (94%), is consistent with the results of a large study of histological changes following paracetamol overdose, in which 98% of patients showed histological evidence of liver damage, but only 44% of patients showed an abnormality of plasma AST activity [James et al 1975]. The increases in GST concentration occurred much earlier, and were considerably more abnormal than the abnormalities of aminotransferase activity. Similarly, GST concentration fell rapidly after peak values were

reached, in contrast with the activities of the aminotransferases; plasma half-life of GST B_1B_1 and B_2B_2 was calculated to be approximately one hour [Beckett et al 1985b]. Therefore, the rapid release of GST into plasma following hepatocellular damage, and its relatively short plasma half-life, allows early detection of injury and early recognition of when resolution begins.

Measurement of plasma GST concentration has proved less valuable in monitoring the course of acute viral hepatitis. Sherman and colleagues (1983a) measured GST concentration and AST activity in sequential plasma samples to examine the changes in liver function that occurred in 68 patients with acute viral hepatitis. At the time of presentation 67 patients had increased GST concentrations, and AST activities were increased in all patients. In patients positive for hepatitis B surface antigen, GST concentration had returned to normal values within 8 weeks, when 20% of patients still had increased plasma AST levels. Similarly, in patients with non-B hepatitis, 62% had persistently increased AST activity when GST concentration had returned to normal. The rapid disappearance of GST from plasma was attributed to its short plasma half-life, which it was concluded, weakened its use as a diagnostic test in acute viral hepatitis. GST measurement may, however, be a useful prognostic indicator, as its disappearance from plasma correlates with recovery from the acute illness [Beckett

& Hayes 1987].

Patients with chronic active hepatitis have consistently increased GST concentrations [Tsuru et al 1978] which correlate well with abnormal liver histology [Sherman et al 1983a]. Results from patients with cirrhosis are somewhat equivocal, some workers finding increased GST activity [Tsuru et al 1978], while other investigators have reported normal values [Adachi et al 1980] even with coexisting raised plasma AST activity [Sherman et al 1983a]. On the other hand, GST is a useful test in detecting subclinical liver damage in hyperthyroidism and in hypothyroid patients receiving thyroxine replacement therapy [Beckett et al 1985c]. GST also appear to be readily released into the peripheral circulation in patients with primary hepatocellular carcinoma, but not in those with hepatic metastases or with primary extra-hepatic tumours [Ohmi & Arias 1981].

GST have been investigated also in experimentally-induced chemical hepatotoxicity. Aniya and Anders (1985b) studied the effects of several hepatotoxins on hepatic and serum GST activity in rats. A marked decrease in hepatic GST activity, and an increase in serum GST activity, was observed after administration of CCl_4 or bromobenzene. These alterations in GST activity were dependent upon pretreatment with phenobarbitone or ethanol. Hepatic damage caused by bromobenzene and CCl_4 is associated with active metabolite formation via

cytochrome P-450, and is potentiated by phenobarbitone or ethanol treatment. It was suggested that reactive metabolites produced from bromobenzene and CCl_4 biotransformation contributed to the observed alterations in GST activity. The increase in serum GST activity was accompanied by an increase in both serum AST activity and serum bilirubin concentration. As with other studies measuring serum GST activity, the poor sensitivity of the enzymic assay and the potential inhibition of GST activity by increased serum bilirubin confuse the interpretation of this data.

LIVER ENZYME TESTS AND ANAESTHESIA

Although minor changes in liver biochemical tests are considered to be common after general anaesthesia, the incidence of mild hepatic damage which is clinically undetected is not known [Cousins 1980], and few studies have examined prospectively the incidence of such abnormalities.

Standard liver enzyme tests

Despite the relatively low specificity of the aminotransferases for minor degrees of hepatocellular damage, ALT and AST have often been used as markers of liver damage after anaesthesia. However, using such measurements in man, several prospective studies of liver function after halothane anaesthesia have provided

conflicting results. Some studies have demonstrated a greater frequency of increased serum aminotransferase activity in patients receiving repeated halothane anaesthetics than in those receiving non-halothane anaesthesia [Trowell, Peto & Smith 1975; Wright et al 1975; Fee et al 1979]. In contrast, Allen and Downing (1977) could demonstrate only minimal changes in aminotransferase activities, while McEwan (1976) found fewer disturbances of liver function after halothane than after non-halothane anaesthesia.

The differing results from these studies require further consideration. In the study by Fee and colleagues (1979), the difference in frequency of increased enzymic activity between patients who received halothane and those who received enflurane disappears when obese patients and those exposed to halothane in the preceding 6 weeks are excluded. In their investigations of biochemical liver disturbance after anaesthesia, McEwan (1976) studied 41 patients and Trowell, Peto and Smith (1975) studied only 39; these numbers are too small to allow interpretation of their observations with confidence. The remaining studies by Wright and coworkers (1975), and Allen and Downing (1977), appear to disagree upon the effect of repeated halothane anaesthesia on serum aminotransferase activities, despite examining patients under similar circumstances. However, Wright and colleagues measured serum aminotransferase activities every 3 to 5 days after

operation for two weeks or more, whereas Allen and Downing measured AST only up to 72 hours after operation. Only two patients in the former study had developed increased aminotransferase activities by the 3rd postoperative day; in the remaining 13 patients AST increased only in the second week after operation. It is possible, therefore, that Allen and Downing might have recorded a greater frequency of increased AST activity had they continued measurement for a longer period. It seems likely that the inconsistencies in these studies may reflect difficulties in defining a sensitive indicator of mild hepatocellular dysfunction.

Aminotransferase activities after operation are influenced also by the nature of the surgery. Clarke, Doggart and Lavery (1976) measured the activities of ALT and AST in patients undergoing four different types of surgical procedure - minimal surgery such as cystoscopy; body surface operations such as herniorrhaphy; gastric surgery; and biliary surgery. No abnormalities were detected after minimal surgical procedures lasting up to one hour. After body surface operations, aminotransferase activities showed minor, transient derangements, while both gastric and biliary surgery resulted in more marked alterations which resolved by the 4th day after surgery. ALT and AST activities increased within one hour of starting surgery, although the investigators considered that these changes were probably not related to liver function.

Schemel (1976) has highlighted a further problem in using aminotransferase activities to diagnose liver dysfunction after anaesthesia. He found that 11 of 7620 healthy patients with no intercurrent disease scheduled for elective surgery had clearly abnormally increased AST, ALT or LDH activities before operation. Surgery was deferred in these patients. All eleven patients proved to have overt liver pathology by further testing, and three subsequently developed jaundice. It is interesting to speculate whether this biochemical evidence of liver damage would have been attributed to the anaesthetic agent had surgery proceeded.

Specific liver enzyme tests

Thompson and Friday (1978) measured serum OCT activity in two comparable groups of adult male patients anaesthetised with halothane or enflurane for vagotomy and pyloroplasty, and demonstrated a biphasic increase in OCT activity in both groups. OCT activity increased significantly in the afternoon following operation, and again on the 5th to 7th postoperative day, when compared with values before operation. These changes tended to parallel increases in ALT activity, although ALT activity did not exceed 60 U l^{-1} in any patient. Despite studying only 24 patients, minimal but reversible increases in OCT activity were demonstrated in 75% of subjects after halothane and enflurane anaesthesia, although no difference was observed between the two agents.

Another group of investigators estimated the activities of seven liver enzymes, including ASAL, before and after cholecystectomy in patients anaesthetised with either halothane or methoxyflurane, or with nitrous oxide and analgesic supplements [Kalow, Rogoman & Sims 1976]. They found that ASAL activity increased after operation in around 50% of patients in each anaesthetic group. Its activity was maximal by the 6th postoperative day and remained abnormally elevated for up to 10 days. However, the type of anaesthetic technique employed did not influence the magnitude of the average increase in ASAL, AST or ALT in the period after operation, suggesting perhaps, that the surgery itself exerted a greater effect on liver enzyme activities than the choice of anaesthetic technique.

Glutathione S-transferase

Aniya and Anders (1985a) injected chloroform into the peritoneal cavity of rats pretreated with phenobarbitone, and measured hepatic cytosolic and serum GST activity by enzymic assay. A marked increase in serum GST activity was observed 5 hours after chloroform treatment. This was accompanied by a decrease in hepatic GST activity, suggesting a leakage of hepatic GST into the peripheral circulation. Serum activity had returned to near control values by 24 hours. Treatment of rats with SKF 525A or cysteine prevented the chloroform-induced increase in serum GST activity. As these agents inhibit the covalent binding of chloroform metabolites to

macromolecules, it was proposed that the leakage of GST into serum resulted from chloroform metabolites becoming bound covalently to liver proteins. It is interesting that pretreatment with SKF 525A or cysteine has been observed to inhibit the development of hepatic necrosis after halothane anaesthesia in the phenobarbitone-hypoxia rat model [Jee et al 1980].

Two groups of workers have examined the effect of halothane on hepatic GST activity in rats. Dale and Nilsen (1984) measured GST activity in the livers of rats exposed to repeated subanaesthetic concentrations of halothane and enflurane. Repeated exposure to concentrations of 50-1000 ppm were used to avoid the effects of general anaesthesia and to increase the metabolised fraction of the inhaled anaesthetics. GST activity in hepatic cytosol decreased to around 85% of control values only in rats exposed to the higher concentrations of halothane or enflurane for several days. Ivanetich, Thumser and Harrison (1988) demonstrated that multiple halothane anaesthetics (1.25 MAC for one hour on 3 alternate days) initially decreased, and subsequently increased, total liver cytosolic GST activity in vivo. Maximal depression occurred 10 days after the first halothane administration; maximal activation was apparent some 15 days later. Isoflurane, enflurane and methoxyflurane appeared to inhibit hepatic GST activity in a time-dependent manner similar to halothane. It is not

known if these agents similarly activate GST as this was not investigated. No significant alteration in GST activity was noted after a single halothane anaesthetic (1.25 MAC for 3 hours). The effect of halothane on the activity of several hepatic GST isoenzymes in vitro was examined also. GST isoenzymes 3-3, 3-4 and 4-4, which correspond immunochemically to human neutral GST, exhibited reduced activity, while that of isoenzyme 1-2 increased; the activity of isoenzyme 1-1 (YaYa, rat ligandin) remained unchanged throughout. Therefore, in the rat, it would seem that the reduction in hepatic GST activity after halothane anaesthesia results from disappearance of neutral, and not basic, GST.

This and the previous study have some important limitations. In neither study was the serum activity of GST measured; it is therefore not possible to comment on whether or not the reduction in hepatic GST activity resulted from leakage of specific transferases into serum. Furthermore, in both studies the activity of GST was measured using CDNB as substrate. The variation in specific activity of different isoenzymes with CDNB makes it difficult to obtain sufficient sensitivity to measure accurately individual GST isoenzyme activity. The finding that activation of hepatic GST activity could occur after halothane exposure has not been confirmed by other studies, which have shown no increase in hepatic GST activity after administration of halothane or other potential hepatotoxins [Aniya & Anders 1985a, 1985b; Dale

& Nilsen 1984]. This activation must be of questionable physiological significance as the process was readily reversible, and exhibited only low extents of activation, even with massive concentrations of halothane. Therefore, the measurement of hepatic and/or serum GST activity does not provide much useful information in assessing the changes in hepatocellular integrity which occur following general anaesthesia.

Plasma GST concentrations have been measured by specific radioimmunoassays to investigate hepatocellular integrity after halothane anaesthesia in man. In a pilot study, Hussey and coworkers (1986a) measured GST B_1 and B_2 subunits in 28 patients undergoing general anaesthesia with halothane. In 23 patients, GST concentration was increased 1 to 3 hours after the end of anaesthesia, compared with the concentration before operation. In most of these instances GST concentration had decreased to control values by the following day. However, in three patients a much larger secondary increase was noted 24 hours after the end of anaesthesia. Thirteen patients (46%) had abnormally increased GST concentrations after operation, whereas no significant changes in plasma ALT activity were noted.

Similar changes in GST concentration were observed in a larger study by Allan and colleagues (1987). They measured the plasma concentrations of GST B_1B_1 in 71 patients undergoing minor urological surgery, who were

randomised to receive either halothane or isoflurane in 70% nitrous oxide, or halothane in 100% oxygen. GST concentrations did not change significantly in patients who received isoflurane, but they increased in most patients in both groups who received halothane. Concentrations had usually reached a peak between 3 and 6 hours after anaesthesia, and had returned to normal by 24 hours in all but five of the 54 patients who received halothane. The occurrence of abnormal GST concentrations after anaesthesia was greater in patients who received halothane in nitrous oxide than in those who received halothane in oxygen alone (35% compared with 24%), but this difference did not achieve statistical significance; no patient who received isoflurane developed an abnormal GST concentration.

There appear to be two distinct phases of increased GST concentration following halothane anaesthesia; one occurs consistently within 3 to 6 hours of anaesthesia, while the second occurs at 24 hours in a small number of patients. The earlier phase may be due to relative hepatic hypoxia resulting from halothane-induced depression of hepatic blood flow, while it is possible that the second, less frequent, phase results from metabolism of halothane to toxic metabolites [Hussey et al 1986a; Allan et al 1987]. It is unlikely that pre-existing liver pathology was responsible for the changes in GST concentration after anaesthesia, as in both studies patients with clinical or biochemical

evidence of liver dysfunction were excluded from analysis, as were those who admitted to a high alcohol intake. As basic GST are also present in small amounts in human kidney, it is possible, although unlikely, that renal release of GST may have contributed to the increased plasma concentrations. Although the changes in median GST concentrations observed after anaesthesia were small, some individual increases were large, and abnormal concentrations were recorded frequently, particularly in patients who received halothane. It would appear, therefore, that a short, single halothane exposure can produce transient hepatic damage shown by release of GST B_1B_1 into plasma, while isoflurane seems to be free of this potential.

In summary, measurement of plasma GST concentration by radioimmunoassay has much to offer in the investigation of acute, toxic liver injury. It provides a sensitive, specific and reproducible index of hepatocellular integrity and its concentration correlates well with the histological severity of disease. In addition, its short plasma half-life of around 90 minutes allows early detection of damage and its resolution. As GST are distributed mainly in the centrilobular region of hepatocytes, its measurement may be of considerable value in the investigation of halothane-induced hepatotoxicity, as this disorder is generally associated with centrilobular necrosis. The value of plasma GST

measurement is at present largely offset by the length of time required to produce reliable, precise measurements of plasma concentration; by RIA, it takes more than 48 hours. Further problems include the limited availability of GST standards, radio-label and antisera. Until the methods of assay are simplified and greatly speeded up there is no possibility that GST could replace aminotransferase measurement in the routine investigation of liver disease. It does, however, offer a unique opportunity for the further investigation of acute, drug-induced liver damage.

This thesis is based on an investigation of the effect of volatile anaesthetic agents on hepatocellular integrity in man, and subsequent attempts to modify such effects, using plasma concentration of GST B_1B_1 as a highly sensitive and organ-specific index of liver damage.

CHAPTER 3

THE EFFECT OF ANAESTHESIA WITH HALOTHANE, ENFLURANE OR ISOFLURANE ON PLASMA GST CONCENTRATION

CHAPTER 3

THE EFFECT OF ANAESTHESIA WITH HALOTHANE, ENFLURANE OR ISOFLURANE ON PLASMA GST CONCENTRATION

It has been demonstrated that significant increases in plasma GST B_1B_1 concentration occur after halothane anaesthesia, but not after anaesthesia with isoflurane [Allan et al 1987], suggesting that halothane is associated with transient, mild, hepatocellular dysfunction whereas isoflurane is not. As previous studies had examined GST changes in patients undergoing surgery of widely varying type and duration, and as the effect of enflurane anaesthesia on GST concentration was not known, it was decided to investigate the changes in plasma GST B_1B_1 concentration in patients administered halothane, enflurane and isoflurane for surgical procedures that were standard in type and duration.

METHODOLOGY

Patient selection

Patients aged between 18 and 70 years in ASA groups I or II undergoing body-surface operations such as varicose vein surgery or inguinal hernia repair were studied. Patients who had received a general anaesthetic in the previous 6 months, or who had pre-existing clinical or biochemical evidence of liver disease were excluded. Patients taking regular medication likely to interfere with liver function were excluded also. Subjects were

allocated according to a previously prepared, randomised list to receive either halothane, enflurane or isoflurane in an otherwise identical anaesthetic technique.

Anaesthetic technique

No premedication was given. Anaesthesia was induced with intravenous thiopentone 4-6 mg kg^{-1}, and maintained with the volatile agent vaporised in 33% oxygen and 67% nitrous oxide. All patients breathed spontaneously from a non-rebreathing anaesthetic system. After induction, intravenous diamorphine in a total dose of 0.07 mg kg^{-1} was titrated in increments to allow anaesthesia to be maintained with an inspired concentration of 1.0-1.5% for halothane, and 1.0-3.0% for enflurane and isoflurane.

Samples

Blood samples for measurement of GST B_1B_1 concentration were taken before induction of anaesthesia (pre), and 3, 6 and 24 hours after the end of anaesthesia. Plasma concentration of bilirubin and activities of ALT, GGT and alkaline phosphatase (ALP) were measured before anaesthesia and 24 hours after anaesthesia. Samples were separated by centrifugation and the resultant serum was stored at -20°C before measurement of GST.

Assay procedures

Concentrations of bilirubin and activities of ALT, GGT and ALP were measured using a Sequential Multiple Analysis with Computer (SMAC) System II (Technicon

Instrument Corporation, Basingstoke, UK). The normal ranges defined by the laboratory which performed these investigations are: bilirubin 2-17 μmol l^{-1}; ALT 10-40 U l^{-1}; GGT 10-55 U l^{-1} (males), 5-35 U l^{-1} (females); ALP 40-100 U l^{-1}. The concentration of GST B_1B_1 in plasma was measured by specific radioimmunoassay [Beckett & Hayes 1984], the reference range being 0.7-4.0 μg l^{-1} (derived from healthy, volunteer blood donors). The interassay coefficient of variation for the GST B_1B_1 RIA was less than 10% and the intra-assay coefficient of variation was less than 5% over the range 2-40 μg l^{-1}.

Statistical analysis

Patient and anaesthetic characteristics in each group were compared using the Kruskal-Wallis test. Changes in GST concentrations within each group were compared using the Friedman test. The Wilcoxon signed rank test was used to compare GST concentrations at 3, 6 and 24 hours after anaesthesia with preoperative values in each group: to eliminate multiple testing errors, the Kruskal-Wallis test was used to compare the changes in GST concentration observed in the three groups at 3 hours after the end of anaesthesia. The incidence of abnormal values in each group was compared using a 4-fold table Chi-squared test. Comparison of conventional liver function tests after operation with preoperative values for each group used the Wilcoxon rank sum test.

RESULTS

One hundred and one patients were studied: 30 received halothane, 41 were administered enflurane and 30 received isoflurane. Patients whose preoperative plasma concentration of GST, or activities of ALT, GGT or ALP exceeded the upper limit of the reference range were excluded from analysis. As idiopathic hyperbilirubinaemia occurs in varying degrees in 5-10% of the population [Stoelting, Dierdorf & McCammon 1988], and as serum bilirubin concentration is not a sensitive indicator of hepatocellular dysfunction [Gitnick 1981], patients who had an increased serum concentration of bilirubin before operation with no other abnormal liver biochemical test were included in analysis.

The remaining 70 patients from whom data were analysed comprised 22 who received halothane (11 M, 11 F), 30 who received enflurane (16 M, 14 F) and 18 who were given isoflurane (10 M, 8 F). Comparisons between the groups revealed no significant differences in age, weight, height, alcohol consumption and duration of anaesthesia (Table 3.1). Significant changes occurred in plasma concentrations of GST after operation in all three groups (halothane $p<0.05$; enflurane $p<0.0001$; isoflurane $p<0.01$), and significant differences in these changes were observed between the groups ($p<0.05$, Tables 3.2 - 3.5). GST concentration was significantly increased at 3 hours after the end of anaesthesia in the group that

received halothane ($p<0.01$), and at 3 and 6 hours after anaesthesia in the group that received enflurane ($p<0.0001$ and $p<0.05$ respectively). No increase in GST concentration was observed after anaesthesia in patients who received isoflurane. In contrast, GST concentration was significantly reduced at 24 hours in this group; a decrease was not apparent in the other two groups. Four patients who received halothane, and three who were administered enflurane, exhibited large secondary increases in GST concentration, which exceeded the reference range, at 24 hours after anaesthesia. One of these patients who had received halothane also had increased serum ALT activity (116 U l^{-1}) at this time. Many patients, particularly those who received halothane or enflurane, developed concentrations of GST which exceeded the upper limit of the reference range; abnormal GST concentrations occurred in 50% of patients following halothane anaesthesia, 20% following enflurane and 11% after isoflurane. The incidence of abnormal GST concentrations after halothane anaesthesia was significantly greater than after anaesthesia with enflurane or isoflurane ($p<0.01$ and $p<0.05$, respectively); there was no significant difference between enflurane and isoflurane anaesthesia. No significant overall changes in the concentration of bilirubin or the activities of ALT, GGT or ALP occurred as a result of anaesthesia (Tables 3.6 - 3.9).

Table 3.1

Mean values (SD) for patient and anaesthetic characteristics for patients receiving halothane, enflurane and isoflurane who had normal preoperative liver biochemistry

	Halothane (n=22)	Enflurane (n=30)	Isoflurane (n=18)
Age (years)	42 (17)	50 (13)	40 (16)
Weight (kg)	70 (14)	71 (15)	69 (12)
Height (cm)	170 (10)	171 (12)	170 (11)
Alcohol (U day^{-1})	1.1 (1.4)	0.8 (1.2)	1.3 (1.4)
Duration of anaesthesia (min)	45 (16)	46 (14)	48 (21)

Table 3.3

Individual values for GST concentration (µg l^{-1}) for patients receiving enflurane

	PRE	3 h	6 h	24 h
1	2.2	2.9	2.9	2.0
2	2.4	3.0	2.8	2.3
3	2.3	3.0	2.8	2.0
4	3.6	3.4	3.1	1.4
5	3.2	3.9	3.7	2.6
6	3.4	3.6	3.2	2.6
7	1.6	1.6	1.6	1.4
8	2.5	3.9	2.6	2.7
9	1.4	2.1	1.9	1.7
10	1.9	2.5	2.7	1.0
11	1.7	2.2	5.5 *	1.2
12	2.3	3.0	3.6	4.3 *
13	1.9	2.6	2.4	1.9
14	2.1	2.4	2.4	2.3
15	0.9	1.2	1.6	1.3
16	3.7	4.0	3.0	5.8 *
17	3.4	4.3 *	3.9	2.8
18	2.5	2.7	2.2	2.2
19	2.1	2.7	3.0	3.0
20	3.2	3.8	3.0	2.2
21	1.3	1.3	1.5	1.1
22	2.2	2.2	2.0	2.1
23	3.0	2.8	2.9	4.8 *
24	2.8	3.1	2.8	3.0
25	0.9	1.8	3.4	1.2
26	2.6	3.1	3.2	2.7
27	3.2	3.9	2.9	1.7
28	3.0	3.1	2.9	3.2
29	2.8	4.5 *	4.3 *	3.5
30	1.5	1.6	1.9	1.4
Excluded from analysis				
31	7.7 *	14.2 *	7.7 *	3.2
32	4.5 *	4.0	4.2 *	2.0
33	5.1 *	6.0 *	4.4 *	9.0 *
34	3.1	3.4	3.4	2.5
35	5.0 *	4.7 *	4.3 *	2.1
36	3.7	3.5	4.8 *	15.0 *
37	3.2	---	2.2	3.2
38	4.8 *	5.7 *	4.6 *	2.9
39	9.5 *	9.6 *	6.2 *	5.6 *
40	5.0 *	6.3 *	5.2 *	3.4
41	3.2	4.1 *	2.8	1.8

* represents a value which exceeds the upper limit of the reference range

Table 3.3

Individual values for GST concentration (μg l^{-1}) for patients receiving enflurane

	PRE	3 h	6 h	24 h
1	2.2	2.9	2.9	2.0
2	2.4	3.0	2.8	2.3
3	2.3	3.0	2.8	2.0
4	3.6	3.4	3.1	1.4
5	3.2	3.9	3.7	2.6
6	3.4	3.6	3.2	2.6
7	1.6	1.6	1.6	1.4
8	2.5	3.9	2.6	2.7
9	1.4	2.1	1.9	1.7
10	1.9	2.5	2.7	1.0
11	1.7	2.2	5.5 *	1.2
12	2.3	3.0	3.6	4.3 *
13	1.9	2.6	2.4	1.9
14	2.1	2.4	2.4	2.3
15	0.9	1.2	1.6	1.3
16	3.7	4.0	3.0	5.8 *
17	3.4	4.3 *	3.9	2.8
18	2.5	2.7	2.2	2.2
19	2.1	2.7	3.0	3.0
20	3.2	3.8	3.0	2.2
21	1.3	1.3	1.5	1.1
22	2.2	2.2	2.0	2.1
23	3.0	2.8	2.9	4.8 *
24	2.8	3.1	2.8	3.0
25	0.9	1.8	3.4	1.2
26	2.6	3.1	3.2	2.7
27	3.2	3.9	2.9	1.7
28	3.0	3.1	2.9	3.2
29	2.8	4.5 *	4.3 *	3.5
30	1.5	1.6	1.9	1.4
Excluded from analysis				
31	7.7 *	14.2 *	7.7 *	3.2
32	4.5 *	4.0	4.2 *	2.0
33	5.1 *	6.0 *	4.4 *	9.0 *
34	3.1	3.4	3.4	2.5
35	5.0 *	4.7 *	4.3 *	2.1
36	3.7	3.5	4.8 *	15.0 *
37	3.2	---	2.2	3.2
38	4.8 *	5.7 *	4.6 *	2.9
39	9.5 *	9.6 *	6.2 *	5.6 *
40	5.0 *	6.3 *	5.2 *	3.4
41	3.2	4.1 *	2.8	1.8

* represents a value which exceeds the upper limit of the reference range

Table 3.4

Individual values for GST concentration (µg l^{-1}) for patients receiving isoflurane

	PRE	3 h	6 h	24 h
1	2.3	2.5	2.7	1.6
2	2.7	2.0	1.5	2.4
3	3.7	4.4 *	3.6	2.4
4	2.0	2.5	2.1	1.4
5	1.9	2.1	2.1	1.8
6	3.3	3.3	3.1	2.4
7	2.9	2.5	2.7	1.7
8	2.5	3.0	2.3	2.3
9	1.4	1.5	1.7	1.8
10	3.6	3.6	2.6	2.5
11	3.2	2.6	2.6	2.1
12	2.9	2.3	4.0	2.3
13	3.8	2.8	2.3	1.6
14	3.5	3.6	3.8	3.4
15	2.4	3.1	4.1 *	1.9
16	1.8	1.8	2.1	2.0
17	1.7	2.1	2.1	1.8
18	3.3	3.9	2.9	1.6
Excluded from analysis				
19	6.3 *	8.3 *	5.0 *	4.6 *
20	6.9 *	5.3 *	4.3 *	26.0 *
21	4.9 *	4.6 *	3.9	2.4
22	6.3 *	7.2 *	3.1	3.8
23	4.4 *	3.7	2.5	2.8
24	8.8 *	14.7 *	7.3 *	5.7 *
25	8.0 *	7.4 *	6.8 *	4.3 *
26	4.2 *	4.2 *	3.5	1.8
27	8.0 *	6.1 *	4.6 *	3.7
28	4.3 *	3.6	5.1 *	5.0 *
29	5.7 *	5.2 *	4.2 *	4.5 *
30	8.2 *	4.4 *	3.1	2.6

* represents a value which exceeds the upper limit of the reference range

Table 3.5

Medians (interquartile range) of GST concentrations ($\mu g\ l^{-1}$) for patients receiving halothane, enflurane and isoflurane

	PRE	3 h	6 h	24 h
Halothane	2.9	3.4 **	3.3	2.5
	(2.3-3.4)	(2.6-4.1)	(2.2-4.0)	(1.8-3.5)
Enflurane	2.4	3.0 ***	2.9 *	2.3
	(1.9-3.0)	(2.2-3.9)	(2.4-3.2)	(1.4-2.8)
Isoflurane	2.9	2.6	2.6	2.0 *
	(2.0-3.3)	(2.1-3.3)	(2.1-3.1)	(1.7-2.4)

* $p<0.05$; ** $p<0.01$; *** $p<0.0001$
compared with preoperative concentration

Table 3.6

Conventional liver biochemical tests before and after operation in patients receiving halothane

	PREOP				POSTOP			
	Bil	ALT	GGT	ALP	Bil	ALT	GGT	ALP
1	9	15	12	85	11	18	9	80
2	11	11	10	39	--	--	--	--
3	11	<10	21	80	9	10	<5	88
4	8	18	12	66	11	12	21	71
5	7	<10	7	64	7	10	8	62
6	14	17	7	49	21 *	15	<5	44
7	12	11	10	84	17	14	7	68
8	11	<10	5	64	9	12	5	49
9	11	14	9	66	10	19	12	61
10	10	26	30	73	8	26	31	63
11	6	22	23	100	9	19	22	87
12	14	16	25	78	16	24	26	78
13	17	23	6	51	21 *	23	5	46
14	16	18	<5	65	6	12	5	55
15	9	16	11	57	10	116 *	52 *	70
16	8	13	8	76	10	11	7	66
17	9	<10	21	88	13	<10	21	78
18	18 *	16	16	84	19 *	16	20	66
19	13	13	15	81	13	13	12	74
20	9	19	11	79	9	16	10	71
21	14	23	15	95	26 *	20	18	75
22	9	16	7	69	7	14	9	63
Excluded from analysis								
23	12	36	24	61	18 *	30	25	36
24	11	37	45	81	17	35	47	75
25	13	60 *	7	80	11	39	8	76
26	9	17	18	56	9	10	21	53
27	11	26	35	102 *	9	21	23	81
28	27 *	13	18	103 *	29 *	15	18	93
29	15	24	17	53	9	18	12	39
30	19 *	13	7	125 *	34 *	30	15	123 *

* represents a value which exceeds the upper limit of the reference range

Table 3.7

Conventional liver biochemical tests before and after operation in patients receiving enflurane
(* abnormally elevated result)

	PREOP				POSTOP			
	Bil	ALT	GGT	ALP	Bil	ALT	GGT	ALP
1	17	14	16	82	22 *	11	10	88
2	7	--	--	46	8	12	8	44
3	12	--	--	64	9	13	7	59
4	18 *	12	12	53	15	<10	12	44
5	7	14	12	41	9	29	7	46
6	14	--	--	80	15	24	47	82
7	9	<10	22	55	9	<10	10	56
8	9	12	17	53	14	13	14	81
9	14	<10	11	68	13	<10	7	65
10	13	11	12	81	20 *	16	12	79
11	13	13	11	98	21 *	22	11	83
12	6	14	31	100	7	16	32	110
13	13	14	19	39	13	16	17	47
14	13	18	10	64	9	14	9	63
15	8	<10	7	58	9	<10	<5	66
16	10	15	25	68	9	10	18	67
17	20 *	20	29	67	--	--	--	--
18	13	13	7	64	9	<10	<5	64
19	15	16	27	92	25 *	13	25	84
20	11	33	41	85	14	28	31	84
21	7	<10	16	91	9	11	11	85
22	15	14	19	63	19 *	15	21	68
23	11	25	27	74	--	--	--	--
24	18 *	11	<5	57	20 *	14	10	43
25	10	<10	<5	51	15	10	<5	48
26	8	29	6	42	--	--	--	--
27	34 *	18	8	61	--	--	--	--
28	12	19	43	64	8	16	8	71
29	9	11	--	61	7	<10	8	71
30	11	11	<5	79	11	13	8	68
Excluded from analysis								
31	8	30	42	119 *	12	26	30	102 *
32	9	13	12	103 *	13	<10	11	89
33	16	41 *	25	44	11	34	22	42
34	--	--	--	--	7	10	--	81
35	10	29	41	63	12	22	37	68
36	12	21	56 *	89	16	37	52	84
37	11	35	20	79	7	27	15	81
38	11	32	53	66	10	25	--	67
39	9	40	50 *	90	11	32	38 *	92
40	17	18	16	67	14	19	17	80
41	--	--	--	--	--	--	--	--

Table 3.8

Conventional liver biochemical tests before and after operation in patients receiving isoflurane

	PREOP				POSTOP			
	Bil	ALT	GGT	ALP	Bil	ALT	GGT	ALP
1	10	<10	12	50	11	11	10	39
2	12	23	22	93	16	19	20	91
3	8	27	37	78	10	22	35	75
4	23 *	23	17	84	11	14	15	79
5	16	15	13	69	13	12	--	56
6	8	12	15	63	11	13	6	54
7	9	18	26	66	6	16	22	60
8	7	14	8	55	8	<10	12	65
9	19 *	--	7	57	17	<10	7	51
10	7	25	24	63	10	22	21	59
11	16	20	7	70	15	14	8	59
12	6	10	11	83	4	<10	12	80
13	11	14	9	73	9	15	8	69
14	13	18	49	100	10	12	38	85
15	8	<10	6	54	10	<10	9	53
16	15	10	8	35	16	10	9	33
17	11	21	14	58	12	13	11	40
18	13	22	23	92	--	--	--	--
Excluded from analysis								
19	10	37	38	56	11	31	33	52
20	10	39	12	113 *	18 *	76 *	28	103 *
21	17	12	12	124 *	23 *	11	11	103 *
22	16	27	36	94	8	22	25	87
23	--	--	--	--	14	20	39	63
24	22 *	68 *	21	87	25 *	51 *	18	83
25	12	33	18	64	15	37	24	73
26	14	27	47	69	14	18	39	55
27	12	32	27	60	16	32	21	63
28	8	23	13	71	8	14	12	72
29	17	26	30	116 *	11	20	24	96
30	6	13	6	67	6	10	8	64

* represents a value which exceeds the upper limit of the reference range

Table 3.9

Median values (range) of conventional liver function tests for patients receiving halothane, enflurane and isoflurane

	Bilirubin ($\mu g\ l^{-1}$)	ALT ($U\ l^{-1}$)	GGT ($U\ l^{-1}$)	ALP ($U\ l^{-1}$)
Halothane				
PRE	11 (6-18)	16 (<10-26)	11 (<5-30)	76 (39-100)
24 h	10 (6-26)	15 (<10-116)	10 (<5-52)	68 (44-88)
Enflurane				
PRE	12 (6-34)	14 (<10-33)	16 (<5-43)	64 (39-100)
24 h	13 (7-25)	13 (<10-29)	10 (<5-47)	68 (43-110)
Isoflurane				
PRE	11 (6-23)	18 (<10-27)	14 (6-49)	69 (35-100)
24 h	11 (4-17)	13 (<10-22)	12 (6-38)	59 (33-91)

DISCUSSION

This study has demonstrated marked differences in the effect of three volatile anaesthetic agents on plasma GST B_1B_1 concentration after anaesthesia. These differences were not attributable to patient selection, as the three groups were comparable with respect to factors such as age, sex, alcohol intake, and type and duration of surgery. Significant increases in GST concentration were observed at approximately 3-6 hours after anaesthesia with halothane and enflurane, but not after isoflurane. These findings are in agreement with the results obtained from a previous study, in which halothane and isoflurane only were studied [Allan et al 1987]. Although the changes in plasma GST concentration were small, a large number of abnormal concentrations were recorded, particularly in the group which received halothane. In addition, secondary and more marked increases in GST concentrations were seen 24 hours after anaesthesia in a few of the patients who had received halothane and enflurane, although this was not observed in patients administered isoflurane.

How do these observations relate to the mechanisms which have been proposed to account for hepatotoxicity caused by volatile anaesthetic agents? The data presented provide evidence for two distinct phases of impaired hepatocellular integrity after anaesthesia with halothane and enflurane, but not with isoflurane. The first phase

ocurred in the majority of patients within 3-6 hours of administration of the agent. This is likely to be caused by the direct depressant effect of these agents on hepatic blood flow resulting in relative tissue hypoxia. In contrast, administration of isoflurane, which preserves hepatic blood flow to a much greater extent than halothane and enflurane, did not cause an increase in GST concentration. The second phase occurred 24 hours after anaesthesia, when large secondary increases in GST concentration were observed occasionally. This latter phase may result from the production of toxic metabolites. The changes in GST concentrations seen at this time support evidence for the existence of the mild subclinical form of halothane hepatitis that has been defined by other workers [Touloukian & Kaplowitz 1981; Neuberger & Williams 1984; Pohl & Gillette 1982; Brown & Gandolfi 1987]. It is interesting that the frequency of abnormal GST concentrations seen after halothane, enflurane and isoflurane correlates directly with the extents of metabolism and incidence of reported clinical hepatic dysfunction for these drugs.

It has been suggested privately that the changes in GST concentration after anaesthesia may not reflect mild hepatocellular damage, but rather may result from a circadian variation in plasma GST concentration, or from reduced GST clearance. Aldridge and colleagues (1991) have measured sequential plasma concentrations of GST B_1B_1 in 30 healthy volunteers in a 24 hour period. The

volunteers fasted overnight, and on the following day blood was sampled at 0900 for measurement of GST concentration. Further samples were taken 3, 6 and 24 hours after this initial sample, time intervals that correspond to those of the present study of patients undergoing anaesthesia. GST concentration did not increase at any time; in contrast to the changes observed in the present study, GST was significantly reduced at 3 hours ($p<0.05$, Wilcoxon signed rank test; Table 3.10). The increases in GST concentration observed after halothane and enflurane anaesthesia are, therefore, unlikely to have resulted from diurnal variation in plasma GST concentration.

Table 3.10

Medians (interquartile range) of GST concentrations ($\mu g\ l^{-1}$) for nonanaesthetised volunteers

Initial fasting	3 h	6 h	24 h
1.9 (1.4-2.6)	1.7 * (1.3-2.1)	1.9 (1.4-2.1)	1.9 (1.5-2.6)

* $p<0.05$ compared with initial fasting concentration

To examine the second possibility that reduced GST clearance was the cause of the observed increases in GST concentration, serum amylase activity was measured in the

samples obtained from 10 patients who received halothane, and in whom GST was increased at 3 hours after induction of anaesthesia. If reduced clearance was responsible for this increase in GST concentration, then amylase activities should also be increased. In fact, in all 10 patients serum amylase activity was lower at 3 hours after anaesthesia compared with preoperative values, suggesting that clearance is not reduced [personal observations]. Therefore, it would appear that increases in plasma GST concentration after anaesthesia do reflect a mild degree of hepatocellular damage, and do not result from diurnal variation or reduced clearance.

Nineteen of the 70 patients entered into analysis exhibited an abnormal plasma concentration of GST after operation; in contrast, abnormal ALT activity was observed in only one patient. This discrepancy does not mean that GST measurement is too sensitive for the detection of minor hepatocellular damage. Rather, it may simply reflect the much more rapid release of GST into the circulation after damage has occurred, and also the different half-lives of GST and ALT in plasma (about 90 minutes and 48 hours respectively). A greater number of abnormal values for ALT activity might have been observed if samples had been taken 48-72 hours after induction of anaesthesia. This was not undertaken as many patients had returned home by that time.

In compliance with the study programme, patients who had

increased concentration of GST or abnormal activities of ALT or GGT before operation were excluded from analysis. It could be argued that a degree of bias was introduced into the results by selecting "normal" patients. However, an examination of GST concentrations after operation in the 31 patients so excluded, revealed a pattern of GST change similar to that seen in those patients who were entered into analysis.

Changes in plasma GST concentration clearly demonstrate the stages of hepatotoxicity which may follow anaesthesia. Their measurement may help in the understanding of the mechanisms involved in volatile agent-induced liver dysfunction and should allow assessment of therapeutic manoeuvres predicted to minimise such toxicity. The following three chapters describe the measurement of plasma GST B_1B_1 concentration to investigate further three of the proposed aetiologies for the development of halothane hepatitis, namely altered cellular calcium homeostasis, biotransformation and regional hepatic hypoxia.

CHAPTER 4

THE EFFECT OF NICARDIPINE ON PLASMA GST CONCENTRATION AFTER HALOTHANE ANAESTHESIA

CHAPTER 4

THE EFFECT OF NICARDIPINE ON PLASMA GST CONCENTRATION AFTER HALOTHANE ANAESTHESIA

The role of calcium in mediating or propagating ischaemic cell injury, and the concept that increased concentrations of intracellular calcium constitute the final common pathway of cell injury have been reviewed [Cheung et al 1986; Thomas & Reed 1989; Farber 1982]. Gelman and Van Dyke (1988) have introduced a similar "calciogenic" hypothesis for the development of liver toxicity associated with volatile anaesthetics. They postulated that damage occurs as a result of disruption of mechanisms which maintain cellular calcium homeostasis, and that the difference in toxicity between agents might result from a different ability of anaesthetics to disrupt intracellular calcium homeostasis. In support of this hypothesis it is known that a number of hepatotoxins, including halothane, can disrupt intracellular calcium homeostasis resulting in excessive accumulation of cytosolic calcium [Moore 1980; Moore et al 1985; Brattin et al 1984; Zucker, Diamond & Berman 1982; Long & Moore 1986]. Furthermore, in the guinea pig model of halothane hepatotoxicity, large increases in total hepatic calcium content occur after exposure to halothane. The increase in liver calcium occurs before significant histological abnormality is observed, and is related to the severity of the necrosis [Farrell et al 1988].

The administration of a calcium channel blocker before the initiation of cell injury may, therefore, prevent a large secondary influx of calcium into the cell, and limit the severity of cellular damage. The calcium channel blocker nifedipine has a cell protective effect in ischaemic myocardial injury in man [Stone et al 1980] and dogs [Henry et al 1978], probably by increasing collateral perfusion in the ischaemic area. Similarly, the administration of flunarizine to rats, and nimodipine to dogs, can reduce the extent of experimentally-induced ischaemic brain damage [Deshpande & Wieloch 1986; Steen et al 1983]. The phenothiazine chlorpromazine, which also inhibits calcium fluxes, has been found to confer a degree of protection against ischaemic liver injury in rats [Chien et al 1977].

Several workers have demonstrated that the administration of a calcium channel blocker can reduce the extent of hepatic necrosis in rats exposed to hepatotoxic agents. Landon, Naukam and Sastry (1986) found that nifedipine, verapamil and chlorpromazine prevented or reduced both the development of centrilobular necrosis and the increase in liver cell calcium resulting from the administration of five hepatotoxic agents including chloroform. Garay, Burnette and Annesley (1984) demonstrated that pretreatment with oral nicardipine could significantly reduce the extent of liver damage after administration of the hepatotoxins carbon tetrachloride or d-galactosamine: other calcium channel

blockers such as nifedipine or verapamil were unable to prevent the subsequent liver damage. More recently, Goto and coworkers (1990) provided evidence that oral administration of nifedipine or flunarizine before anaesthesia could reduce the incidence of halothane-induced hepatotoxicity in the phenobarbitone-hypoxia rat model.

Nicardipine is a "second generation" dihydropyridine calcium antagonist which is structurally related to nifedipine. It is a potent peripheral, cerebral and coronary vasodilator with no negative inotropism, and is without effect on myocardial conduction [Freedman & Waters 1987]. It has proved effective in the treatment of exertional angina, vasospastic angina and essential hypertension [Freedman & Waters 1987], and has been shown to have a cardioprotective effect in animal ischaemia models [Alps, Calder & Wilson 1985; Endo et al 1985]. Both oral and intravenous formulations of nicardipine have been made; when given intravenously, nicardipine requires to be given as an infusion because bolus injection is relatively ineffective [Campbell, Kelman & Hillis 1985].

Because nicardipine reduced the extent of chemically-induced liver damage in rats, it was decided to assess its possible protective effect on hepatic function after halothane anaesthesia in man.

METHODOLOGY

Patient selection

Eighty patients aged 18-70 years, of ASA class I or II, who were to have minor orthopaedic surgery such as arthroscopy, gave informed consent to participate in the study. Patients who had undergone halothane anaesthesia within the preceding 3 months were excluded. Those who were receiving regular oral medication other than thiazide diuretics or benzodiazepines, and those who gave a history of parenchymal liver disease, contact with hepatitis or recent blood transfusion were excluded also, as were patients whose average daily intake of alcohol exceeded 3 units, and those whose weight was greater than 120% of that expected for a patient of the same age, sex and height. Subjects were allocated, according to a previously prepared, randomised, sex-stratified list, to receive either an intravenous infusion of nicardipine or a comparable infusion of normal saline.

Infusion plan

The infusion was designed to achieve a stable plasma nicardipine concentration of about 120 ng ml^{-1} after one hour of infusion, based on an estimated plasma clearance rate of nicardipine of 600 ml min^{-1} for an adult subject and an elimination rate constant of 0.2 h^{-1} [Graham et al 1985]. The contents of a syringe which contained nicardipine 5 mg in normal saline 60 ml were infused from a syringe pump at a rate of 1 ml min^{-1} into a closed vial

that contained nicardipine 20 mg in 20 ml volume, via a fine-bore needle to encourage mixing within the vial. The infusion was led from this vial to the patient, a technique similar to that described by Riddell and coworkers (1984), and illustrated schematically below.

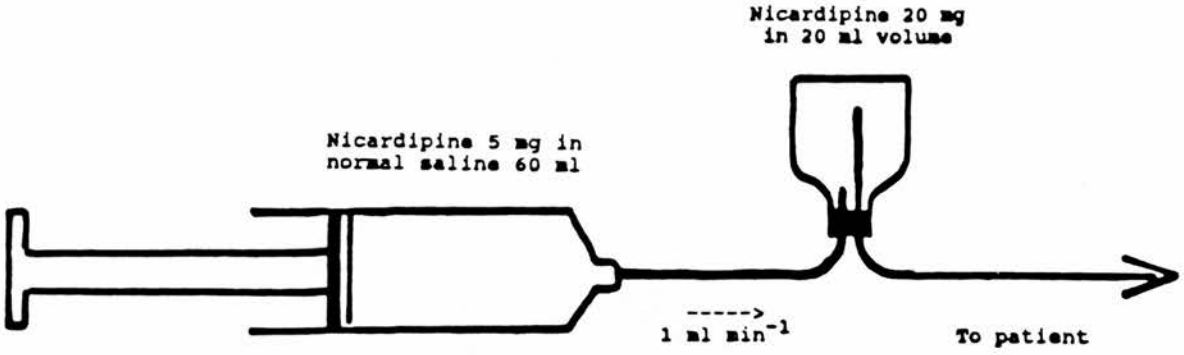

Figure 4.1 Schematic representation of solution delivery in exponentially decreasing concentration

A patient allocated to have nicardipine would have received, in the first hour of this treatment, a combination of a steady infusion of nicardipine 5 mg and an exponentially decreasing infusion with a total of nicardipine 20 mg, of which 95% would have been administered in that time. A constant infusion of nicardipine in normal saline (0.5 mg ml^{-1}) was given subsequently for a further 5 hours at a rate of 5 mg h^{-1}.

The infusion was started one hour before induction of anaesthesia. The electrocardiograph was monitored continuously and systemic arterial pressure was measured non-invasively and automatically for the duration of the

infusion. The infusion was discontinued if the systolic arterial pressure became less than 75% of the preinfusion value, or if tachycardia >150 beat min^{-1} or disorders of cardiac rhythm occurred.

Anaesthetic technique

Premedication was with oral temazepam 20 mg 2 hours before operation. Anaesthesia was induced with intravenous thiopentone 4-6 mg kg^{-1}, and suxamethonium 1 mg kg^{-1} was administered to enable tracheal intubation if this was necessary. Anaesthesia was maintained with halothane and 66% nitrous oxide in oxygen. Ventilation was spontaneous in all patients. Maximum duration of anaesthesia was restricted to 90 minutes. Pain relief after operation was provided by intramuscular diamorphine, oral dihydrocodeine or soluble aspirin as necessary; metoclopramide was prescribed to relieve nausea.

Samples and measurement

End-tidal concentrations of halothane (Penlon halothane meter) and carbon dioxide (Hewlett-Packard 47210A capnometer) were recorded every 5 minutes for the duration of anaesthesia. The carbon dioxide sensor was attached to the mount of the mask in non-intubated patients. Patients were withdrawn from the study if their end-tidal carbon dioxide partial pressure ($P_E{\cdot}CO_2$) exceeded 8 kPa. The mean of the end-tidal concentrations of halothane and carbon dioxide was

determined for each patient, and a time-weighted halothane average calculated (mean end-tidal halothane concentration ^{x} duration of anaesthesia in minutes).

Blood was sampled before starting the infusion, for GST B_1B_1 concentration and conventional liver biochemistry. Further samples for measurement of GST concentration were taken 1, 3, 6 and 24 hours after induction of anaesthesia. Conventional liver biochemical tests were also measured in the sample taken at 24 hours. Alkaline phosphatase was not used as an index of liver function in this study as patients undergoing orthopaedic surgery might have increased serum ALP activity from bone. Blood sampling for nicardipine assay was performed after one hour of infusion (ie, at the end of the exponential infusion) and at 30 minutes, 1, 2, 3 and 6 hours after induction of anaesthesia.

Assay procedures

Activities of ALT and GGT, and concentrations of bilirubin were measured using a Sequential Multiple Analysis with Computer (SMAC) System II. The normal ranges defined by the laboratory which performed these investigations are detailed in the previous chapter. The plasma concentration of GST B_1B_1 was measured by specific radioimmunoassay as in earlier studies. With greater experience of the assay in man, and using a larger volunteer population, the reference range for GST B_1B_1 concentration was redefined as 0.7-4.2 $\mu g\ l^{-1}$. The

interassay coefficient of variation and the intra-assay coefficient of variation for the GST radioimmunoassay remained unchanged from earlier studies (<10% and <5% respectively). Samples for nicardipine assay were separated by centrifugation and the resultant serum stored at -20°C until assay was performed by the standard high pressure liquid chromatography method [Wu, Massey & Kushinsky 1984]. Analysis of nicardipine-containing serum was performed by Syntex Pharmaceuticals Ltd, Palo Alto, California.

Statistical analysis

The patient characteristics, the mean end-tidal halothane and carbon dioxide concentrations, the duration of anaesthesia, and the time-weighted halothane average for each group were compared using Student's t test. The Wilcoxon signed rank test examined changes in GST concentration from the preoperative values at 1, 3, 6 and 24 hours after induction of anaesthesia in each group. Differences in GST changes at these times between the two groups, and postoperative conventional liver function tests relative to preoperative values, were compared using the Wilcoxon rank sum test. Correlation of changes in GST concentration with total dose of halothane administered, duration of anaesthesia and mean end-tidal carbon dioxide concentration was with Spearman's rank coefficient. The same test was used also to investigate the relation between GST changes and plasma nicardipine concentration for males and females separately.

Fisher's exact test compared the incidence of abnormal GST values in each group.

RESULTS

Eighty patients were entered into the study: 42 received nicardipine (27 M, 15 F) and 38 received placebo (26 M, 12 F). The infusion was discontinued in 11 patients: eight had received nicardipine and on each occasion the infusion was stopped because of hypotension, while the infusion was discontinued also in three patients who received normal saline - two because of tachycardia and one whose operation was cancelled. These patients were excluded from subsequent analysis. In addition, data were not analysed in patients who had an increased concentration of GST, or abnormal activities of ALT or GGT in the sample taken before operation. As in the study described in the previous chapter, patients who had an increased serum bilirubin concentration before operation with no other abnormal liver biochemical test were included in analysis.

The remaining 53 patients from whom data were analysed comprised 29 who received nicardipine (17 M, 12 F) and 24 who were given placebo (13 M, 11 F). The groups were comparable in respect of age, height, weight, % expected weight, daily alcohol intake, dose of thiopentone received, and mean end-tidal concentrations

of halothane and carbon dioxide (Table 4.1). In no patient did end-tidal carbon dioxide exceed 8 kPa, and only four patients had values greater than 6 kPa. Duration of anaesthesia was significantly longer in patients who received nicardipine than in those who received placebo ($p<0.01$), and consequently, the time-weighted halothane average was greater in that group ($p<0.05$, Table 4.1).

The individual plasma GST B_1B_1 concentrations for patients receiving placebo and nicardipine infusions are shown in Tables 4.2 and 4.3. GST concentration increased significantly from preoperative values at 3 and 6 hours after induction in each group, and a significant reduction was observed at 24 hours in the group that had received nicardipine (Table 4.4). Administration of nicardipine resulted in a greater increase in GST concentration at 3 hours after induction than did placebo ($p<0.05$): this difference was attributable almost entirely to males receiving nicardipine, in whom a greater increase in GST concentration occurred at 3 hours than occurred in females receiving nicardipine (Figure 4.1). The plasma concentration of nicardipine was significantly lower in males than in females after the first hour of the infusion and at 2 hours after induction of anaesthesia ($p<0.05$ both times, Table 4.5). At these times the plasma concentration of nicardipine was inversely related to the increase in GST at 3 hours ($p<0.01$ after one hour of infusion; $p<0.05$ at 2 hours

after induction, Figure 4.2). The magnitude of change in GST concentration after operation was not related to total dose of halothane administered (time-weighted halothane average), duration of anaesthesia, or mean end-tidal carbon dioxide concentration in either group.

Four of the patients who received placebo (17%) had a plasma GST concentration after anaesthesia which exceeded the upper limit of the reference range compared with ten patients who received nicardipine (34%), but this difference was not statistically significant (p=0.124).

The concentrations of bilirubin and the activities of ALT and GGT after anaesthesia were not significantly different compared with the values before operation. No patient exhibited abnormal activity of ALT or GGT after operation, although in four patients, one of whom received placebo, serum bilirubin concentration increased significantly after anaesthesia (Tables 4.6 - 4.8).

Table 4.1

Mean values (SD) for patient and anaesthetic characteristics in patients receiving placebo and nicardipine infusions who had normal preoperative liver biochemistry

	Placebo	Nicardipine
Age (years)	34 (12)	30 (8)
Height (cm)	171 (10)	174 (11)
Weight (kg)	68 (9)	71 (13)
% expected weight	100 (12)	103 (12)
Alcohol (U day^{-1})	1.4 (0.8)	1.6 (1.1)
Thiopentone (mg)	414 (63)	428 (86)
[Halothane] (%)	1.1 (0.3)	1.0 (0.2)
$P_E{\cdot}CO_2$ (kPa)	4.9 (0.8)	5.1 (0.7)
Duration of anaesthesia (min)	39 (11)	54 (21)
Time-weighted halothane average	40.3 (13.7)	53.1 (21.5)

Table 4.2

Individual plasma GST concentrations (µg l^{-1}) for patients receiving placebo in whom the infusion was completed

	PRE	1 h	3 h	6 h	24 h
1	3.9	4.2	4.1	4.1	12.0 *
2	3.1	2.0	2.7	5.1 *	2.3
3	2.0	1.6	2.4	2.4	1.5
4	2.5	2.1	2.6	2.2	2.0
5	2.5	1.8	2.5	2.8	2.8
6	2.8	3.2	3.3	2.7	1.7
7	0.5	0.6	2.3	2.0	3.5
8	1.6	1.4	1.9	2.5	1.5
9	2.4	3.0	3.8	3.0	1.7
10	3.4	3.3	3.2	3.3	2.4
11	3.0	3.1	3.5	3.5	2.7
12	2.3	2.6	2.3	3.5	2.0
13	2.2	2.7	2.4	2.0	1.8
14	1.8	1.5	1.7	1.7	1.7
15	2.8	2.6	3.4	3.8	2.6
16	1.6	1.3	1.5	1.4	1.4
17	1.4	2.1	2.0	2.2	1.8
18	1.8	1.6	2.9	2.6	2.5
19	2.2	2.4	3.6	3.3	1.7
20	1.7	1.6	1.7	1.6	1.4
21	2.3	3.5	2.8	3.1	2.0
22	1.7	1.2	2.2	2.8	2.1
23	2.1	2.1	3.0	2.2	8.6 *
24	4.2	2.7	4.6 *	2.8	1.2
Excluded from analysis					
25	5.9 *	4.7 *	4.7 *	4.9 *	2.5
26	5.9 *	6.7 *	6.9 *	7.1 *	4.2
27	27.6 *	24.7 *	29.2 *	17.4 *	6.3 *
28	10.9 *	7.6 *	9.4 *	7.8 *	7.4 *
29	4.8 *	3.6	3.3	3.4	2.4
30	5.5 *	4.7 *	6.2 *	6.2 *	---
31	5.1 *	4.1	4.1	4.1	2.8
32	6.6 *	4.3 *	6.0 *	4.4 *	1.7
33	5.6 *	5.3 *	6.8 *	5.3 *	3.8
34	6.8 *	5.5 *	6.7 *	5.0 *	5.5 *
35	6.2 *	5.0 *	6.0 *	3.6	1.7

* represents a value which exceeds the upper limit of the reference range

Table 4.3

Individual plasma GST concentrations (μg l^{-1}) for patients receiving nicardipine in whom the infusion was completed

	PRE	1 h	3 h	6 h	24 h
1	3.2	3.0	4.7 *	3.6	3.4
2	2.1	1.9	2.8	4.2	1.8
3	1.9	1.7	2.8	5.0 *	1.5
4	0.9	1.1	1.4	1.9	0.9
5	3.0	2.6	3.2	2.0	1.5
6	2.8	2.8	3.2	4.3 *	2.3
7	2.3	2.0	2.9	2.1	1.5
8	1.8	2.0	3.2	2.4	1.6
9	2.4	2.4	2.8	2.1	3.4
10	2.7	2.3	4.2	3.6	5.8 *
11	3.5	4.0	5.5 *	3.9	2.1
12	4.1	3.5	4.6 *	3.5	3.0
13	1.3	1.2	1.2	1.4	1.7
14	2.6	2.0	2.2	2.5	1.2
15	3.6	2.9	4.2	4.4 *	6.3 *
16	1.3	1.4	2.0	2.2	1.4
17	1.6	1.8	2.4	3.2	1.8
18	2.6	2.9	3.4	3.9	1.4
19	1.5	1.8	2.2	2.1	1.4
20	1.3	1.0	1.6	1.5	1.4
21	2.5	2.9	3.2	3.0	2.6
22	2.1	1.6	2.3	1.7	1.8
23	2.7	2.7	4.4 *	3.5	2.0
24	2.2	1.8	2.6	2.2	1.8
25	2.8	3.5	4.9 *	2.7	1.3
26	3.9	4.0	4.7 *	2.8	3.5
27	2.0	2.1	4.1	2.2	1.7
28	2.4	2.0	2.3	2.0	1.4
29	1.2	1.3	2.7	2.2	1.3
Excluded from analysis					
30	7.8 *	7.0 *	7.5 *	7.3 *	3.6
31	8.7 *	7.4 *	8.7 *	6.2 *	8.5 *
32	5.1 *	3.5	5.8 *	3.5	2.7
33	5.4 *	4.4 *	4.8 *	3.8	2.1
34	4.6 *	3.9	6.4 *	3.7	3.7

* represents a value which exceeds the upper limit of the reference range

Table 4.4

Medians (interquartile range) of GST concentrations ($\mu g\ l^{-1}$) for patients receiving placebo and nicardipine infusions

	PRE	1 h	3 h	6 h	24 h
Placebo					
	2.2	2.1	2.7 **	2.8 **	2.0
	(1.8-2.8)	(1.6-2.8)	(2.2-3.4)	(2.2-3.3)	(1.7-2.6)
Nicardipine					
	2.4	2.0	2.9 **	2.5 *	1.7 *
	(1.7-2.8)	(1.7-2.9)	(2.3-4.2)	(2.1-3.6)	(1.4-2.4)

* $p<0.05$; ** $p<0.01$
compared with preoperative concentration

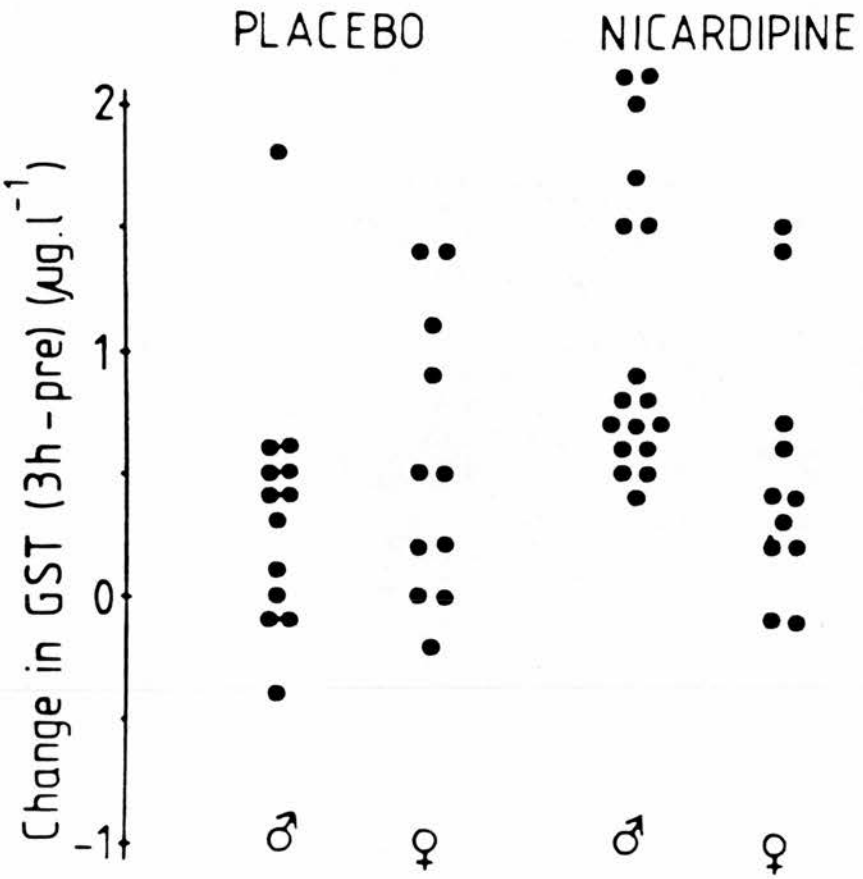

Figure 4.1

Individual changes in plasma GST concentrations, relative to preoperative values, at 3 hours after induction, showing male and female subjects separately.
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Table 4.5

Medians (range) for plasma nicardipine concentrations (ng ml^{-1}) in male and female patients

	Male		Female
End of exponential infusion	115.2 (61.0-149.5)	*	149.8 (87.5-196.0)
30 minutes	90.8 (67.4-159.3)		107.7 (52.5-158.2)
1 hour	90.3 (72.1-117.3)		99.4 (77.0-166.6)
2 hours	79.7 (64.9-118.6)	*	105.4 (75.7-149.2)
3 hours	80.6 (63.2-128.3)		103.8 (59.5-139.6)
6 hours	35.5 (20.2-58.5)		34.8 (18.8-54.0)

* $p<0.05$ between sexes

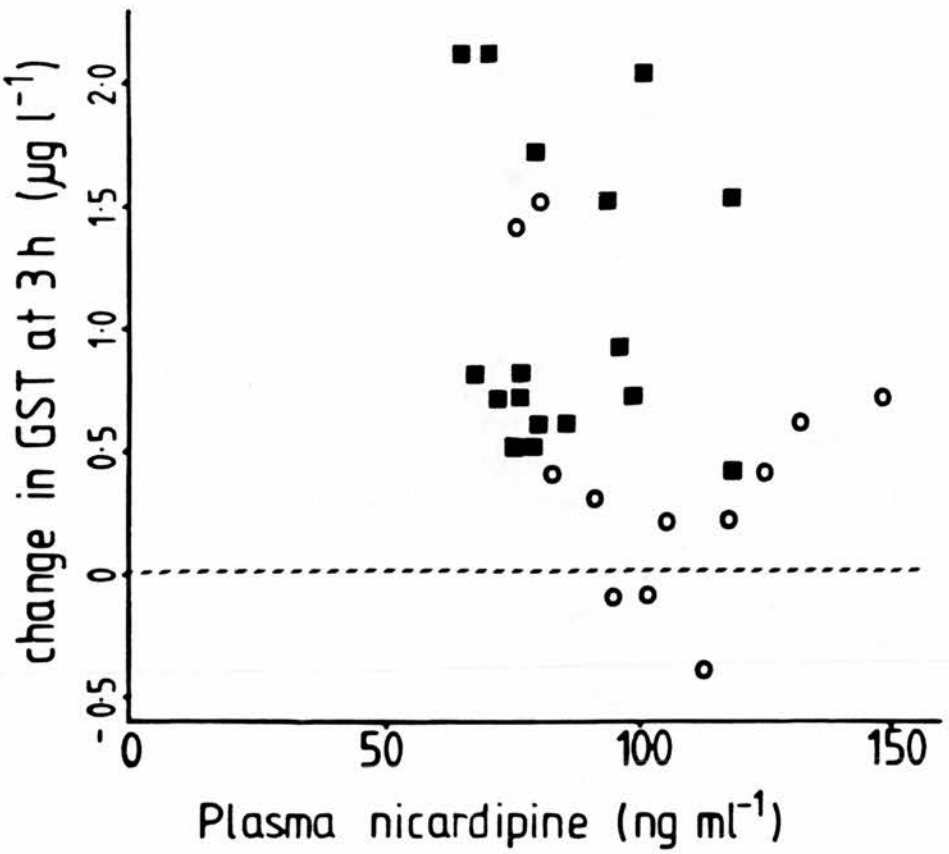

Figure 4.2

Relationship between the plasma concentration of nicardipine achieved 2 hours after induction of anaesthesia, and the change in GST that occurred at 3 hours after induction of anaesthesia.
■ = male patients; o = female patients. There is a negative linear correlation ($r=-0.43$, $p<0.05$) for the patients taken together.
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Table 4.6

Conventional liver biochemical tests before and after operation in patients receiving placebo in whom the infusion was completed

	PREOP			POSTOP		
	Bil	ALT	GGT	Bil	ALT	GGT
1	5	23	20	6	25	15
2	12	19	17	14	19	16
3	13	18	6	11	10	<5
4	12	<10	14	10	13	10
5	20 *	22	19	17	19	11
6	10	15	6	14	12	7
7	13	20	13	8	14	<5
8	10	10	15	9	<10	8
9	11	<10	6	8	<10	<5
10	6	30	19	8	23	15
11	8	25	15	7	27	26
12	12	<10	<5	11	<10	<5
13	13	17	5	15	23	<5
14	5	--	12	7	12	11
15	15	16	17	7	10	13
16	14	11	<5	11	<10	8
17	14	<10	10	18 *	<10	9
18	14	15	12	16	<10	9
19	11	14	<5	12	10	<5
20	9	<10	15	13	<10	13
21	6	19	8	8	12	8
22	15	10	44	13	14	41
23	9	14	8	12	26	10
24	12	40	30	15	31	22
Excluded from analysis						
25	12	40	--	16	24	24
26	9	20	10	7	34	14
27	8	88 *	78 *	16	53 *	74 *
28	6	108 *	119 *	13	85 *	84 *
29	10	26	22	6	20	22
30	12	37	126 *	--	--	--
31	--	--	--	13	33	45
32	13	36	12	13	36	10
33	12	48 *	78 *	14	36	67 *
34	10	75 *	50 *	11	53 *	45
35	9	45 *	14	26	37	12

* represents a value which exceeds the upper limit of the reference range

Table 4.7

Conventional liver biochemical tests before and after operation in patients receiving nicardipine in whom the infusion was completed

	PREOP			POSTOP		
	Bil	ALT	GGT	Bil	ALT	GGT
1	35 *	24	18	51 *	23	23
2	8	10	14	8	10	14
3	17	18	13	23 *	15	15
4	12	<10	10	36 *	10	8
5	4	10	6	6	10	7
6	5	18	16	9	<10	16
7	20 *	19	22	19 *	18	21
8	10	<10	7	15	<10	9
9	10	29	12	5	30	7
10	18 *	14	13	18 *	10	10
11	7	34	19	7	24	19
12	14	19	20	8	14	12
13	22 *	17	8	23 *	<10	6
14	8	14	12	8	<10	10
15	11	20	28	6	19	20
16	15	10	10	8	<10	--
17	21 *	<10	10	28 *	14	8
18	13	18	15	24 *	17	15
19	37 *	18	9	50 *	12	10
20	12	19	5	17	11	<5
21	11	16	14	9	12	12
22	6	26	11	6	16	--
23	11	19	9	11	<10	6
24	7	17	8	5	<10	7
25	21 *	30	36	18 *	29	35
26	10	34	21	8	23	18
27	19 *	13	11	22 *	<10	11
28	8	<10	16	7	<10	13
29	9	17	14	8	16	15
Excluded from analysis						
30	8	39	31	18 *	51 *	36
31	15	51 *	159 *	18 *	43 *	151 *
32	8	28	16	11	23	15
33	8	31	22	12	26	21
34	12	28	9	10	26	6

* represents a value which exceeds the upper limit of the reference range

Table 4.8

Medians (range) of conventional liver biochemical tests before and after anaesthesia for patients receiving placebo and nicardipine infusions

	Placebo	Nicardipine
Bilirubin (μmol l^{-1})		
PRE	12 (5-20)	11 (4-37)
24 h	11 (6-18)	9 (5-51)
ALT (U l^{-1})		
PRE	15 (<10-40)	18 (<10-34)
24 h	12 (<10-31)	12 (<10-30)
GGT (U l^{-1})		
PRE	13 (<5-44)	13 (5-36)
24 h	10 (<5-41)	12 (<5-35)

DISCUSSION

This study has confirmed that transient GST release occurs following anaesthesia with halothane in man, as has been demonstrated previously [previous chapter; Allan et al 1987; Hussey et al 1986a]. Although the changes in median concentrations of GST were small, abnormally increased GST concentrations were observed in 14 of 53 patients after anaesthesia; ten of these patients had received nicardipine. Administration of nicardipine was associated with a greater release of GST after operation than was placebo, resulting mostly from an exaggerated GST response in males, but not females, receiving nicardipine. Plasma concentration of nicardipine at the end of the exponential infusion and at 2 hours after induction of anaesthesia was significantly greater in females than in males, and this was related to a lesser increase in GST concentration at 3 hours. The larger increase in GST at 3 hours in males receiving nicardipine may reflect the combined effects of halothane and nicardipine causing a greater reduction in hepatic perfusion than halothane alone. The greater plasma concentrations achieved in females perhaps conferred a degree of protection against this combined effect, but possibly were still insufficient to prevent release of GST into the circulation. Although plasma concentrations of nicardipine throughout the infusion were compatible with those required to give a clinically relevant effect [Debbas, Jackson & Turner 1985], it may

be that even greater plasma concentrations of nicardipine are required for hepatoprotection after halothane anaesthesia. Such concentrations may, however, cause undesirable cardiovascular side effects. This study was unable, therefore, to demonstrate that nicardipine, in the dose administered, prevented this type of liver dysfunction in man.

The finding that nicardipine conferred no hepatoprotection is at variance with animal studies. However, the dose of nicardipine administered to rats [Garay, Annesley & Burnette 1984] was 25 to 50 times greater than the total dose given to patients in the present study. As suggested above, larger doses of nicardipine given to man might also have the same protective effect, but at the cost of increased cardiovascular side effects. Other workers have shown that the severity of halothane-induced hepatic damage in rats is related to total halothane dosage [Jee et al 1980], but this has not been demonstrated in humans. In the present study, patients who received nicardipine received a greater total dose of halothane (time-weighted halothane average), and, therefore, might have received a greater hepatic insult, than patients given placebo. However, the magnitude of GST increase after operation could not be related to total dose of halothane received, suggesting that this difference between the groups is unlikely to have influenced the present findings.

There exists remarkable similarity between the pharmacological effects of volatile anaesthetics and calcium channel blocking drugs [Jones 1984]. The depressant effects on sino-atrial node conduction by halothane, enflurane and isoflurane have been found to be similar to those of the calcium channel blockers verapamil and diltiazem [Bosnjak & Kampine 1983]. It has been demonstrated that halothane and enflurane have a degree of calcium channel blocking activity [Lynch, Vogel & Sperelakis 1981; Lynch et al 1982] while isoflurane appeared to have a greater effect on intracellular calcium kinetics [Lynch 1986]. Furthermore, the negative inotropic and vasodilatory effects of the volatile anaesthetics are similar to those of calcium channel blockers [Merin 1987].

If halothane has calcium channel blocking activity, is it logical to propose that the administration of a second calcium channel blocker might reduce the extent of liver injury? Current evidence proposes that there are at least four different types of calcium entry channel [Schramm & Towart 1985]. These have been designated leak channels, stretch-sensitive channels, potential-dependent channels and receptor-dependent channels. The two former channels are unlikely to be classical gated channels but probably represent mechanisms of calcium entry termed channels for convenience. The latter mechanisms have been defined more clearly; the existence of the potential-dependent

channel as an ion-specific gated channel is now beyond dispute [Schramm & Towart 1985]. Specific calcium channel blockers are a heterogeneous group of drugs with diverse chemical structures [Jenkins & Scoates 1985; Reves et al 1982]. This heterogeneity of structure implies that there is no one receptor which mediates the effects of the calcium channel blockers. Snyder and Reynolds (1985) have suggested that at least two, and possibly three, distinct receptors for calcium channel blocking drugs exist, all of which are associated with the potential-dependent channel. This raises the possibility that volatile anaesthetics exert their effect on cellular calcium regulation via a mechanism which is distinct from that of the specific calcium channel blockers. In addition, it has been proposed that the effects of the volatile anaesthetics may be related to their actions upon intracellular handling of calcium, and not necessarily upon calcium entry channels [Lynch 1984]. It would appear, therefore, that prevention of calcium entry into the cell is more important than altered intracellular handling of calcium in reducing the severity of cellular injury. The administration of a specific calcium channel blocker which reduces cellular calcium ingress might, therefore, be expected to limit the severity of liver damage after anaesthesia with halothane. The findings from this study appear to indicate that this hypothesis is not proven, suggesting that halothane-associated hepatotoxicity might have some other mechanism.

CHAPTER 5

THE EFFECT OF CIMETIDINE ON PLASMA GST CONCENTRATION AFTER HALOTHANE ANAESTHESIA

CHAPTER 5

THE EFFECT OF CIMETIDINE ON PLASMA GST CONCENTRATION AFTER HALOTHANE ANAESTHESIA

Toxic metabolic products resulting from halothane biotransformation are thought to be a possible mechanism of halothane hepatotoxicity [Cousins et al 1987a; de Groot & Noll 1983]. Halothane is metabolised in liver microsomes via both oxidative [Cohen et al 1975] and reductive pathways [Sharp, Trudell & Cohen 1979]. This biotransformation is dependent on the mixed-function oxidase enzyme (MFOE) system, of which cytochrome P-450 is the single most important component [Van Dyke 1973]. The H_2-receptor antagonist cimetidine has been reported to interact with liver microsomal enzymes [Desmond et al 1980a]; binding of its imidazole system to the haem moiety of cytochrome P-450 results in inhibition of cytochrome P-450-dependent MFOEs [Bauman & Kimelblatt 1982]. Consequently, cimetidine can impair the metabolism of drugs that are metabolised by the MFOE system. Drugs reported to be affected in this way include benzodiazepines [Desmond et al 1980b; Klotz & Reimann 1980], β-blockers [Heagerty et al 1981; Feely, Wilkinson & Wood 1981], lignocaine [Feely et al 1982], theophylline [Jackson et al 1981; Reitberg, Bernhard & Schentag 1981], warfarin [Serlin et al 1979], verapamil [Loi et al 1985] and anticonvulsants [Hetzel et al 1981; Telerman-Toppet, Duret & Coërs 1981]. Cimetidine has been shown to inhibit halothane metabolism in vitro [Loesch, Siegers & Younes 1987], and animal experiments

suggest also that cimetidine reduces halothane biotransformation and consequently lessens the severity of hepatic injury [Plummer et al 1984; Wood et al 1986]. Therefore, it was thought reasonable to assess the influence of cimetidine on the release of GST after halothane anaesthesia, in case halothane metabolism could be altered to reduce toxicity.

METHODOLOGY

Patient selection and anaesthetic technique

Informed consent was obtained from patients aged 16-70 years, ASA class I or II, undergoing minor orthopaedic surgery. Reasons for exclusion were the same as those detailed in the previous chapter. Patients were assigned randomly to one of two groups, to receive either cimetidine 800 mg orally the evening before operation with a further 800 mg given at the time of premedication, or equivalent placebo tablets. The technique of anaesthesia was exactly similar to that described in Chapter 4.

Samples, measurement and assay procedures

End-tidal concentrations of halothane (Penlon halothane meter) and carbon dioxide (Hewlett-Packard 47210A capnometer) were recorded every 5 minutes throughout anaesthesia, and a time-weighted halothane average was calculated for each patient. Blood samples were taken

for GST assay before operation and at 3, 6 and 24 hours after induction of anaesthesia. Measurements of conventional liver biochemical tests were made on the day before and the day after anaesthesia. Measurements of GST B_1B_1 and bilirubin concentrations, and ALT and GGT activities were as described in Chapter 4.

Statistical analysis

Student's t test compared the demographic data and anaesthetic characteristics for each group. The GST changes from the preoperative values at 3, 6 and 24 hours after induction were compared using the Wilcoxon signed rank test; the Wilcoxon rank sum test examined differences in GST changes at these times between the groups, and conventional liver function tests before and after anaesthesia. Spearman's rank coefficient was used to determine whether the GST changes could be related to the total dose of halothane administered. The incidence of abnormal GST values in each group was compared using Fisher's exact test.

RESULTS

Fifty-three patients were studied; 26 received placebo (16 M, 10 F) and 27 received cimetidine (18 M, 9 F). Eight patients were excluded from analysis: three were withdrawn because of excessive duration of anaesthesia (>90 minutes), three had abnormal preoperative liver

biochemistry, one refused to have further blood samples taken and surgery was cancelled in one. Data from the remaining 45 patients were analysed: 24 had received placebo (14 M, 10 F) and 21 cimetidine (14 M, 7 F).

The groups were comparable in respect of age, height, weight, % expected weight and alcohol intake (Table 5.1). The mean dose of thiopentone, and the mean end-tidal concentrations of halothane and carbon dioxide were similar in the two groups. The total dose of halothane administered, reflected in the time-weighted halothane average, appeared greater in the placebo group, but this failed to achieve statistical significance (p=0.064) (Table 5.1).

The plasma GST B_1B_1 concentrations for each patient receiving placebo and cimetidine are shown in Tables 5.2 and 5.3. GST increased significantly at 3 hours after induction of anaesthesia in both groups, and at 6 hours in the group which had received cimetidine (Table 5.4); the administration of cimetidine did not influence the magnitude of increase in GST after anaesthesia.
Although some patients in both groups showed an increase in GST concentration which exceeded the upper limit of the reference range, there was no significant difference in the frequency of such abnormal values between the two groups (p=0.266). One patient who had received placebo exhibited a large secondary increase in GST concentration at 24 hours after induction of anaesthesia, but no

patient who had received cimetidine showed a comparable change. There was no correlation between GST increase and total dose of halothane administered, or mean end-tidal carbon dioxide concentration. Concentrations of bilirubin and activities of ALT and GGT after anaesthesia were not significantly different relative to preoperative values in each group (Tables 5.5 - 5.7). In particular no patient developed an abnormally increased activity of ALT or GGT after operation.

Table 5.1

Mean values (SD) for patient and anaesthetic characteristics in patients receiving placebo and cimetidine who had normal preoperative liver biochemistry

	Placebo	Cimetidine
Age (years)	33 (12)	32 (13)
Height (cm)	171 (7)	173 (11)
Weight (kg)	68 (11)	70 (11)
% expected weight	99 (13)	101 (13)
Alcohol (U day^{-1})	1.5 (1.0)	1.5 (1.2)
Thiopentone (mg)	412 (78)	425 (59)
[Halothane] (%)	1.2 (0.3)	1.0 (0.3)
$P_E{}'CO_2$ (kPa)	5.1 (0.7)	5.2 (0.8)
Duration of anaesthesia (min)	46 (17)	43 (20)
Time-weighted halothane average	53.6 (24.2)	41.1 (20.1)

Table 5.2

Individual plasma GST concentrations (µg l^{-1}) for patients receiving placebo

	PRE	3 h	6 h	24 h
1	3.3	3.5	3.3	2.7
2	3.0	3.7	3.2	2.1
3	1.6	2.3	2.0	1.7
4	1.9	4.1	3.6	2.6
5	2.7	3.1	2.7	2.1
6	3.1	3.0	3.7	3.1
7	2.8	3.7	3.4	1.9
8	4.0	4.7 *	3.1	4.5 *
9	2.3	2.8	2.9	2.5
10	2.8	3.0	3.3	2.9
11	2.0	1.0	1.4	3.6
12	2.2	2.5	1.1	2.5
13	2.3	3.7	4.3 *	---
14	3.5	3.1	2.6	1.4
15	2.5	2.9	3.0	1.9
16	3.7	5.2 *	5.0 *	2.5
17	1.6	2.3	2.1	1.3
18	3.9	5.0 *	4.1	3.7
19	3.6	3.6	2.9	2.8
20	1.9	2.8	3.1	1.7
21	1.8	2.6	2.8	1.8
22	3.1	2.4	2.8	2.4
23	3.2	4.4 *	3.1	2.9
24	2.3	2.5	2.4	9.6 *
Excluded from analysis				
25	3.1	---	---	---
26	5.3 *	8.0 *	6.8 *	2.8

* represents a value which exceeds the upper limit of the reference range

Table 5.3

Individual plasma GST concentrations (μg l^{-1}) for patients receiving cimetidine

	PRE	3 h	6 h	24 h
1	3.6	3.3	1.6	2.1
2	2.4	2.6	4.1	2.0
3	1.8	2.8	2.2	2.2
4	2.0	2.6	3.0	2.0
5	3.8	4.9 *	4.2	1.9
6	1.5	2.2	2.7	1.8
7	2.1	2.6	2.3	2.1
8	2.5	3.6	6.8 *	3.3
9	4.2	6.5 *	5.1 *	6.9 *
10	4.0	4.3 *	3.6	3.1
11	3.6	4.4 *	3.6	2.9
12	2.2	2.9	3.2	2.2
13	3.1	3.3	2.3	1.6
14	3.2	3.3	3.3	2.4
15	3.0	3.7	2.8	---
16	4.2	8.5 *	7.0 *	3.0
17	1.7	2.6	2.8	1.3
18	1.9	3.8	2.6	1.9
19	4.2	5.4 *	3.5	5.0 *
20	2.3	2.5	6.1 *	3.3
21	2.1	2.6	2.7	2.2
Excluded from analysis				
22	6.1 *	6.6 *	5.0 *	3.3
23	3.3	---	---	---
24	16.2 *	20.3 *	17.4 *	27.1 *
25	1.1	---	---	---
26	2.0	---	---	---
27	---	---	---	---

* represents a value which exceeds the upper limit for the reference range

Table 5.4

Medians (interquartile range) of GST concentrations ($\mu g\ l^{-1}$) for patients receiving placebo and cimetidine

	PRE	3 h	6 h	24 h
Placebo	2.7	3.1 **	3.0	2.5
	(2.1-3.2)	(2.6-3.7)	(2.6-3.4)	(1.9-2.9)
Cimetidine	2.5	3.3 **	3.2 *	2.2
	(2.0-3.7)	(2.6-4.4)	(2.6-4.2)	(2.0-3.0)

* $p<0.05$; ** $p<0.01$
compared with preoperative concentration

Table 5.5

Conventional liver biochemical tests before and after operation in patients receiving placebo

	PREOP			POSTOP		
	Bil	ALT	GGT	Bil	ALT	GGT
1	8	10	8	9	13	7
2	9	12	19	15	12	16
3	12	<10	8	14	<10	10
4	42 *	15	11	74 *	20	11
5	25 *	23	14	23 *	<10	11
6	9	29	42	19	28	38
7	14	16	15	20 *	<10	10
8	7	34	31	6	31	22
9	4	11	6	9	17	8
10	5	<10	6	9	16	8
11	11	26	8	14	19	8
12	12	<10	14	12	10	15
13	16	12	17	--	--	--
14	11	22	20	11	12	17
15	5	13	11	15	<10	15
16	5	31	22	9	<10	18
17	12	11	15	21 *	14	15
18	9	18	33	7	23	29
19	11	22	11	15	24	10
20	8	21	8	5	12	7
21	25 *	17	27	26 *	11	--
22	8	<10	10	6	<10	11
23	9	18	20	8	15	19
24	8	11	15	13	28	17
Excluded from analysis						
25	10	23	24	11	19	21
26	--	--	--	--	--	--

* represents a value which exceeds the upper limit of the reference range

Table 5.6

Conventional liver biochemical tests before and after operation in patients receiving cimetidine

	PREOP			POSTOP		
	Bil	ALT	GGT	Bil	ALT	GGT
1	12	<10	9	--	--	--
2	26 *	<10	9	18 *	<10	6
3	15	10	14	19 *	14	15
4	5	10	21	7	<10	19
5	38 *	20	23	79 *	14	22
6	17	19	8	9	18	--
7	8	<10	7	11	14	5
8	5	23	45	6	25	42
9	12	28	45	9	24	34
10	11	<10	17	12	<10	20
11	19 *	14	34	15	14	34
12	18 *	16	21	15	16	21
13	20 *	11	14	43 *	15	15
14	8	40	18	6	40	16
15	14	<10	10	--	--	--
16	19 *	33	45	22 *	24	28
17	12	18	9	16	<10	6
18	7	13	10	7	<10	11
19	9	40	40	11	36	40
20	12	<10	18	14	25	19
21	12	15	18	--	--	--
Excluded from analysis						
22	9	47 *	35	13	43 *	30
23	11	117 *	79 *	15	210 *	73 *
24	--	--	--	--	--	--
25	--	--	--	--	--	--
26	--	--	--	--	--	--
27	--	--	--	--	--	--

* represents a value which exceeds the upper limit of the reference range

Table 5.7

Medians (range) of conventional biochemical liver tests for patients receiving placebo and cimetidine

	Placebo	Cimetidine
Bilirubin (μmol l^{-1})		
PRE	9 (4-42)	12 (5-38)
24 h	13 (5-74)	14 (6-79)
ALT (U l^{-1})		
PRE	16 (<10-34)	14 (<10-40)
24 h	13 (<10-31)	15 (<10-40)
GGT (U l^{-1})		
PRE	15 (6-42)	18 (7-45)
24 h	15 (7-38)	19 (5-42)

DISCUSSION

This study was unable to demonstrate that cimetidine influenced GST B_1B_1 release into the circulation up to 24 hours after halothane anaesthesia. No patient who received cimetidine exhibited a large secondary increase (greater than twice the upper limit of the reference range) in GST concentration at 24 hours, although it would be necessary to study many more patients to be certain that cimetidine prevented this less frequent increase. The present study provides yet further confirmation that halothane anaesthesia is associated with a transient increase in plasma GST concentration after operation. Once again the changes in median GST concentration were not large. However, these changes are considered significant as abnormally increased GST concentrations after anaesthesia occurred in 14 of 45 patients.

Halothane biotransformation occurs in vitro and in vivo and this takes place in the microsomal fraction of the liver [Van Dyke 1973]. The binding of halothane metabolites to rat liver microsomes is enhanced by agents that induce cytochrome P-450, such as phenobarbitone [Van Dyke 1973] and polychlorinated biphenyl [Van Dyke & Gandolfi 1976]; it is reduced by enzyme inhibitors such as metyrapone [Gandolfi et al 1980] and SKF 525A [Van Dyke & Wood 1973]. In rats exposed to halothane, Jee and colleagues (1980) demonstrated increased

hepatotoxicity after induction of cytochrome P-450, and showed that significant protection was afforded by pretreatment with enzyme inhibitors. Similarly, cimetidine has been shown to offer protection against halothane hepatotoxicity in rats, although the reports differ on the identity of the pathway of halothane metabolism inhibited [Plummer et al 1984; Wood et al 1986]. Using measurements of exhaled metabolites CDF and CTF, and of urinary fluoride excretion, Plummer and colleagues (1984) suggested that reductive metabolism was inhibited by cimetidine. In contrast, Wood and coworkers (1986) demonstrated inhibition of oxidative metabolism by cimetidine: using urinary fluoride excretion as the only indicator of reductive metabolism, however, they were unable to demonstrate inhibition of this pathway, but acknowledged the limitations of this method alone.

Other animal studies have indicated that the two pathways of halothane biotransformation may display different levels of inhibition. Fiserova-Bergerova (1984) demonstrated in rats that isoflurane inhibited the oxidative biotransformation of halothane to a far greater extent than the reductive pathway. If this were the case with cimetidine in the present study, then reductive metabolism of halothane may have continued with subclinical damage as indicated by increased plasma GST concentration. However, evidence suggests that cimetidine can inhibit both pathways [Plummer et al 1984;

Wood et al 1986].

The influence of cimetidine on the metabolism of enflurane in man has been investigated also. Yeager and colleagues (1986) measured serum fluoride concentrations in patients given a total dose of cimetidine 600 mg before enflurane anaesthesia, but could not demonstrate significant alteration in enflurane biotransformation. Similarly, Oikkonen, Rosenberg and Saarnivaara (1989) found that premedication with cimetidine 800 mg did not inhibit the metabolism of enflurane in seven patients undergoing major ear surgery. The negative influence of cimetidine on enflurane metabolism would be explained if enflurane and cimetidine interact differently with the MFOE system. Alternatively, this may simply reflect the low extent of enflurane metabolism which might make it difficult to detect an effect from a competitive inhibitor such as cimetidine.

In the present study, cimetidine was not shown to reduce liver damage associated with halothane anaesthesia (as indicated by GST release), an observation which is at variance with animal studies. Possible reasons for this difference include other potential effects of cimetidine such as those on hepatic blood flow, and perhaps inadequate dosage of cimetidine.

Original evidence suggested that cimetidine reduced hepatic blood flow. Such a reduction in flow and

resultant ischaemia could contribute to the release of GST. Feely, Wilkinson and Wood (1981) reported a 25% reduction in hepatic blood flow, when measured by indocyanine green (ICG) clearance, in normal volunteers given a single 600 mg dose of cimetidine by mouth. Others have described a similar reduction in liver blood flow measured by this technique [Garg et al 1982; Feely & Wood 1983]. In contrast, other workers who measured ICG clearance have reported cimetidine to have no effect on liver blood flow [Daneshmend et al 1984], or even to increase it [Loi et al 1985]. Such variability in results suggests that this indirect experimental procedure is not a reliable method of monitoring hepatic blood flow. In addition, the use of the ICG clearance technique has been criticised by Groszmann (1983), who concluded that unless the degree of extraction of the indicator by the liver is accurately determined it is incorrect to assume that clearance is directly proportional to blood flow. This statement raises doubts about the results of all of the early ICG clearance studies, including those of Feely (1981,1983) and Garg (1982), where no correction factor was applied. Further investigation has failed to demonstrate that cimetidine reduces liver blood flow using various techniques, such as clearance of galactose [Henderson et al 1983] or sorbitol [Arditi et al 1983], and more direct methods including the use of an electromagnetic blood flow meter [Tyden, Thulin & Nyberg 1983], an ultrasound doppler system or cineangiography [Ohnishi et al 1985].

Thus there is no convincing evidence that cimetidine has any effect on hepatic blood flow.

The doses of cimetidine given in the rat studies [Plummer et al 1984; Wood et al 1986] were enormous (120 and 180 mg kg^{-1}), and it is obviously not possible to administer such large doses to man. Most human studies which have shown that cimetidine impairs metabolism of other drugs have involved giving a larger total dose of cimetidine than that given in the present study. This usually involved pretreatment with cimetidine 1000 mg or 1200 mg daily for 2-14 days [Loi et al 1985; Jackson et al 1981; Desmond et al 1980b; Heagerty et al 1981; Feely, Wilkinson & Wood 1981; Henderson et al 1983]. However, Klotz, Arvela and Rosenkranz (1985) have shown that a single oral dose of cimetidine 800 mg is sufficient to interfere with the pharmacokinetics of midazolam administered concurrently. This single dose achieved a mean maximum plasma concentration of cimetidine of 2.53 µg ml^{-1} which is within the therapeutic range evaluated by Somogyi and Gugler (1983). In addition, cimetidine 1000 mg was found to increase steady-state plasma propranolol concentrations after only one day of medication, in contrast to pretreatment with ranitidine [Reimann, Klotz & Frölich 1982]. Furthermore, cimetidine in total doses of 1500 mg and 800 mg respectively, has been shown to reduce clearance of the local anaesthetic agents lignocaine and bupivacaine, and to increase toxicity of lignocaine given as an

intravenous infusion [Feely et al 1982; Noble, Smith & Dundas 1987]. The data from these studies suggest that the dose of cimetidine used in the present study (1600 mg) is sufficient to alter the metabolism of other drugs administered concurrently.

If it can be assumed that the dose of cimetidine administered was sufficient to reduce halothane metabolism, data from the present study suggest that products of halothane metabolism are not responsible for the early release of GST described previously [Hussey et al 1986; Allan et al 1987], although many more patients would require to be studied to be confident that cimetidine prevents the secondary phase of GST release at 24 hours.

CHAPTER 6

THE EFFECT OF MODE OF VENTILATION ON PLASMA GST CONCENTRATION AFTER INDUCED HYPOTENSION DURING HALOTHANE OR ISOFLURANE ANAESTHESIA

CHAPTER 6

THE EFFECT OF MODE OF VENTILATION ON PLASMA GST CONCENTRATION AFTER INDUCED HYPOTENSION DURING HALOTHANE OR ISOFLURANE ANAESTHESIA

Hepatic hypoxia has been proposed as a possible mechanism of halothane-induced liver damage [Gelman 1987; Gelman, Fowler & Smith 1984a]. In rats, reduction in the inspired oxygen concentration during halothane anaesthesia leads to increased reductive metabolism of halothane and more severe liver injury [Cousins et al 1979; McLain, Sipes & Brown 1979; Ross, Daggy & Cardell 1979]. There is no evidence that in humans inspired hypoxia is a contributing factor to halothane hepatotoxicity: the importance attached to maintenance of inspired oxygen and arterial oxygenation make such an association unlikely. However, volatile anaesthetic agents depress oxygen availability to the liver, partly as a result of decreased hepatic blood flow [Gelman 1987], and it is possible that these changes may be exacerbated by the effect of surgery [Gelman 1976], leading to global or regional hepatic hypoxia.

In man, total liver blood flow is derived from two sources, the hepatic artery and the portal vein. Under most circumstances there exists reciprocity between these sources, such that an increase in flow through one circuit leads to increased resistance to flow through the other circuit, tending to maintain a constant total blood flow through the liver. Anaesthetics can alter the

splanchnic circulation by different mechanisms acting on the various factors controlling splanchnic blood flow. These mechanisms include alterations in systemic haemodynamics, changes in sympathetic tone, the release of hormones and other endogenous substances, and changes in oxygen requirement.

Specific volatile anaesthetic agents have different effects on the hydrodynamic interaction between portal and hepatic arterial vascular beds. Halothane, enflurane and isoflurane all decrease portal blood flow; hepatic arterial flow is generally increased or preserved with isoflurane, and to a lesser extent with enflurane, and is decreased with halothane [Gelman 1987]. During halothane anaesthesia, cardiac output is the main determinant of portal blood flow (the coefficient of correlation between portal blood flow and cardiac output, r=0.97), and to a lesser extent, a determinant of hepatic arterial blood flow (r=0.74) [Gelman, Fowler & Smith 1984a]. The extent to which halothane, enflurane and isoflurane each reduce total liver blood flow closely parallels the incidence of reported clinical liver dysfunction for these agents.

Other factors which may affect hepatic blood flow include the mode of ventilation and reduced systemic arterial pressure. Positive pressure ventilation is associated with a reduction and redistribution of cardiac output. Splanchnic blood flow is decreased [Epstein et al 1966;

Cooperman, Warden & Price 1968], and blood supply to the liver is reduced to a greater degree than the decrease in cardiac output [Geiger, Georgieff & Lutz 1986]. There is little, if any, pressure-dependent autoregulation of blood flow exhibited by either hepatic arterial or portal vascular beds, with the result that liver blood flow and arterial pressure have an approximately linear relationship [Richardson & Withrington 1981]. Positive pressure ventilation and hypotension may, therefore, exacerbate liver damage associated with halothane anaesthesia.

As halothane may cause hepatic damage by reducing liver blood flow, it was decided to assess the effect of the mode of ventilation on plasma GST B_1B_1 concentration in patients undergoing deliberate hypotension during anaesthesia with halothane or isoflurane.

METHODOLOGY

Patient selection

Sixty patients aged 15 to 70 years of ASA class I or II, who were to undergo elective ENT surgery for which deliberate hypotension was required, were enrolled into the study. Reasons for exclusion were the same as those detailed in Chapter 4. The patients came from two different wards; those from one ward received intermittent positive pressure ventilation (IPPV) and

those from the other ward were allowed to breathe spontaneously (SV). Within each group patients were allocated randomly to receive either halothane or isoflurane.

Anaesthetic technique

All patients received diazepam 10 mg orally 1 hour before operation. Anaesthesia was induced with intravenous thiopentone 4-6 mg kg^{-1}. Thereafter, patients allocated to receive artificial ventilation received fentanyl 100 µg and d-tubocurarine 0.5 mg kg^{-1}. Following tracheal intubation, ventilation was commenced using a Penlon Nuffield 400 series ventilator connected to a circle system into which fresh gas was supplied at a flow rate of 4 l min^{-1}. All patients received a preset tidal volume of 7 ml kg^{-1} at a frequency of 13 breath min^{-1}. To the fresh gas supply was added either halothane 1% or isoflurane 1.5-2% (vaporiser setting). After induction of anaesthesia, patients who were to breathe spontaneously received d-tubocurarine 5 mg and suxamethonium 1 mg kg^{-1} to enable tracheal intubation. Anaesthesia was maintained with either halothane 3-5% or isoflurane 3-5% (vaporiser setting) delivered in a fresh gas flow of 1.5 l min^{-1} via a circle system. The fractional inspired oxygen concentration was maintained between 0.5 and 0.6 in all patients.

A cannula was inserted percutaneously into a radial artery after induction of anaesthesia to permit

continuous measurement of arterial pressure. Within 10 minutes of commencing anaesthesia, hypotension was induced with an intravenous infusion of trimetaphan 50 mg and sodium nitroprusside (SNP) 12.5 mg in 5% dextrose 500 ml, infused at a rate of 3-20 ml min^{-1} to maintain a mean arterial pressure between 40 and 55 mm Hg [Simpson et al 1987]. Analgesia after operation was provided with intramuscular papaveretum 10-20 mg, and prochlorperazine 12.5 mg was prescribed to relieve nausea.

Samples, measurement and assay procedures

Blood samples for GST B_1B_1 radioimmunoassay were taken before induction of anaesthesia and at 1, 3, 6 and 24 hours after the end of anaesthesia. Plasma concentration of bilirubin and activities of ALT and GGT were measured in the samples obtained before anaesthesia and at 24 hours. Plasma from the samples was stored at -20°C before measurement of GST. Concentrations of GST and bilirubin, and ALT and GGT activities were measured as described in Chapter 4. Arterial tensions of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) were measured during stable hypotension.

Statistical analysis

As in the preceding studies, Student's t test examined differences in patient and anaesthetic characteristics, and the Wilcoxon rank sum and signed rank tests were used as appropriate to compare GST changes and differences

between groups. Kendall rank correlation was used to determine whether GST changes could be related to arterial oxygen and carbon dioxide tensions, duration of anaesthesia, duration of hypotension and total dose of SNP and trimetaphan administered. Fisher's exact test compared the incidence of abnormal GST concentrations in patients who received IPPV and in those who breathed spontaneously.

RESULTS

Sixty patients were studied; 28 were artificially ventilated (14 halothane, 14 isoflurane) and 32 breathed spontaneously (14 halothane, 18 isoflurane). Data were not analysed in 12 patients who had abnormal values of GST, ALT or GGT before operation. The remaining 48 patients comprised 25 who received IPPV (13 halothane (9 M, 4 F), 12 isoflurane (6 M, 6 F)) and 23 who breathed spontaneously (11 halothane (5 M, 6 F), 12 isoflurane (7 M, 5 F)).

Within the four groups patients were equally matched for age, height, weight and alcohol consumption. No significant differences were observed in anaesthetic characteristics between patients given halothane or isoflurane who received the same mode of ventilation (Table 6.1). However, when the modes of ventilation alone are compared, patients who received IPPV had a

significantly lower mean arterial carbon dioxide tension than those in the SV group (4.8 kPa compared with 7.0 kPa, $p<0.001$). The mean duration of anaesthesia was also slightly, but significantly, shorter in ventilated patients (49 minutes compared with 59 minutes, $p<0.05$).

Significant increases in GST concentration from preoperative values were observed after anaesthesia in all 4 groups. GST concentration was increased significantly at 1 hour in patients who received isoflurane in the SV group and in both groups of patients who received IPPV, and at 3 hours in patients anaesthetised with halothane in both SV and IPPV groups. A significant reduction in GST at 24 hours was observed in both groups who received IPPV (Tables 6.2 - 6.4). No significant difference in GST changes was demonstrated between patients who had received halothane or those who had received isoflurane, irrespective of the mode of ventilation. Therefore, the results obtained using the two volatile agents were combined, to allow an assessment of the effect of mode of ventilation on plasma GST concentration after anaesthesia. Patients who received IPPV had a significantly greater increase in GST at 1 hour after anaesthesia than did those who breathed spontaneously ($p<0.01$, Table 6.5). The quantitative change in GST concentration was unrelated to any of the other measured variables.

In the majority of patients in whom increases in GST were

noted, the GST values remained within the normal range. Eight patients (4 halothane, 4 isoflurane) in the group which received IPPV had GST concentrations that exceeded the upper limit of the reference range. Abnormally increased GST concentrations occurred in three patients who breathed spontaneously, two of whom had received isoflurane. The mode of ventilation did not significantly influence the incidence of abnormal values of GST ($p=0.075$).

Three patients who received IPPV and halothane developed an abnormally increased serum bilirubin concentration after anaesthesia. No other abnormalities in conventional liver biochemical tests were noted (Tables 6.6 - 6.8).

Table 6.1

Mean values (SD) for patient and anaesthetic characteristics in patients in all four groups

	IPPV		SV	
	Hal	Iso	Hal	Iso
Age (years)	36 (18)	35 (12)	37 (13)	38 (14)
Height (cm)	171 (10)	167 (8)	166 (12)	167 (9)
Weight (kg)	72 (13)	71 (11)	64 (13)	64 (12)
Alcohol ($u\ day^{-1}$)	0.8 (1.3)	1.2 (1.7)	1.8 (2.0)	1.2 (1.2)
Duration of anaesthesia (min)	52 (22)	44 (16)	55 (16)	61 (21)
Duration of hypotension (min)	34 (17)	24 (16)	34 (13)	33 (12)
Dose of SNP/ trimetaphan (ml)	155 (219)	174 (146)	220 (206)	202 (204)
PaO_2 (kPa)	24 (11)	27 (9)	28 (8)	34 (7)
$PaCO_2$ (kPa)	4.9 (1.0)	4.7 (1.2)	6.8 (0.8)	7.1 (1.2)

Table 6.2

Individual plasma GST concentrations (μg l^{-1}) for patients undergoing deliberate hypotension, anaesthetised with halothane or isoflurane, who received intermittent positive pressure ventilation (IPPV)

	PRE	1 h	3 h	6 h	24 h
Halothane					
1	2.2	3.6	2.4	2.1	1.8
2	2.0	2.0	1.9	1.6	1.8
3	2.5	2.8	3.0	5.7 *	2.4
4	2.2	3.9	2.7	2.4	1.5
5	1.8	4.6 *	3.4	2.5	1.5
6	3.5	4.2	3.7	2.6	3.7
7	1.9	2.9	2.8	2.1	1.6
8	3.2	5.3 *	5.0 *	4.3 *	3.1
9	2.3	4.9 *	3.3	2.9	1.5
10	1.0	1.7	1.6	1.3	0.9
11	2.3	3.9	2.7	3.7	1.8
12	1.9	1.8	1.5	1.3	1.0
13	2.1	2.8	2.3	1.9	2.8
Excluded from analysis					
14	6.8 *	6.2 *	6.9 *	4.8 *	3.9
Isoflurane					
1	1.7	2.8	2.4	2.1	1.7
2	1.2	2.8	2.2	1.9	2.0
3	4.2	4.1	3.5	2.7	2.2
4	2.8	5.7 *	3.6	3.1	1.8
5	2.3	3.2	2.3	2.5	1.8
6	2.5	3.6	2.9	2.6	1.8
7	4.2	7.7 *	5.4 *	4.4 *	2.8
8	1.5	2.4	2.4	1.6	1.1
9	4.2	4.5 *	4.1	3.2	4.2
10	3.1	4.8 *	3.5	3.2	2.0
11	3.1	3.6	2.8	2.1	2.5
12	1.7	2.8	1.9	1.4	1.4
Excluded from analysis					
13	5.6 *	7.4 *	5.6 *	3.1	3.7
14	2.9	4.2	3.0	2.5	2.5

* abnormal result

Table 6.3

Individual plasma GST concentrations (μg l^{-1}) for spontaneously ventilating (SV) patients undergoing deliberate hypotension, anaesthetised with halothane or isoflurane

	PRE	1 h	3 h	6 h	24 h
Halothane					
1	2.0	2.0	2.0	2.8	2.6
2	2.8	3.4	3.6	3.7	3.0
3	3.1	3.0	3.8	2.8	2.3
4	2.8	3.6	3.8	2.8	2.2
5	2.0	2.0	2.2	2.6	2.1
6	3.3	3.3	3.6	3.3	2.3
7	2.5	2.4	3.1	2.7	2.2
8	3.5	3.5	4.6 *	4.5 *	4.2
9	2.0	1.9	2.2	2.7	1.3
10	1.2	1.7	1.9	1.5	2.0
11	2.5	3.8	2.2	1.8	1.6
Excluded from analysis					
12	5.6 *	5.1 *	4.5 *	4.8 *	3.6
13	5.4 *	5.5 *	6.5 *	3.8	2.2
14	4.6 *	4.9 *	5.7 *	2.6	4.0
Isoflurane					
1	2.5	2.4	2.7	2.1	2.1
2	2.0	2.3	2.3	2.3	1.5
3	2.3	2.3	2.0	1.9	2.0
4	1.0	1.8	2.2	1.2	1.2
5	3.3	4.7 *	11.6 *	16.1 *	5.4 *
6	3.6	4.0	4.4 *	3.1	4.5 *
7	2.9	3.7	3.2	2.9	2.4
8	1.8	2.6	2.6	1.7	1.5
9	2.3	2.3	2.2	2.0	1.8
10	2.3	4.2	3.1	2.7	1.7
11	2.8	3.5	2.6	2.1	2.3
12	1.7	2.1	1.5	1.1	0.9
Excluded from analysis					
13	10.9 *	13.2 *	11.5 *	6.7 *	3.2
14	5.4 *	4.9 *	4.0	4.7 *	3.8
15	4.8 *	5.3 *	3.9	3.0	2.4
16	8.0 *	9.3 *	8.8 *	7.1 *	3.2
17	3.4	3.6	2.9	2.0	2.0

* abnormal result

Table 6.4

Median values (1st-3rd quartile) of plasma GST B_1B_1 concentration ($\mu g\ l^{-1}$) for patients in all four groups.

	PRE	1 h	3 h	6 h	24 h
IPPV					
Halothane	2.2	3.7 **	2.7 *	2.7	1.7 *
	(1.9-2.3)	(2.0-4.6)	(2.3-3.0)	(1.9-2.4)	(0.5-2.4)
Isoflurane	2.6	3.6 **	2.8	2.5	1.9 *
	(1.7-4.2)	(2.8-4.8)	(2.3-3.6)	(1.9-3.2)	(1.7-2.5)
SV					
Halothane	2.5	3.0	3.1 *	2.8	2.2
	(2.0-3.1)	(2.0-3.5)	(2.2-3.8)	(2.6-3.3)	(2.0-2.6)
Isoflurane	2.3	2.5 *	2.6	2.1	1.9
	(1.8-2.9)	(2.3-4.0)	(2.2-3.2)	(1.7-2.9)	(1.5-2.4)

* $p<0.05$; ** $p<0.01$
compared with preoperative concentration

Table 6.5

Median values (1st-3rd quartile) of plasma GST concentrations (μg l^{-1}) for patients receiving halothane and isoflurane, combined to allow an assessment of effect of mode of ventilation

	PRE	1 h	3 h	6 h	24 h
IPPV	2.3	3.6 *	2.8	2.5	1.8
	(1.9-3.1)	(2.8-4.5)	(2.3-3.5)	(1.9-3.1)	(1.5-2.4)
SV	2.5	2.6	2.6	2.7	2.2
	(2.0-2.9)	(2.1-3.6)	(2.2-3.6)	(1.9-2.9)	(1.6-2.4)

* $p<0.01$ between modes of ventilation

Table 6.6

Conventional liver biochemical tests in patients undergoing deliberate hypotension, who received intermittent positive pressure ventilation (IPPV)

	PREOP			POSTOP		
	Bil	ALT	GGT	Bil	ALT	GGT
Halothane						
1	10	20	15	13	19	16
2	11	18	8	11	19	15
3	16	20	9	13	11	6
4	13	13	<5	14	<10	6
5	11	<10	10	21 *	12	10
6	9	26	31	11	24	37
7	6	13	10	8	15	10
8	7	19	16	10	22	14
9	53 *	14	7	71 *	13	7
10	10	26	20	6	23	17
11	13	30	8	20 *	26	8
12	10	15	11	13	10	7
13	15	20	9	26 *	24	9
Excluded from analysis						
14	9	25	12	12	23	14
Isoflurane						
1	12	10	13	10	13	18
2	11	10	7	11	17	23
3	13	12	13	11	15	12
4	6	18	8	7	16	7
5	28 *	<10	12	26 *	12	12
6	18 *	22	12	13	<10	8
7	11	17	22	12	15	15
8	9	20	15	10	19	11
9	16	26	14	10	25	20
10	7	15	18	5	15	13
11	11	23	14	14	28	15
12	11	21	12	11	22	11
Excluded from analysis						
13	12	48 *	49	11	38	38
14	8	35	74 *	5	37	67 *

* abnormal result

Table 6.7

Conventional liver biochemical tests in spontaneously ventilating (SV) patients undergoing deliberate hypotension (* denotes an abnormal result)

	PREOP			POSTOP		
	Bil	ALT	GGT	Bil	ALT	GGT
Halothane						
1	10	15	17	10	14	14
2	8	19	15	9	15	7
3	19 *	20	21	18 *	21	21
4	25 *	20	17	17	23	17
5	14	11	16	16	17	16
6	11	27	17	9	26	13
7	16	17	19	13	16	19
8	9	14	15	8	23	15
9	17	29	17	14	25	12
10	9	20	19	8	17	16
11	11	23	12	12	13	12
Excluded from analysis						
12	8	42 *	6	6	23	<5
13	10	25	33	14	25	32
14	10	22	95 *	11	32	94 *
Isoflurane						
1	11	15	15	6	18	6
2	18 *	14	<5	19 *	<10	8
3	9	13	9	7	13	9
4	8	<10	5	7	<10	<5
5	13	25	14	9	28	13
6	9	19	18	7	18	19
7	20 *	14	9	16	10	7
8	13	16	16	13	13	13
9	9	23	8	9	16	8
10	12	33	14	14	19	11
11	6	27	17	7	28	15
12	9	21	20	11	26	19
Excluded from analysis						
13	12	41 *	46	12	36	41
14	12	42 *	58 *	10	40	51
15	9	14	10	7	13	8
16	12	75 *	40	8	57 *	39
17	11	62 *	33	10	29	26

Table 6.8

Medians (range) of conventional liver biochemical tests before and after anaesthesia for patients receiving halothane and isoflurane, combined to allow an assessment of effect of mode of ventilation

	IPPV		SV	
Bilirubin (μmol l^{-1})				
PRE	11	(6-53)	11	(6-25)
24 h	11	(5-71)	10	(6-19)
ALT (U l^{-1})				
PRE	18	(<10-30)	19	(<10-33)
24 h	16	(<10-28)	17	(<10-28)
GGT (U l^{-1})				
PRE	12	(<5-31)	16	(<5-21)
24 h	12	(6-37)	13	(<5-21)

DISCUSSION

The major finding from this study was that patients who received IPPV in association with deliberate hypotension during halothane or isoflurane anaesthesia displayed a significantly greater increase in plasma GST B_1B_1 concentration at 1 hour after the end of anaesthesia than patients who breathed spontaneously. In contrast to earlier studies in normotensive patients [Chapter 3; Allan et al 1987], the present study failed to show any difference between the effects of halothane and isoflurane on changes in GST concentration.

Some of the methodology in this study is unfortunately open to criticism, especially the method by which patients were allocated to different modes of ventilation, the administration of fentanyl to patients who received IPPV but not SV, and the lack of measurement of inspired or end-tidal concentrations of halothane and isoflurane. Patients were not allocated randomly to different modes of ventilation other than by ward of origin. However, no relevant factors influenced the choice of admission of patients to the two wards, and no significant differences between the groups were observed in patient characteristics or type of operations performed. The slightly shorter duration of anaesthesia in patients who received IPPV simply reflects the speed of the surgical team. The administration of fentanyl to patients who received IPPV, but not to those who breathed

spontaneously, is unlikely to have influenced the present findings. Gelman, Dillard and Bradley (1987) found that hepatic arterial and portal blood flows, and hepatic oxygen supply were very well maintained during fentanyl anaesthesia, in contrast to their findings during halothane or isoflurane administration. The criticism that inspired or end-tidal volatile concentrations were not measured, and therefore that the anaesthetics were probably not administered in equipotent doses, is more difficult to answer. This objection would be meaningful if the development of halothane-associated liver damage in man was dose-dependent. However, there is no evidence to support this hypothesis, although Jee and coworkers (1980) found that the severity of hepatic damage caused by halothane in rats was related to total halothane dosage. Therefore, although more accurate measurement of concentration of volatile agent administered might have strengthened the study, omitting to do so is unlikely to have significantly influenced the findings or their interpretation. These criticisms notwithstanding, the findings from this study provide further valuable information in the investigation of possible mechanisms of halothane-induced liver damage.

It is likely that the transient hepatic dysfunction demonstrated in the present study resulted from alterations in liver blood flow. Possible reasons for the observed difference in GST concentration between the modes of ventilation include the effects on liver blood

flow of positive pressure ventilation and arterial carbon dioxide tension.

Total liver blood flow is reduced by positive pressure ventilation to a greater extent than the concomitant reduction in cardiac output [Geiger, Georgieff & Lutz 1986]. It is possible that deliberate hypotension could exaggerate these changes, but the effects of hypotension on liver blood flow are difficult to predict. Animal studies have reported controversial data on splanchnic and hepatic arterial blood flows during SNP-induced hypotension; these blood flows have been shown to be either increased [Ross & Cole 1973; Miller & Delaney 1981; Gelman & Ernst 1978], decreased [Wang, Liu & Katz 1977], or unchanged [Sivarajan, Amory & McKenzie 1985; Fan et al 1980]. Hepatic arterial blood flow has been reported to either decrease [Skivolocki, Pace & Thomford 1972], remain unchanged [Sivarajan 1986], or increase temporarily [Plewes & Farhi 1985] during hypotension induced by trimetaphan. The hepatic circulation has not been studied extensively in humans during controlled hypotension because of the difficulties of measuring precisely liver blood flow, although Chauvin and coworkers (1985) found no reduction in total hepatic blood flow during SNP-induced hypotension in neurosurgical patients. The effect of a combined infusion of SNP and trimetaphan on liver blood flow has not been reported. Total hepatic blood flow is closely related to changes in cardiac output during SNP-induced

hypotension. Liver blood flow is reduced only when cardiac output decreases, possibly because the adaptations of hepatic arterial resistances are quantitatively too small to compensate for the decrease in portal blood flow induced by reduced cardiac output [Chauvin et al 1985]. Wildsmith and colleagues (1983) found in patients who underwent controlled hypotension with combined SNP and trimetaphan infusion, that cardiac output was reduced in those who received IPPV but not in those who breathed spontaneously. As these patients were studied in circumstances very similar to those of the present study, it is likely that cardiac output, and by inference, liver blood flow, was reduced to a greater extent in patients receiving IPPV than in those who breathed spontaneously. This might account for the observed difference in plasma GST concentration at 1 hour between the modes of ventilation.

The mean $PaCO_2$ measured at stable hypotension was significantly greater in patients who were breathing spontaneously than in those who received IPPV, but this did not significantly influence the increase in GST concentration at 1 hour after the end of anaesthesia. Hypercapnia is usually accompanied by a substantial increase in liver blood flow [Hughes et al 1979; Thomson et al 1983] with a consequent increase in hepatic oxygen supply. These increases, although potentially beneficial to the hepatic parenchyma, are balanced by an increase in hepatic oxygen consumption, probably

resulting from an increase in sympathetic discharge [Thomson et al 1983]. The hepatic oxygen supply/demand ratio thus remains unaltered. More recent work in beagles, however, has suggested that mild hypercapnia ($PaCO_2$ 7.9 kPa) is associated with a depression of hepatic function, in spite of the increases in liver blood flow [Fujita et al 1989]. It would therefore be expected that increased $PaCO_2$ would be associated with an increase in plasma GST concentration. The present study, however, has demonstrated that the increase in GST concentration is not related to $PaCO_2$. This tends to suggest that the greater increase in GST concentration at 1 hour after the end of anaesthesia in patients who received IPPV is more likely to be due to the effect of IPPV on liver blood flow and not due to the effect of $PaCO_2$ on splanchnic haemodynamics.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS FOR THE USE OF HALOTHANE

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CONCLUSIONS

It is now generally accepted that two types of halothane-induced liver damage exist. A mild form manifest by elevated biochemical liver tests, which may occur in up to 20% of patients exposed to halothane, is thought to be due to toxic products of halothane biotransformation, possibly determined by genetic factors, or to hepatic hypoxia resulting from an imbalance between hepatic oxygen supply and demand; altered cellular calcium balance is another possible mechanism. The much rarer fulminant form is associated with the appearance of a specific circulating antibody and is thought to result from an immune reaction. Precise definition of the mechanism(s) responsible for damage is complicated because no diagnostic test is available for either form of toxicity. Evidence exists supporting the involvement of each of the currently proposed aetiologies. For example, the extents of metabolism of halothane, enflurane and isoflurane correlate directly with the incidence of reported clinical hepatic dysfunction for these drugs, as does the degree to which each of these agents interferes with the balance between hepatic oxygen supply and demand, the extent to which each of them stimulates the release of calcium from stores in hepatocytes, and their ability to

form immunoreactive protein adducts.

Much of the work supporting each of the proposed mechanisms has been performed in animal models often requiring the presence of demanding conditional factors which do not pertain during clinical anaesthesia in man. Therefore, data from these animal studies may not be applicable to the problem of human hepatotoxicity. In addition, human studies which have attempted to investigate biochemical liver dysfunction after halothane anaesthesia have suffered from the lack of a suitably sensitive indicator of mild hepatocellular damage. The studies in this thesis have investigated the more common subclinical type of liver dysfunction using plasma GST B_1B_1 concentration as a highly sensitive and specific index of liver damage.

The four studies upon which this thesis is based have shown consistently that halothane anaesthesia is associated with increased plasma concentrations of GST B_1B_1 after operation. Enflurane is also associated with increased concentrations of GST after anaesthesia, although not to the same extent as with halothane, while isoflurane appears to have little effect on GST concentration. Several patients in each study developed GST values which exceeded the upper limit of the reference range, and some of these values were considerably increased. When the four studies are combined, 118 patients who received general anaesthesia

with halothane, enflurane or isoflurane did not undergo any therapeutic manoeuvre designed to alter the release of GST after operation; these patients comprise those in all three groups in Chapter 3, and those who received placebo in Chapters 4 and 5. The incidence of abnormal GST concentrations observed in these patients is shown in Table 7.1.

Table 7.1

Incidence of abnormal GST concentrations in patients who underwent spontaneously breathing, normotensive, general anaesthesia

	Patients with abnormal GST concentration			
	At 3, 6 or 24h		At 24 h	
	n	%	n	%
Halothane	21/70	30	8/69	11.6
Enflurane	6/30	20	3/30	10
Isoflurane	2/18	11.1	0/18	0

Almost one-third of patients who underwent routine, spontaneously ventilating halothane anaesthesia developed an abnormal GST concentration after anaesthesia; the incidence was much lower in, admittedly fewer, patients after enflurane or isoflurane anaesthesia. As diurnal variation in GST concentration has not been demonstrated in volunteers, and as clearance of GST is unlikely to be impaired, the increases observed after anaesthesia are

considered to reflect impaired hepatocellular integrity.

Measurement of plasma GST B_1B_1 concentration has revealed two, probably distinct, phases of toxicity. The initial phase occurs at 3-6 hours after anaesthesia, and the second phase occurs at 24 hours. In all four studies plasma GST concentration increased significantly from preoperative values at 3 or 6 hours in spontaneously breathing patients anaesthetised with halothane. In contrast, administration of isoflurane to patients breathing spontaneously did not result in significant increases in GST concentration at either 3 or 6 hours, although when arterial pressure was deliberately reduced, GST increased significantly at 1 hour after the end of anaesthesia in patients who received isoflurane. It is not clear whether this increase reflects the combined influence of isoflurane and deliberate hypotension as GST concentrations were not measured at 1 hour in normotensive patients receiving isoflurane. Enflurane was administered in one study only, but as with halothane, GST concentration increased at 3 to 6 hours in the great majority of patients.

The second phase of toxicity occurs at 24 hours after anaesthesia, when much greater increases were observed in a small number of patients. For the eleven patients described in Table 7.1 who had increased GST at 24 hours, the median value of GST at that time was 9.1 $\mu g\ l^{-1}$ (range 4.5-16.5) in those who received halothane, and

4.8 μg l^{-1} (range 4.3-5.8) for those who received enflurane. This later increase at 24 hours does not occur as consistently as the earlier increase at 3 to 6 hours; in one study no patient who received halothane developed an increased GST concentration at 24 hours, whereas almost 1 in 5 patients who received halothane in another study developed an increased GST concentration at this time. It would be necessary, therefore, to study many more patients to ascertain the true incidence of this secondary increase in GST concentration.

How have these studies assisted in our knowledge and understanding of the mechanisms responsible for halothane-induced liver damage? The incidence of abnormal GST concentrations after exposure to each of the volatile agents was found to parallel their reported ability to cause hepatotoxicity (halothane > enflurane > isoflurane). Thus it was established that measurement of plasma GST B_1B_1 concentration might be valuable in the investigation of subclinical liver damage after volatile anaesthesia. Subsequently, three of the proposed aetiologies for halothane hepatitis, namely altered hepatocellular calcium homeostasis, toxic products of halothane biotransformation and hepatic hypoxia, were investigated in more detail. This involved respectively, the administration of the calcium channel blocker nicardipine, the administration of cimetidine, which reduces the metabolism of several drugs including halothane, and the use of deliberate hypotension and

intermittent positive pressure ventilation, during which liver blood flow is reduced.

Perioperative administration of nicardipine by intravenous infusion did not prevent or reduce the release of GST into the peripheral circulation after halothane anaesthesia at either 3 to 6 hours or at 24 hours, and nor did it result in a lesser frequency of abnormal GST values; rather it resulted in a significantly greater increase in GST concentration at 3 hours than did halothane alone, although this occurred only in male patients. This suggests, therefore, that altered calcium homeostasis is probably not the major mechanism involved, although a supporting role in the pathogenesis of halothane-induced liver damage cannot be excluded.

Similarly, the administration of cimetidine by mouth before halothane anaesthesia did not influence either the magnitude of increase in plasma GST concentration or the frequency of abnormally increased GST values after anaesthesia, although no patient who had received cimetidine developed a large secondary increase in GST at 24 hours. The findings from this study would tend to support the view that products of halothane metabolism are not responsible for the early increase in GST concentration. The role of halothane biotransformation in causing the secondary increase in GST concentration cannot be excluded; many more patients would require to

be studied to be certain that cimetidine prevented this less frequent increase.

The technique of IPPV and concurrent hypotension resulted in a greater increase in GST concentration at 1 hour after anaesthesia than did the same technique in which patients breathed spontaneously, irrespective of the volatile agent administered. The mode of ventilation did not influence the increases in GST concentration at any other time. It is most likely that the greater increase in GST concentration following IPPV resulted from the greater depression of liver blood flow which occurs during IPPV than during spontaneous ventilation.

These studies provide evidence supporting the view that a different mechanism is responsible for each phase of toxicity recognised in the 24 hours after anaesthesia. The early phase up to 6 hours most probably results from an imbalance between liver oxygen supply and demand, although the role of altered hepatocellular calcium homeostasis cannot be excluded, whereas the secondary phase at 24 hours may result from toxic products of halothane metabolism. The four studies described in this thesis, therefore, support the proposal that the aetiology of the mild form of halothane-induced liver dysfunction is multifactorial, in which products of biotransformation, altered calcium homeostasis and hepatic hypoxia all participate.

The measurement of plasma GST concentration can therefore help our understanding of the mechanisms responsible for initiating halothane-induced liver injury, and may allow assessment of therapeutic manoeuvres which might prevent or reduce the severity of liver injury.

RECOMMENDATIONS FOR THE USE OF HALOTHANE

The preparation of this thesis has involved an extensive survey of the literature on the problem of halothane hepatotoxicity, and this has enabled an understanding of the arguments for and against the continued use of halothane as an anaesthetic agent. It therefore seems not unreasonable to present a personal opinion on the current place of halothane in anaesthetic practice. It should be stressed, however, that the discussion and recommendations which follow arise not from the investigations measuring plasma GST concentration, but rather from impressions gained from surveying the literature.

For more than 30 years, halothane has enjoyed a reputation as a reliable and usually safe anaesthetic which can be used in many clinical circumstances. More recently however, there has been considerable controversy regarding the continued use of halothane as an anaesthetic agent [Bennetts 1986; Blogg 1986; Weis & Engelhardt 1989]. Much of the discussion has been far

removed from any scientific basis and as a result has bordered on the irrational. There is no doubt that, on rare occasions, halothane is associated with severe liver damage which can prove fatal. One apparently obvious solution to the problem of hepatotoxicity is not to use halothane. However, the problems associated with halothane anaesthesia must be kept in perspective, and the hazards of alternative methods must be considered if halothane is to be eliminated.

The risks of halothane anaesthesia cannot be considered apart from the overall risks of anaesthesia. The incidence of death from the most unfavourable studies on halothane-induced liver failure is approximately 1 in 10,000. This is the same as the overall mortality due to anaesthesia reported by Lunn and Mushin (1982) and is similar to the incidence of serious neurological sequelae following epidural blockade [Usubiaga 1975; Scott & Hibbard 1990]. The incidence of severe morbidity after adverse reactions to intravenous agents in the United Kingdom is estimated to be around 1 in 5000 [Noble & Yap 1989], and more patients die from hypersensitivity reactions to anaesthetic drugs in general than from halothane hepatitis [Nimmo 1980].

What, then, of alternatives to halothane? Neither enflurane nor isoflurane are known to be superior to halothane in relation to the safety of anaesthesia. Both these agents have the potential to cause liver

damage under certain conditions in animals, although their hepatotoxicity in humans has not yet been proven. Enflurane and isoflurane both undergo considerably less metabolism than halothane, and it is alleged that this reduces their potential for toxicity. However, a common mechanism may link the hepatotoxicity induced by these three agents. It is proposed that acyl metabolites of the volatile anaesthetics (possibly trifluoroacetic acid for halothane and isoflurane) may become covalently bound to hepatic proteins and act as antigens, thus precipitating an immune reaction [Christ et al 1988b]. Although enflurane and isoflurane have less immunogenic potential than halothane, it cannot be considered trivial since the degree of haptenic alteration necessary to provoke an immune response in a sensitised individual is not known.

Metabolism of enflurane causes an increase in serum fluoride ion concentration which is potentially nephrotoxic [Mazze, Calverley & Smith 1977], especially if the patient is obese or receiving isoniazid [Brown & Gandolfi 1987; Mazze, Woodruff & Heerdt 1982]; production of fluoride is much less with halothane and isoflurane [Baden & Rice 1986]. Enflurane reduces the cerebral convulsion threshold and may precipitate seizures even in those with no history of epilepsy [Joas, Stevens & Eger 1971; Steen & Michenfelder 1979; Jenkins & Milne 1984]; frank convulsions have also been reported with isoflurane [Harrison 1986; Hymes 1985; Poulton & Ellingson 1984].

Isoflurane is a potent coronary vasodilator, and has been implicated in causing myocardial ischaemia by diverting flow away from areas of borderline perfusion towards areas that are already perfused adequately, a phenomenon known as "coronary steal" [Reiz et al 1983; Buffington et al 1987]. Although this is important probably only in high inspired concentrations, isoflurane is almost certainly dangerous for some patients with coronary artery disease under some conditions [Becker 1987]. A further problem with isoflurane is its pungent odour which makes it unpleasant to breathe. This would appear to be responsible for the high incidence of laryngospasm and arterial oxygen desaturation seen during inhalation induction with isoflurane [Phillips, Brimacombe & Simpson 1988]. Therefore, despite the perceived advantage of greater resistance to metabolism, neither enflurane nor isoflurane appears to be significantly closer to the ideal inhalation anaesthetic agent than halothane.

More recently sevoflurane and desflurane (I-653) have attracted interest as possible alternatives to the volatile anaesthetic agents in current use. Sevoflurane is potent, pleasant to inhale, non-flammable in clinical concentrations, and allows rapid and smooth induction of anaesthesia [Holaday & Smith 1981]. It is metabolised to approximately the same extent as enflurane, but is degraded substantially (31% at 37°C) in the presence of soda lime, raising questions about its potential toxicity [Strum, Johnson & Eger 1987]. Indeed, sevoflurane has

been shown to cause hepatic injury in animal models [Strum et al 1987; Lind et al 1989]. There has been little attempt to promote the widespread international use of sevoflurane, suggesting that it offers little or no advantage over the currently available agents.

Desflurane, which is undergoing evaluation for clinical use, represents an interesting development in the quest for the ideal inhalation anaesthetic agent [Miller & Greene 1990]. It permits more rapid induction and emergence, and greater sensitivity of control of anaesthetic depth than all the other volatile anaesthetic agents [Heijke & Smith 1990]. It undergoes minimal biotransformation [Koblin et al 1988; Koblin et al 1989] and is more stable in the presence of soda lime than any of the presently used volatile anaesthetics [Eger, Johnson & Ferrell 1987]. To date there are no reports of liver damage associated with the administration of desflurane either in animals [Eger et al 1987; Eger, Johnson & Ferrell 1987; Holmes et al 1989] or in humans [Jones et al 1990b]. However, specialised vaporisation systems and the use of closed circuit anaesthesia are essential due to its high volatility and expense, and these present practical difficulties in its use. It would be premature to speculate on the cost/benefit ratio of desflurane compared with isoflurane or enflurane at such an early stage in its development.

Total intravenous anaesthesia is another alternative to

halothane, although protagonists of the technique appear to be more concerned about the problem of anaesthetic gas pollution than about toxicity of volatile anaesthetics [Wright & Dundee 1982]. Although the technique is valuable in certain clinical situations, it is not without limitations. These include the lack of an ideal agent despite the introduction of propofol, difficulty in controlling depth of anaesthesia, enormous individual variation in dose requirements [White 1989], and an increased risk of awareness during anaesthesia. Furthermore, intravenous anaesthesia with Althesin or propofol was shown to result in liver enzyme changes similar to those seen after halothane anaesthesia [Sear, Prys-Roberts & Dye 1983], and altered liver biochemical tests have been reported after the administration of thiopentone [Dundee 1955], methohexitone [Bittrich, Kane & Mosher 1963] and ketamine [Dundee et al 1980]. Therefore, intravenous anaesthesia does not appear to solve the problem of hepatotoxicity associated with anaesthesia.

Halothane is more potent than enflurane and isoflurane and is less irritant to the respiratory tract. These properties may confer an important advantage when there is a need to deepen the level of anaesthesia quickly, as inability to effectively control the depth of anaesthesia presents an immediate threat to a patient's life in circumstances such as regurgitation or airway obstruction. Therefore, when overall patient safety is

considered, it would seem that the risk/benefit ratio for halothane compares favourably with that for alternative volatile agents and the technique of intravenous anaesthesia. Halothane has deserved its prominence in anaesthesia for more than 30 years; it would be most unfortunate if it were to disappear because of the protestations of non-anaesthetists whose understanding of the balance of risks in anaesthetic practice is probably deficient by anaesthetists' standards.

What, then, is a "safe" interval between halothane anaesthetics? The Committee on Safety of Medicines (CSM) originally recommended that repeated halothane anaesthesia should be avoided within 28 days. This recommendation was based on the findings of Inman and Mushin (1974; 1978) who analysed cases of alleged halothane hepatotoxicity reported to CSM. Of 251 cases considered, 82% involved multiple exposures to halothane, and 75% of these multiple exposures involved at least two halothane anaesthetics within 28 days. However, neither the physical status of the patient nor the severity of the surgical illness were taken into account, and the recommendation appears to have been nothing other than a cautious guess. More recently, and again without firm evidence, CSM advised that the minimum interval between halothane anaesthetics should be increased to 3 months, and that a history of unexplained jaundice or pyrexia in a patient following exposure to halothane contraindicated its future use in that patient [Committee on Safety of

Medicines 1986]. These recommendations are thought by many to impose severe limitations on the choice of volatile anaesthetic agent, which might in certain clinical situations be harmful to patients [Adams et al 1986]. In addition, because CSM depends on a system of open reporting, the reliability of the figures upon which such recommendations were made has been questioned [Spence 1987a]. There can be little argument that unexplained jaundice following a previous exposure to halothane contraindicates its future use in that patient. Unexplained pyrexia however, is hopelessly superficial: many patients develop fever after operation, irrespective of the anaesthetic used, and its value as a sign of sensitisation to halothane is of doubtful significance [Dykes 1971].

It is not possible to define a "safe" interval for all patients; halothane hepatotoxicity may occur after a single exposure to the drug, whereas other cases have been reported several years after the previous exposure [Neuberger & Williams 1984]. If the fulminant form of halothane hepatitis results from immunological sensitisation then there can be no "safe" interval for patients so predisposed.

In these days of increasing litigation, it would seem prudent to adopt the following guidelines for the administration of halothane:

1. A history of unexplained jaundice following previous

exposure to halothane contraindicates its future use in that patient; unexplained pyrexia requires further definition.

2. A minimum interval between halothane anaesthetics of 3 months should apply for most adult patients, realising that for some patients there is no "safe" period. The interval of 3 months is not appropriate for children under the age of 14 years, in whom the risk of halothane hepatotoxicity is extremely remote, and for whom halothane provides many advantages for overall safety.

3. If, on consideration of the available options and the patient's condition, halothane is to be administered within 3 months from a previous exposure, the appropriate reasons for the choice of halothane should be documented in the case notes.

4. If halothane is to be avoided for fear of provoking liver damage, the alternative inhalation agent should be delivered from an anaesthetic machine to which a halothane vaporiser has not been attached, in order to prevent the development of liver injury from inapparent circuit contamination with halothane: that this is not the practice in most operating theatres serves only to underline the double standards which are applied currently to the use of halothane.

Halothane is a safe and useful anaesthetic agent. As with all drugs, however, its use can result in adverse effects. An understanding of the mechanisms of halothane-induced hepatotoxicity, and the factors predisposing to it, will enable full advantage to be taken of the desirable properties of this agent while minimising the risks.

REFERENCES

REFERENCES

Adachi Y, Horii K, Takahashi Y, Tanihata M, Ohba Y, Yamamoto T (1980). Serum glutathione S-transferase activity in liver diseases. Clinica Chimica Acta 106: 243-255.

Adams AP, Campbell D, Clarke RSJ, Downing JW, Healy TEJ, Hull CJ, Hutton P, Jones JG, Norman J, Payne JP, Robson Sir Gordon, Spence AA, Vickers MD (1986). Halothane and the liver. British Medical Journal 293: 1023.

Aldridge LM, Ray DC, Drummond GB, Howie AF, Beckett GJ (1991). Variation of plasma glutathione S-transferase concentration in healthy volunteers. British Journal of Anaesthesia (in press).

Allan LG, Hussey AJ, Howie J, Beckett GJ, Smith AF, Hayes JD, Drummond GB (1987). Hepatic glutathione S-transferase release after halothane anaesthesia: open randomised comparison with isoflurane. Lancet 1: 771-774.

Allen PJ, Downing JW (1977). A prospective study of hepatocellular function after repeated exposures to halothane or enflurane in women undergoing radium therapy for cervical cancer. British Journal of Anaesthesia 49: 1035-1039.

Allen HL, Metcalf DW (1964). A search for halothane liver complications. Anesthesia and Analgesia 43: 159-162.

Alps BJ, Calder C, Wilson A (1985). Nicardipine in models of myocardial infarction. British Journal of Clinical Pharmacology 20: 29s-49s.

Aniya Y, Anders MW (1985a). Chloroform-induced alteration of glutathione S-transferase activity. Biochemical Pharmacology 34: 249-255.

Aniya Y, Anders MW (1985b). Alteration of hepatic glutathione S-transferases and release into serum after treatment with bromobenzene, carbon tetrachloride, or N-nitrosodimethylamine. Biochemical Pharmacology 34: 4239-4244.

Arditi M, Cravetto C, Molino G, Pera A, Ponti V (1983). The effect of chronic treatment with gastric antisecretory drugs on functional liver plasma flow. Gastroenterology 84: 1092.

Asada M, Galambos JT (1963). Sorbitol dehydrogenase and hepatocellular injury: An experimental and clinical study.
Gastroenterology 44: 578-587.

Atallah MM, Geddes IC (1973). Metabolism of halothane during and after anaesthesia in man.
British Journal of Anaesthesia 45: 464-470.

Awasthi YC, Dao DD, Saneto RP (1980). Interrelationship between anionic and cationic forms of glutathione S-transferases of human liver.
Biochemistry Journal 191: 1-10.

Baden JM, Rice SA (1986). Metabolism and toxicity of inhaled anesthetics. In: Miller RD, ed. Anesthesia. New York: Churchill Livingstone Inc.: 701-744.

Bass NM, Kirsch RE, Tuff SA, Saunders SJ (1977). Radioimmunoassay of ligandin.
Biochimica et Biophysica Acta 494: 131-143.

Bass NM, Kirsch RE, Tuff SA, Saunders SJ (1978). Radioimmunoassay of plasma ligandin. A sensitive index of experimental hepatocellular necrosis.
Gastroenterology 75: 589-594.

Bauman JH, Kimelblatt BJ (1982). Cimetidine as an inhibitor of drug metabolism: Therapeutic implications and review of the literature.
Drug Intelligence and Clinical Pharmacy 16: 380-386.

Becker GL, Fiskum G, Lehninger AL (1980). Regulation of free Ca^{2+} by liver mitochondria and endoplasmic reticulum.
Journal of Biological Chemistry 225: 9009-9012.

Becker LC (1987). Is isoflurane dangerous for the patient with coronary artery disease?
Anesthesiology 66: 259-261.

Beckett GJ, Chapman BJ, Dyson EH, Hayes JD (1985a). Plasma glutathione S-transferase measurements after paracetamol overdose: evidence for early hepatocellular damage.
Gut 26: 26-31.

Beckett GJ, Dyson EH, Chapman BJ, Templeton AJ, Hayes JD (1985b). Plasma glutathione S-transferase measurements by radioimmunoassay: a sensitive index of hepatocellular damage in man.
Clinica Chimica Acta 146: 11-19.

Beckett GJ, Hayes JD (1984). Development of specific radioimmunoassays for the measurement of human hepatic basic and N/A2b glutathione S-transferase.
Clinica Chimica Acta 141: 267-273.

Beckett GJ, Hayes JD (1987). Plasma glutathione S-transferase measurements and liver disease in man. Journal of Clinical Biochemistry and Nutrition 2: 1-24.

Beckett GJ, Kellett HA, Gow SM, Hussey AJ, Hayes JD, Toft AD (1985c). Raised plasma glutathione S-transferase values in hyperthyroidism and in hypothyroid patients receiving thyroxine replacement: evidence for hepatic damage. British Medical Journal 291: 427-431.

Belfrage S, Ahlgren I, Axelson S (1966). Halothane hepatitis in an anaesthetist. Lancet 2: 1466-1467.

Benjamin SB, Goodman ZD, Ishak KG, Zimmerman HJ, Irey NS (1985). The morphologic spectrum of halothane-induced hepatic injury: Analysis of 77 cases. Hepatology 5: 1163-1171.

Bennetts FE (1986). Halothane and the liver. The problem revisited. Proceedings of a symposium at Bristol University Medical School. Bristol: Sir Humphrey Davy Department of Anaesthesia.

Bentley JB, Vaughan RW, Gandolfi AJ, Cork RC (1982). Halothane biotransformation in obese and nonobese patients. Anesthesiology 57: 94-97.

Berman ML, Kuhnert L, Phythyon JM, Holaday DA (1983). Isoflurane and enflurane-induced hepatic necrosis in triiodothyronine-pretreated rats. Anesthesiology 58: 1-5.

Bittrich NM, Kane AV'R, Mosher RE (1963). Methohexital and its effect on liver function tests. A comparative study. Anesthesiology 24: 81-90.

Blackburn WR, Ngai SH, Lindenbaum J (1964). Morphologic changes in hepatic necrosis following halothane anesthesia in man. Anesthesiology 25: 270-283.

Blake DA, Cascorbi HF, Rozman RS, Meyer FJ (1969). Animal toxicity of 2,2,2-trifluoroethanol. Toxicology and Applied Pharmacology 15: 83-91.

Blogg CE (1986). Halothane and the liver: the problem revisited and made obsolete. British Medical Journal 292: 1691-1692.

Board PG (1981). Biochemical genetics of glutathione S-transferase in man. American Journal of Human Genetics 33: 36-43.

Booth J, Boyland E, Sims P (1961). An enzyme from rat liver catalysing conjugations with glutathione. Biochemistry Journal 79: 516-524.

Bosnjak ZJ, Kampine JP (1983). Effects of halothane, enflurane, and isoflurane on the SA node. Anesthesiology 58: 314-321.

Böttiger LE, Dalén E, Hallén B (1976). Halothane-induced liver damage: An analysis of the material reported to the Swedish Adverse Drug Reaction Committee 1966-1973. Acta Anaesthesiologica Scandinavica 20: 40-46.

Boyer TD (1989). The glutathione S-transferases: An update. Hepatology 9: 486-496.

Brattin WJ, Pencil SD, Waller RL, Glende EA, Recknagel RO (1984). Assessment of the role of calcium ion in halocarbon hepatotoxicity. Environmental Health Perspectives 57: 321-323.

Brensilver HL, Kaplan MM (1975). Significance of elevated liver alkaline phosphatase in serum. Gastroenterology 68: 1556-1562.

Brody GL, Sweet RB (1963). Halothane anesthesia as a possible cause of massive hepatic necrosis. Anesthesiology 24: 29-37.

Brown BR (1981). Pharmacogenetics and the halothane hepatitis mystery. Anesthesiology 55: 93-94.

Brown BR (1985). Halothane hepatitis revisited. New England Journal of Medicine 313: 1347-1348.

Brown BR, Gandolfi AJ (1987). Adverse effects of volatile anaesthetics. British Journal of Anaesthetics 59: 14-23.

Brown BR, Sipes IG (1977). Biotransformation and hepatotoxicity of halothane. Biochemical Pharmacology 26: 2091-2094.

Brown BR, Sipes IG, Sagalyn AM (1974). Mechanisms of acute hepatic toxicity: chloroform, halothane, and glutathione. Anesthesiology 41: 554-561.

Buffington CW, Romson JL, Levine A, Duttlinger NC, Huang AH (1987). Isoflurane induces coronary steal in a canine model of chronic coronary occlusion. Anesthesiology 66: 280-292.

Bunker JP, Blumenfeld CM (1963). Liver necrosis after halothane anesthesia. Cause or coincidence? New England Journal of Medicine 268: 531-534.

Burnap TK, Galla SJ, Vandam LD (1958). Anesthetic, circulatory and respiratory effects of Fluothane. Anesthesiology 19: 307-320.

Burrows S, Feldman W, McBride F (1975). Serum gamma-glutamyl transpeptidase. Evaluation in screening of hospitalized patients. American Journal of Clinical Pathology 64: 311-314.

Callis AH, Brooks SD, Roth TP, Gandolfi AJ, Brown BR (1987). Characterization of a halothane-induced humoral immune response in rabbits. Clinical and Experimental Immunology 67: 343-351.

Campanini RZ, Tapia RA, Sarnat W, Natelson S (1970). Evaluation of serum argininosuccinate lyase (ASAL) concentrations as an index to parenchymal liver disease. Clinical Chemistry 16: 44-53.

Campbell BC, Kelman AW, Hillis WS (1985). Noninvasive assessment of the haemodynamic effects of nicardipine in normotensive subjects. British Journal of Clinical Pharmacology 20: 55s-61s.

Carney FMT, Van Dyke RA (1972). Halothane hepatitis: A critical review. Anesthesia and Analgesia 51: 135-160.

Carrigan TW, Straughen WJ (1987). A report of hepatic necrosis and death following isoflurane anesthesia. Anesthesiology 67: 581-583.

Cascorbi HF, Blake DA, Helrich M (1970). Differences in the biotransformation of halothane in man. Anesthesiology 32: 119-123.

Cascorbi HF, Vesell ES, Blake DA, Helrich M (1971). Genetic and environmental influence on halothane metabolism in twins. Clinical Pharmacology and Therapeutics 12: 50-55.

Chase RE, Holaday DA, Fiserova-Bergerova V, Saidman LJ, Mack FE (1971). The biotransformation of Ethrane in man. Anesthesiology 35: 262-267.

Chasseaud LF (1979). The role of glutathione and glutathione S-transferases in the metabolism of chemical carcinogens and other electrophilic agents. Advances in Cancer Research 29: 175-274.

Chauvin M, Bonnet F, Montembault C, Lafay M, Curet P, Viars P (1985). Hepatic plasma flow during sodium nitroprusside-induced hypotension in humans. Anesthesiology 63: 287-293.

Cheung JY, Bonventre JV, Malis CD, Leaf A (1986). Calcium and ischemic injury. New England Journal of Medicine 314: 1670-1676.

Chien KR, Abrams J, Pfau RG, Farber JL (1977). Prevention by chlorpromazine of ischemic liver cell death. American Journal of Pathology 88: 539-557.

Christ DD, Kenna JG, Kammerer W, Satoh H, Pohl LR (1988a). Enflurane metabolism produces covalently bound liver adducts recognized by antibodies from patients with halothane hepatitis. Anesthesiology 69: 833-838.

Christ DD, Satoh H, Kenna JG, Pohl LR (1988b). Potential metabolic basis for enflurane hepatitis and the apparent cross-senstization between enflurane and halothane. Drug Metabolism and Disposition 16: 135-140.

Clark AG, Smith JN, Speir TW (1973). Cross specificity in some vertebrate and insect glutathione-transferases with methyl parathion (dimethyl p-nitrophenyl phosphorothionate), 1-chloro-2,4-dinitrobenzene and S-crotonyl-N-acetylcysteamine as substrates. Biochemical Journal 135: 385-392.

Clarke RSJ, Doggart JR, Lavery T (1976). Changes in liver function after different types of surgery. British Journal of Anaesthesia 48: 119-127.

Cohen EN (1969). Metabolism of halothane-2^{14}C in the mouse. Anesthesiology 31: 560-565.

Cohen EN (1971). Metabolism of the volatile anesthetics. Anesthesiology 35: 193-202.

Cohen EN, Hood N (1969). Application of low-temperature autoradiography to studies of the uptake and metabolism of volatile anesthetics in the mouse. Anesthesiology 31: 553-559.

Cohen EN, Trudell JR, Edmunds HN, Watson E (1975). Urinary metabolites of halothane in man. Anesthesiology 43: 392-401.

Cohen GA, Goffinet JA, Donabedian RK, Conn HO (1976). Observations on decreased serum glutamic oxalacetic transaminase (SGOT) activity in azotemic patients. Annals of Internal Medicine 84: 275-280.

Cohen PJ, Chance B (1990). Continuous in vivo measurement of hepatic lipoperoxidation using chemiluminescence: Halothane and chloroform compared.
Anesthesia and Analgesia 70: 296-298.

Combes B, Shore GM, Cunningham FG, Walker FB, Shorey JW, Ware A (1977). Serum gamma-glutamyl transpeptidase activity in viral hepatitis: suppression in pregnancy and by birth control pills.
Gastroenterology 72: 271-274.

Combes B, Stakelum GS (1961). A liver enzyme that conjugates sulfobromophthalein sodium with glutathione.
Journal of Clinical Investigation 40: 981-988.

Committee on Safety of Medicines (1986). Halothane hepatotoxicity. Current Problems No. 18. London: Committee on Safety of Medicines.

Cook TL, Beppu WJ, Hitt BA, Kosek JC, Mazee RI (1975). Renal effects and metabolism of sevoflurane in Fischer 344 rats: An in-vivo and in-vitro comparison with methoxyflurane.
Anesthesiology 43: 70-77.

Cooperman LH, Warden JC, Price HL (1968). Splanchnic circulation during nitrous oxide anesthesia and hypocarbia in normal man.
Anesthesiology 29: 254-258.

Cousins MJ (1980). Halothane hepatitis: What's new?
Drugs 19: 1-6.

Cousins MJ, Gourlay GK, Knights KM, Hall P de la M, Lunam CA, O'Brien P (1987a). A randomised prospective controlled study of the metabolism and hepatotoxicity of halothane in humans.
Anesthesia and Analgesia 66: 299-308.

Cousins MJ, Plummer JL, Hall P de la M (1989). Risk factors for halothane hepatitis.
Australian and New Zealand Journal of Surgery 59: 5-14.

Cousins MJ, Plummer JL, Knights KM, Hall P, Gourlay GK, Jenner M (1987b). Animal models of hepatic injury associated with halogenated anesthetics.
Anesthesia and Analgesia 66: 804.

Cousins MJ, Sharp JH, Gourlay GK, Adams JF, Haynes WD, Whitehead R (1979). Hepatotoxicity and halothane metabolism in an animal model with application for human toxicity.
Anaesthesia and Intensive Care 7: 9-24.

Dale O, Nilsen OG (1984). Glutathione and glutathione S-transferases in rat liver after inhalation of halothane and enflurane.
Toxicology Letters 23: 61-66.

Daneshmend TK, Ene MD, Parker G, Roberts CJC (1984). Effects of chronic oral cimetidine on apparent liver blood flow and hepatic microsomal enzyme activity in man.
Gut 25: 125-128.

Danilewitz MD, Braude BM, Bloch HM, Botha J, Kew MC (1980). Acute hepatitis following enflurane anaesthesia.
British Journal of Anaesthesia 52: 1151-1153.

Dao DD, Partridge CA, Kurosky A, Awasthi YC (1984). Human glutathione S-transferases. Characterization of the anionic forms from lung and placenta.
Biochemical Journal 221: 33-41.

Debbas NMG, Jackson SHD, Turner P (1985). A study of the haemodynamic effects of nicardipine infusion in man.
British Journal of Clinical Pharmacology 19: 563p-564p.

de Groot H, Noll T (1983). Halothane hepatotoxicity: Relation between metabolic activation, hypoxia, covalent binding, lipid peroxidation and liver cell damage.
Hepatology 3: 601-606.

Defalque RJ (1968). The first delayed chloroform poisoning.
Anesthesia and Analgesia 47: 374-375.

Denlinger JK, Lecky JH, Nahrwold ML (1974). Hepatocellular dysfunction without jaundice after enflurane anesthesia.
Anesthesiology 41: 86-87.

Deshpande JK, Wieloch T (1986). Flunarizine, a calcium entry blocker, ameliorates ischemic brain damage in the rat.
Anesthesiology 64: 215-224.

Desmond PV, Patwardhan R, Parker R, Schenker S, Speeg KV (1980a). Effect of cimetidine and other antihistaminics on the elimination of aminopyrine, phenacetin and caffeine.
Life Sciences 26: 1261-1268.

Desmond PV, Patwardhan RV, Schenker S, Speeg KV (1980b). Cimetidine impairs elimination of chlordiazepoxide (Librium) in man. Annals of Internal Medicine 93: 266-268.

Dienstag JL (1980). Halothane hepatitis. Allergy or idiosyncrasy? New England Journal of Medicine 303: 102-104.

Drotman RB, Lawhorn GT (1978). Serum enzymes as indicators of chemically induced liver damage. Drug and Chemical Toxicology 1: 163-171.

Dundee JW (1955). Thiopentone as a factor in the production of liver dysfunction. British Journal of Anaesthesia 27: 14-23.

Dundee JW, Fee JPH, Moore J, McIlroy PDA, Wilson DB (1980). Changes in serum enzyme levels following ketamine infusions. Anaesthesia 35: 12-16.

Dykes MHM (1971). Unexplained postoperative fever. Its value as a sign of halothane sensitization. Journal of the American Medical Association 216: 641-644.

Dykes MHM (1977). Is halothane hepatitis chronic active hepatitis? There may be a need to control the challenge test. Anesthesiology 46: 233-235.

Dykes MHM (1984). Is enflurane hepatotoxic? Anesthesiology 61: 235-237.

Dykes MHM, Walzer SG, Slater EM, Gibson JM, Ellis DS (1965). Acute parenchymatous hepatic disease following general anesthesia. Clinical appraisal of hepatotoxicty following administration of halothane. Journal of the American Medical Association 193: 339-344.

Editorial (1986). Halothane-associated liver damage. Lancet 1: 1251-1252.

Eger EI, Johnson BH, Ferrell LD (1987). Comparison of the toxicity of I-653 and isoflurane in rats: A test of the effect of repeated anesthesia and use of dry soda lime. Anesthesia and Analgesia 66: 1230-1233.

Eger EI, Johnson BH, Strum DP, Ferrell LD (1987). Studies of the toxicity of I-653, halothane, and isoflurane in enzyme-induced, hypoxic rats. Anesthesia and Analgesia 66: 1227-1229.

Eger EI, Smuckler EA, Ferrell LD, Goldsmith CH, Johnson BH (1986). Is enflurane hepatotoxic? Anesthesia and Analgesia 65: 21-30.

Ellis G, Goldberg DM, Spooner RJ, Ward AM (1978). Serum enzyme tests in diseases of the liver and biliary tree. American Journal of Clinical Pathology 70: 248-258.

Endo T, Nejima J, Iida N, Kikuchi K, Hayakawa H (1985). The effectiveness of two different treatments with nicardipine, a new calcium antagonist, on infarct size following coronary artery occlusion in dogs. Journal of the American College of Cardiology 5: 490.

Epstein RM, Deutsch S, Cooperman LH, Clement AJ, Price HL (1966). Splanchnic circulation during halothane anesthesia and hypercapnia in normal man. Anesthesiology 27: 654-661.

Fan F-C, Kim S, Simchon S, Chen RYZ, Schuessler GB, Chien S (1980). Effects of sodium nitroprusside on systemic and regional hemodynamics and oxygen utilization in the dog. Anesthesiology 53: 113-120.

Farber JL (1982). Calcium and the mechanisms of liver necrosis. In: Popper H, Schaffner F, eds. Progress in liver diseases Vol 7. New York: Grune & Stratton: 347-360.

Farrell G, Prendergast D, Murray M (1985). Halothane hepatitis. Detection of a constitutional susceptibility factor. New England Journal of Medicine 313: 1310-1314.

Farrell GC, Mahoney J, Bilous M, Frost L (1988). Altered hepatic calcium homeostasis in guinea pigs with halothane-induced hepatotoxicity. Journal of Pharmacology and Experimental Therapeutics 247: 751-756.

Fassoulaki A, Eger EI (1986). Starvation increases the solubility of volatile anaesthetics in rat liver. British Journal of Anaesthesia 58: 327-329.

Fassoulaki A, Eger EI, Johnson BH, Ferrell LD, Smuckler EA, Harper MH, Eger RR, Cahalan MK (1984). Nitrous oxide, too, is hepatotoxic in rats. Anesthesia and Analgesia 63: 1076-1080.

Fee JPH, Black GW, Dundee JW, McIlroy PDA, Johnston HML, Johnston SB, Black IHC, McNeill HG, Neill DW, Doggart JR, Merrett JD, McDonald JR, Bradley DSG, Haire M, McMillan SA (1979). A prospective study of liver enzyme and other changes following repeat administration of halothane and enflurane. British Journal of Anaesthesia 51: 1133-1141.

Feely J, Wilkinson GR, McAllister CB, Wood AJJ (1982). Increased toxicity and reduced clearance of lidocaine by cimetidine. Annals of Internal Medicine 96: 592-594.

Feely J, Wilkinson GR, Wood AJJ (1981). Reduction of liver blood flow and propranolol metabolism by cimetidine. New England Journal of Medicine 304: 692-695.

Feely J, Wood AJJ (1983). Effect on apparent liver blood flow of histamine-receptor blockers and inhibition of prostaglandin synthesis. Clinical Pharmacology and Therapeutics 33: 91-94.

Fiserova-Bergerova V (1984). Inhibitory effect of isoflurane upon oxidative metabolism of halothane. Anesthesia and Analgesia 63: 399-404.

Freedman DD, Waters DD (1987). "Second generation" dihydropyridine calcium antagonists. Greater vascular selectivity and some unique applications. Drugs 34: 578-598.

Friedman LS, Dienstag JL, Watkins E, Hinkle CA, Spiers JA, Rieder SV, Huggins CE (1987). Evaluation of blood donors with elevated serum alanine aminotransferase levels. Annals of Internal Medicine 107: 137-144.

Fujita Y, Sakai T, Ohsumi A, Takaori M (1989). Effects of hypocapnia and hypercapnia on splanchnic circulation and hepatic function in the beagle. Anesthesia and Analgesia 69: 152-157.

Galambos JT, Wills CE (1978). Relationship between 505 paired liver tests and biopsies in 242 obese patients. Gastroenterology 74: 1191-1195.

Gandolfi AJ, White RD, Sipes IG, Pohl LR (1980). Bioactivation and covalent binding of halothane in vitro: Studies with [^{3}H]- and [^{14}C] halothane. Journal of Pharmacology and Experimental Therapeutics 214: 721-725.

Garay GL, Annesley P, Burnette M (1984). Prevention of experimental liver injury in rats by nicardipine (N), a calcium entry blocker. Gastroenterology 86: 1319.

Garg DC, Weidler DJ, Jallad NS, Eshelman FN (1982). The effects of ranitidine and cimetidine on hepatic blood flow.
Clinical Pharmacology and Therapeutics 31: 228-229.

Geiger K, Georgieff M, Lutz H (1986). Side effects of positive pressure ventilation on hepatic function and splanchnic circulation.
International Journal of Clinical Monitoring and Computing 3: 103-106.

Gelman SI (1976). Disturbances in hepatic blood flow during anesthesia and surgery.
Archives of Surgery 111: 881-883.

Gelman S (1986). Halothane hepatotoxicity - again?
Anesthesia and Analgesia 65: 831-834.

Gelman S (1987). General anesthesia and hepatic circulation.
Canadian Journal of Physiology and Pharmacology 65: 1762-1779.

Gelman S, Dillard E, Bradley EL (1987). Hepatic circulation during surgical stress and anesthesia with halothane, isoflurane or fentanyl.
Anesthesia and Analgesia 66: 936-943.

Gelman S, Ernst EA (1978). Hepatic circulation during sodium nitroprusside infusion in the dog.
Anesthesiology 49: 182-187.

Gelman S, Fowler KC, Smith LR (1984a). Liver circulation and function during isoflurane and halothane anesthesia.
Anesthesiology 61: 726-730.

Gelman S, Fowler KC, Smith LR (1984b). Regional blood flow during isoflurane and halothane anesthesia.
Anesthesia and Analgesia 63: 557-565.

Gelman S, Rimerman V, Fowler KC, Bishop SP, Bradley EL (1984). The effect of halothane, isoflurane, and blood loss on hepatotoxicity and hepatic oxygen availability in phenobarbital-pretreated hypoxic rats.
Anesthesia and Analgesia 63: 965-972.

Gelman S, Van Dyke R (1988). Mechanism of halothane-induced hepatotoxicity: another step on a long path.
Anesthesiology 68: 479-482.

Gitnick G (1981). Assessment of liver function.
Surgical Clinics of North America 61: 197-207.

Goldberg DM (1980). Structural, functional and clinical aspects of gamma-glutamyltransferase.
CRC Critical Reviews in Clinical Laboratory Sciences 12: 1-58.

Goldfarb G, Rogier E, Gebauer C, Lassen C, Bernuau D, Jolis P, Feldmann G (1989). Comparative effects of halothane and isoflurane anesthesia on the ultrastructure of human hepatic cells.
Anesthesia and Analgesia 69: 491-495.

Goto T, Ohwan K, Matsumoto N, Miyazaki T, Murakami Y, Ohhata M, Hori T, Shiota K (1990). Protective effect of calcium channel blockers on the liver against halothane hepatitis in rats.
Masui 39: 204-209.

Gourlay GK, Adams JF, Cousins MJ, Hall P (1981). Genetic differences in reductive metabolism and hepatotoxicity of halothane in three rat strains.
Anesthesiology 55: 96-103.

Gourlay GK, Adams JF, Cousins MJ, Sharp JH (1980). Time-course of formation of volatile reductive metabolites of halothane in humans and an animal model.
British Journal of Anaesthesia 52: 331-336.

Graham DJM, Dow RJ, Hall DJ, Alexander OF, Mroszczak EJ, Freedman D (1985). The metabolism and pharmacokinetics of nicardipine hydrochloride in man.
British Journal of Clinical Pharmacology 20: 23s-28s.

Grégoire S, Smiley RK (1986). Acute hepatitis in a patient with mild factor IX deficiency after anesthesia with isoflurane.
Canadian Medical Association Journal 135: 645-646.

Groszmann RJ (1983). The measurement of liver blood flow using clearance techniques.
Hepatology 3: 1039-1040.

Guthenberg C, Mannervik B (1981). Glutathione S-transferase (transferase π) from human placenta is identical or closely related to glutathione S-transferase (transferase ρ) from erythrocytes.
Biochimica et Biophysica Acta 661: 255-260.

Hargreaves T (1986). The appropriate use of diagnostic services: (vi) Which biochemical liver tests should we use?
Health Bulletin 44: 65-69.

Harper MH, Collins P, Johnson BH, Eger EI, Biava CG (1982). Postanesthetic hepatic injury in rats: Influence of alterations in hepatic blood flow, surgery, and anesthesia time.
Anesthesia and Analgesia 61: 79-82.

Harrison JL (1986). Postoperative seizures after isoflurane anesthesia.
Anesthesia and Analgesia 65: 1235-1236.

Hart SM, Fitzgerald PG (1975). Unexplained jaundice following non-halothane anaesthesia.
British Journal of Anaesthesia 47: 1321-1325.

Hastings KL, Schuman S, Thomas C, Brown AP, Hubbard AK, Gandolfi AJ (1989). Laboratory methods for the diagnosis of halothane hepatitis.
Anesthesiology 71: A235.

Haxholdt OS, Loft S, Clemmensen A, Hjortsø E (1986). Increased hepatic microsomal activity after halothane anaesthesia in children.
Anaesthesia 41: 579-581.

Hayes JD (1984). Purification and characterization of glutathione S-transferases P, S and N. Isolation from rat liver of Yb_1Yn protein, the existence of which was predicted by subunit hybridization in vitro.
Biochemical Journal 224: 839-852.

Hayes JD (1986). Purification and physical characterization of glutathione S-transferase K. Differential use of S-hexylglutathione and glutathione affinity matrices to isolate a novel glutathione S-transferase from rat liver.
Biochemical Journal 233: 789-798.

Hayes JD, Gilligan D, Chapman BJ, Beckett GJ (1983). Purification of human hepatic glutathione S-transferases and the development of a radioimmunoassay for their measurement in plasma.
Clinica Chimica Acta 134: 107-121.

Hayes JD, Mantle TJ (1986a). Use of immuno-blot techniques to discriminate between the glutathione S-transferase Yf, Yk, Ya, Yn/Yb and Yc subunits and to study their distribution in extrahepatic tissues. Evidence for three immunochemically distinct groups of transferase in the rat.
Biochemical Journal 233: 779-788.

Hayes JD, Mantle TJ (1986b). Anomalous electrophoretic behaviour of the glutathione S-transferase Ya and Yk subunits isolated from man and rodents. A potential pitfall for nomenclature.
Biochemical Journal 237: 731-740.

Heagerty AM, Donovan MA, Castleden CM, Pohl JF, Patel L, Hedges A (1981). Influence of cimetidine on pharmacokinetics of propranolol.
British Medical Journal 282: 1917-1919.

Heijke S, Smith G (1990). Quest for the ideal inhalation anaesthetic agent.
British Journal of Anaesthesia 64: 3-6.

Henderson JC, Gordon RA (1964). The incidence of postoperative jaundice with special reference to halothane.
Canadian Anaesthetists Society Journal 11: 453-459.

Henderson JM, Ibrahim SZ, Millikan WJ, Santi M, Warren WD (1983). Cimetidine does not reduce liver blood flow in cirrhosis.
Hepatology 3: 919-922.

Henry PD, Shuchleib R, Borda LJ, Roberts R, Williamson JR, Sobel BE (1978). Effects of nifedipine on myocardial perfusion and ischemic injury in dogs.
Circulation Research 43: 372-380.

Hetzel DJ, Bochner F, Hallpike JF, Shearman DJC, Hann CS (1981). Cimetidine interaction with phenytoin.
British Medical Journal 282: 1512.

Hobbhahn J, Conzen P, Goetz A, Habazettl H, Brendel W, Peter K (1986). Leberperfusion und -oxygenation unter isoflurane.
Anasthesie, Intensivtherapie, Notfallmedizin 21: 85-89.

Hoft RH, Bunker JP, Goodman HI, Gregory PB (1981). Halothane hepatitis in three pairs of closely related women.
New England Journal of Medicine 304: 1023-1024.

Holaday DA, Fiserova-Bergerova V, Latto IP, Zumbiel MA (1975). Resistance of isoflurane to biotransformation in man.
Anesthesiology 43: 325-332.

Holaday DA, Smith FR (1981). Clinical characteristics and biotransformation of sevoflurane in healthy human volunteers.
Anesthesiology 54: 100-106.

Holmes MA, Weiskopf RB, Eger EI, Johnson BH, Rampil IJ (1990). Hepatocellular integrity in swine after prolonged desflurane (I-653) and isoflurane anesthesia: Evaluation of plasma alanine aminotransferase activity
Anesthesia and Analgesia 71: 249-253.

Hubbard AK, Gandolfi AJ, Brown BR (1988a).
Immunological basis of anesthetic-induced hepatotoxicity.
Anesthesiology 69: 814-817.

Hubbard AK, Roth TP, Gandolfi AJ, Brown BR, Webster NR, Nunn JF (1988b). Halothane hepatitis patients generate an antibody response toward a covalently bound metabolite of halothane.
Anesthesiology 68: 791-796.

Hughes M, Powell LW (1970). Recurrent hepatitis in patients receiving multiple halothane anesthetics for radium treatment of carcinoma of the cervix uteri.
Gastroenterology 58: 790-797.

Hughes RL, Campbell D, Fitch W (1980). Effects of enflurane and halothane on liver blood flow and oxygen consumption in the greyhound.
British Journal of Anaesthesia 52: 1079-1086.

Hughes RL, Mathie RT, Campbell D, Fitch W (1979).
Effect of hypercarbia on hepatic blood flow and oxygen consumption in the greyhound.
British Journal of Anaesthesia 51: 289-296.

Hursh D, Gelman S, Bradley EL (1987). Hepatic oxygen supply during halothane or isoflurane anesthesia in guinea pigs.
Anesthesiology 67: 701-706.

Hussey AJ, Howie J, Allan LG, Drummond G, Hayes JD, Beckett GJ (1986a). Impaired hepatocellular integrity during general anaesthesia, as assessed by measurement of plasma glutathione S-transferase.
Clinica Chimica Acta 161: 19-28.

Hussey AJ, Stockman PK, Beckett GJ, Hayes JD (1986b).
Variations in the glutathione S-transferase subunits expressed in human livers.
Biochimica et Biophysica Acta 874: 1-12.

Hymes JA (1985). Seizure activity during isoflurane anesthesia.
Anesthesia and Analgesia 64: 367-368.

Iaizzo PA, Seewald MJ, Powis G, Van Dyke RA (1990). The effects of volatile anesthetics on Ca^{++} mobilization in rat hepatocytes.
Anesthesiology 72: 504-509.

Inman WHW, Mushin WW (1974). Jaundice after repeated exposure to halothane: An analysis of reports to the Committee on Safety of Medicines.
British Medical Journal 1: 5-10.

Inman WHW, Mushin WW (1978). Jaundice after repeated exposure to halothane: a further analysis of reports to the Committee on Safety of Medicines. British Medical Journal 2: 1455-1456.

Ivanetich KM, Thumser AEA, Harrison GG (1988). Halothane: inhibition and activation of rat hepatic glutathione S-transferases. Biochemical Pharmacology 37: 1903-1908.

Jackson JE, Powell JR, Wandell M, Bentley J, Dorr R (1981). Cimetidine decreases theophylline clearance. American Review of Respiratory Disease 123: 615-617.

Jakoby WB (1978). The glutathione S-transferases: A group of multifunctional detoxification proteins. Advances in Enzymology 46: 383-414.

Jakoby WB (1985). Glutathione transferases: An overview. Methods in Enzymology 113: 495-499.

Jakoby WB, Ketterer B, Mannervik B (1984). Glutathione transferases: Nomenclature. Biochemical Pharmacology 33: 2539-2540.

James O, Lesna M, Roberts SH, Pulman L, Douglas AP, Smith PA, Watson AJ (1975). Liver damage after paracetamol overdose. Comparison of liver-function tests, fasting serum bile acids, and liver histology. Lancet 2: 579-581.

Jee RC, Sipes IG, Gandolfi AJ, Brown BR (1980). Factors influencing halothane hepatotoxicity in the rat hypoxic model. Toxicology and Applied Pharmacology 52: 267-277.

Jenkins J, Milne AC (1984). Convulsive reaction following enflurane anaesthesia. Anaesthesia 39: 44-45.

Jenkins LC, Scoates PJ (1985). Anaesthetic implications of calcium channel blockers. Canadian Anaesthetists Society Journal 32: 436-447.

Jenner MA, Plummer JL, Cousins MJ (1990). Halothane reductive metabolism in an adult surgical population. Anaesthesia and Intensive Care 18: 395-399.

Joas TA, Stevens WC, Eger EI (1971). Electroencephalographic seizure activity in dogs during anaesthesia. British Journal of Anaesthesia 43: 739-745.

Johnston CI, Mendelsohn F (1971). Halothane hepatitis in a laboratory technician.
Australian and New Zealand Journal of Medicine 1: 171-173.

Johnstone M (1956). The human cardiovascular response to Fluothane anaesthesia.
British Journal of Anaesthesia 28: 392-410.

Jones RM (1984). Calcium antagonists.
Anaesthesia 39: 747-749.

Jones RM, Cashman JN, Eger EI, Damask MC, Johnson BH (1990a). Kinetics and potency of desflurane (I-653) in volunteers.
Anesthesia and Analgesia 70: 3-7.

Jones RM, Cashman JN, Mant TGK (1990). Clinical impressions and cardiorespiratory effects of a new fluorinated inhalation anaesthetic, desflurane (I-653), in volunteers.
British Journal of Anaesthesia 64: 11-15.

Jones RM, Koblin DD, Cashman JN, Eger EI, Johnson BH, Damask MC (1990b). Biotransformation and hepato-renal function in volunteers after exposure to desflurane (I-653).
British Journal of Anaesthesia 64: 482-487.

Jones WM, Margolis G, Stephen CR (1958). Hepatotoxicity of inhalation anesthetic drugs.
Anesthesiology 19: 715-723.

Kahn RA, Johnson G, Aach RD, Hines A, Ellis FR, Miller WV (1982). The distribution of serum alanine aminotransferase levels in a blood donor population.
American Journal of Epidemiology 115: 929-940.

Kalow B, Rogoman E, Sims FH (1976). A comparison of the effects of halothane and other anaesthetic agents on hepato-cellular function in patients submitted to elective operations.
Canadian Anaesthetists Society Journal 23: 71-79.

Kamisaka K, Habig WH, Ketley JN, Arias IM, Jakoby WB (1975). Multiple forms of human glutathione S-transferase and their affinity for bilirubin.
European Journal of Biochemistry 60: 153-161.

Kaplan MM (1987). Laboratory tests. In: Schiff L, Schiff ER, eds. Diseases of the liver.
Philadelphia: JB Lippincott Co.: 234-246.

Karashima D, Hirokata Y, Shigematsu A, Furukawa T (1977). The in vitro metabolism of halothane (2-bromo-2-chloro-1,1,1-trifluoethane) by hepatic microsomal cytochrome P-450.
Journal of Pharmacology and Experimental Therapeutics 203: 409-416.

Kenna JG, Neuberger J, Mieli-Vergani G, Mowat AP, Williams R (1987). Halothane hepatitis in children.
British Medical Journal 294: 1209-1211.

Kenna JG, Neuberger J, Williams R (1984). An enzyme-linked immunosorbent assay for detection of antibodies against halothane-altered hepatocyte antigens.
Journal of Immunological Methods 75: 3-14.

Kenna JG, Neuberger J, Williams R (1987). Specific antibodies to halothane-induced liver antigens in halothane-associated hepatitis.
British Journal of Anaesthesia 59: 1286-1290.

Kenna JG, Neuberger J, Williams R (1988a). Evidence for expression in human liver of halothane-induced neoantigens recognized by antibodies in sera from patients with halothane hepatitis.
Hepatology 8: 1635-1641.

Kenna JG, Satoh H, Christ DD, Pohl LR (1988b). Metabolic basis for a drug hypersensitivity: Antibodies in sera from patients with halothane hepatitis recognize liver neoantigens that contain the trifluoroacetyl group derived from halothane.
Journal of Pharmacology and Experimental Therapeutics 245: 1103-1109.

Klatskin G, Kimberg DV (1969). Recurrent hepatitis attributable to halothane sensitization in an anesthetist.
New England Journal of Medicine 280: 515-522.

Kline MM (1980). Enflurane-associated hepatitis.
Gastroenterology 79: 126-127.

Klion FM, Schaffner F, Popper H (1969). Hepatitis after exposure to halothane.
Annals of Internal Medicine 71: 467-477.

Klotz U, Arvela P, Rosenkranz B (1985). Effect of single doses of cimetidine and ranitidine on the steady-state plasma levels of midazolam.
Clinical Pharmacology and Therapeutics 38: 652-655.

Klotz U, Reimann I (1980). Delayed clearance of diazepam due to cimetidine.
New England Journal of Medicine 302: 1012-1014.

Koblin DD, Eger EI, Johnson BH, Konopka K, Waskell L (1988). I-653 resists degradation in rats. Anesthesia and Analgesia 67: 534-538.

Koblin DD, Weiskopf RB, Holmes MA, Konopka K, Rampil IJ, Eger EI, Waskell L (1989). Metabolism of I-653 and isoflurane in swine. Anesthesia and Analgesia 68: 147-149.

Kools AM, Bloomer JR (1987). Abnormal liver function tests. How to assess their importance in asymptomatic patients. Postgraduate Medicine 81: 45-51.

Krantz JC, Park CS, Truitt EB, Ling ASC (1958). Anesthesia LVII: A further study of the anesthetic properties of 1,1,1,trifluoro-2,2-bromochlorethane (fluothane). Anesthesiology 19: 38-44.

Landers DF, Becker GL, Wong KC (1989). Calcium, calmodulin, and anesthesiology. Anesthesia and Analgesia 69: 100-112.

Landon EJ, Naukam RJ, Sastry BVR (1986). Effects of calcium channel blocking agents on calcium and centrilobular necrosis in the liver of rats treated with hepatotoxic agents. Biochemical Pharmacology 35: 697-705.

Lewis JH, Zimmerman HJ, Ishak KG, Mullick FG (1983). Enflurane hepatotoxicity. A clinicopathologic study of 24 cases. Annals of Internal Medicine 98: 984-992.

Lind RC, Gandolfi AJ, Brown BR, Hall P de la M (1987). Halothane hepatotoxicity in guinea pigs. Anesthesia and Analgesia 66: 222-228.

Lind RC, Gandolfi AJ, Hall P de la M (1989). The role of oxidative biotransformation of halothane in the guinea pig model of halothane-associated hepatotoxicity. Anesthesiology 70: 649-653.

Lind RC, Gandolfi AJ, Sipes IG, Brown BR (1984). The involvement of endotoxin in halothane-associated liver injury. Anesthesiology 61: 544-550.

Lind RC, Gandolfi AJ, Sipes IG, Brown BR (1985). Comparison of the requirements for hepatic injury with halothane and enflurane in rats. Anesthesia and Analgesia 64: 955-963.

Lind RC, Ghantous HN, Gandolfi AJ, Brown BR (1989). Sevoflurane biotransformation and hepatotoxicity in the guinea pig. Anesthesiology 71: A310.

Lindenbaum J, Leifer E (1963). Hepatic necrosis associated with halothane anesthesia. New England Journal of Medicine 268: 525-530.

Lings S (1988). Halothane related liver affection in an anaesthetist. British Journal of Industrial Medicine 45: 716-717.

Little DM, Barbour CM, Given JB (1958). The effects of fluothane, cyclopropane, and ether anesthesias on liver function. Surgery, Gynecology & Obstetrics 107: 712-718.

Loesch J, Siegers C-P, Younes M (1987). Influence of cimetidine and diethyldithiocarbamate on the metabolism of halothane and methoxyflurane in vitro. Pharmacological Research Communications 19: 395-403.

Loi C-M, Rollins DE, Dukes GE, Peat MA (1985). Effect of cimetidine on verapamil disposition. Clinical Pharmacology and Therapeutics 37: 654-657.

Long RM, Moore L (1986). Inhibition of liver endoplasmic reticulum calcium pump by CCl_4 and release of a sequestered calcium pool. Biochemical Pharmacology 35: 4131-4137.

Lunam CA, Cousins MJ, Hall P de la M (1985). Guinea-pig model of halothane-associated hepatotoxicity in the absence of enzyme induction and hypoxia. Journal of Pharmacology and Experimental Therapeutics 232: 802-809.

Lunam CA, Cousins MJ, Hall P de la M (1986). Genetic predisposition to liver damage after halothane anesthesia in guinea pigs. Anesthesia and Analgesia 65: 1143-1148.

Lund I, Skulberg A, Helle I (1974). Occupational hazard of halothane. Lancet 2: 528.

Lunn JN, Mushin WW (1982). Mortality associated with anaesthesia. London: The Nuffield Provincial Hospitals Trust.

Lynch C (1984). Are volatile anesthetics really calcium entry blockers? Anesthesiology 61: 644-646.

Lynch C (1986). Differential depression of myocardial contractility by halothane and isoflurane in vitro. Anesthesiology 64: 620-631.

Lynch C, Vogel S, Pratila MG, Sperelakis N (1982). Enflurane depression of myocardial slow action potentials. Journal of Pharmacology and Experimental Therapeutics 222: 405-409.

Lynch C, Vogel S, Sperelakis N (1981). Halothane depression of myocardial slow action potentials. Anesthesiology 55: 360-368.

McEwan J (1976). Liver function tests following anaesthesia. British Journal of Anaesthesia 48: 1065-1070.

McIntyre N (1983). The limitations of conventional liver function tests. Seminars in Liver Disease 3: 265-274.

McLain GE, Sipes IG, Brown BR (1979). An animal model of halothane hepatotoxicity: Roles of enzyme induction and hypoxia. Anesthesiology 51: 321-326.

McLaughlin DF, Eger EI (1984). Repeated isoflurane anesthesia in a patient with hepatic dysfunction. Anesthesia and Analgesia 63: 775-778.

Mannervik B (1985). The isoenzymes of glutathione transferase. Advances in Enzymology 57: 357-417.

Mannervik B, Ålin P, Guthenberg C, Jensson H, Tahir MK, Warholm M, Jörnvall H (1985). Identification of three classes of cytosolic glutathione transferase common to several mammalian species: Correlation between structural data and enzymatic properties. Proceedings of the National Academy of Sciences of the United States of America 82: 7202-7206.

Mannervik B, Danielson UH (1988). Glutathione transferases - structure and catalytic activity. CRC Critical Reviews in Biochemistry 23: 283-337.

Mannervik B, Guthenberg C (1981). Glutathione transferase (human placenta). Methods in Enzymology 77: 231-235.

Manohar M, Parks CM (1984). Porcine systemic and regional organ blood flow during 1.0 and 1.5 minimum alveolar concentrations of sevoflurane anesthesia without and with 50% nitrous oxide. Journal of Pharmacology and Experimental Therapeutics 231: 640-648.

Marcus CJ, Habig WH, Jakoby WB (1978). Glutathione transferase from human erythrocytes. Nonidentity with the enzymes from liver.
Archives of Biochemistry and Biophysics 188: 287-293.

Martin JL, Kenna JG, Pohl LR (1990). Antibody assays for the detection of patients sensitized to halothane.
Anesthesia and Analgesia 70: 154-159.

Mazze RI, Calverley RK, Smith NT (1977). Inorganic fluoride nephrotoxicity: Prolonged enflurane and halothane anesthesia in volunteers.
Anesthesiology 46: 265-271.

Mazze RI, Woodruff RE, Heerdt ME (1982). Isoniazid-induced enflurane defluorination in humans.
Anesthesiology 57: 5-8.

Merin RG (1987). Calcium channel blocking drugs and anesthetics: Is the drug interaction beneficial or detrimental?
Anesthesiology 66: 111-113.

Miller ED, Delaney TJ (1981). Blood flow alteration induced by saralasin or sodium nitroprusside in rats.
Anesthesiology 54: 199-203.

Miller ED, Greene NM (1990). Waking up to desflurane: The anesthetic for the '90s?
Anesthesia and Analgesia 70: 1-2.

Moore L (1980). Inhibition of liver-microsome calcium pump by in vivo administration of CCl_4, $CHCl_3$ and 1,1-dichloroethylene (vinylidene chloride).
Biochemical Pharmacology 29: 2505-2511.

Moore M, Thor H, Moore G, Nelson S, Moldéus P, Orrenius S (1985). The toxicity of acetaminophen and N-acetyl-p-benzoquinone imine in isolated hepatocytes is associated with thiol depletion and increased cytosolic Ca^{2+}.
Journal of Biological Chemistry 260: 13035-13040.

Morgenstern R, DePierre JW, Jörnvall H (1985). Microsomal glutathione transferase. Primary structure.
Journal of Biological Chemistry 260: 13976-13983.

Moult PJA, Adjukiewicz AB, Gaylarde PM, Sarkany I, Sherlock S (1975). Lymphocyte transformation in halothane-related hepatitis.
British Journal of Medicine 2: 69-70.

Moult PJA, Sherlock S (1975). Halothane related hepatitis.
Quarterly Journal of Medicine 44: 99-114.

Mukai S, Morio M, Fujii K, Hanaki C (1977). Volatile metabolites of halothane in the rabbit.
Anesthesiology 47: 248-251.

Mushin WW, Rosen M, Bowen DJ, Campbell H (1964). Halothane and liver dysfunction: a retrospective study.
British Medical Journal 2: 329-341.

National Halothane Study (1969). Bunker JP, Forrest WH, Mosteller F, Vandam LD, eds. A study of the possible association between halothane anaesthesia and postoperative hepatic necrosis. Washington DC: US Government Printing Office.

Neuberger J, Gimson AES, Davis M, Williams R (1983). Specific serological markers in the diagnosis of fulminant hepatic failure associated with halothane anaesthesia.
British Journal of Anaesthesia 55: 15-19.

Neuberger JM, Kenna JG, Williams R (1987). Halothane hepatitis: Attempt to develop an animal model.
International Journal of Immunopharmacology 9: 123-131.

Neuberger J, Mieli-Vergani G, Tredger JM, Davis M, Williams R (1981a). Oxidative metabolism of halothane in the production of altered hepatocyte membrane antigens in acute halothane-induced hepatic necrosis.
Gut 22: 669-672.

Neuberger J, Vergani D, Mieli-Vergani G, Davis M, Williams R (1981b). Hepatic damage after exposure to halothane in medical personnel.
British Journal of Anaesthesia 53: 1173-1177.

Neuberger J, Williams R (1984). Halothane anaesthesia and liver damage.
British Medical Journal 289: 1136-1139.

Nimmo WS (1980). Drug reactions and the liver.
British Journal of Anaesthesia 52: 1059-1060.

Nimmo WS, Thompson PG, Prescott LF (1981). Microsomal enzyme induction after halothane anaesthesia.
British Journal of Clinical Pharmacology 12: 433-434.

Noble DW, Smith KJ, Dundas CR (1987). Effects of H-2 antagonists on the elimination of bupivacaine.
British Journal of Anaesthesia 59: 735-737.

Noble DW, Yap PL (1989). Screening for antibodies to anaesthetics.
British Medical Journal 299: 2.

Nomura F, Hatano H, Ohnishi K, Akikusa B, Okuda K (1986). Effects of anticonvulsant agents on halothane-induced liver injury in human subjects and experimental animals.
Hepatology 6: 952-956.

Ohmi N, Arias IM (1981). Ligandinemia in primary liver cell cancer in rat and man.
Hepatology 1: 316-318.

Ohnishi K, Nakayama T, Saito M, Hatano H, Nomura F, Iida S, Okuda K (1985). Effects of cimetidine and ranitidine on splanchnic hemodynamics in patients with chronic liver disease.
American Journal of Gastroenterology 80: 290-293.

Oikkonen M, Rosenberg PH, Saarnivaara L (1989). Cimetidine and ranitidine do not affect enflurane metabolism in surgical patients.
Acta Anaesthesiologica Scandinavica 33: 129-131.

Ona FV, Patanella H, Ayub A (1980). Hepatitis associated with enflurane anesthesia.
Anesthesia and Analgesia 59: 146-149.

Otsuka S, Yamamoto M, Kasuya S, Ohtomo H, Yamamoto Y, Yoshida TO, Akaza T (1985). HLA antigens in patients with unexplained hepatitis following halothane anesthesia.
Acta Anaesthesiologica Scandinavica 29: 497-501.

Paronetto F, Popper H (1970). Lymphocyte stimulation induced by halothane in patients with hepatitis following exposure to halothane.
New England Journal of Medicine 283: 277-280.

Paull JD, Fortune DW (1987). Hepatotoxicity and death following two enflurane anaesthetics.
Anaesthesia 42: 1191-1196.

Peters RL, Edmondson HA, Reynolds TB, Meister JC, Curphey TJ (1969). Hepatic necrosis associated with halothane anesthesia.
American Journal of Medicine 47: 748-764.

Phillips AJ, Brimacombe JR, Simpson DL (1988). Anaesthetic induction with isoflurane or halothane.
Anaesthesia 43: 927-929.

Pilato MA, Salidivia V, Roldan L, Orrego H, Carmichael FJ, Lerman J (1989). Effects of sevoflurane and halothane on hepatic blood flow in adult rats.
Anesthesiology 71: A233.

Plewes JL, Farhi LE (1985). Cardiovascular responses to hemodilution and controlled hypotension in the dog. Anesthesiology 62: 149-154.

Plummer JL, Beckwith ALJ, Bastin FN, Adam JF, Cousins MJ, Hall P (1982). Free radical formation in vivo and hepatotoxicity due to anesthesia with halothane. Anesthesiology 57: 160-166.

Plummer JL, Hall P de la M, Jenner MA, Cousins MJ (1985). Sex differences in halothane metabolism and hepatotoxicity in a rat model. Anesthesia and Analgesia 64: 563-569.

Plummer JL, Wanwimolruk S, Jenner MA, Hall P de la M, Cousins MJ (1984). Effects of cimetidine and ranitidine on halothane metabolism and hepatotoxicity in an animal model. Drug Metabolism and Disposition 12: 106-110.

Pohl LR, Gillette JR (1982). A perspective on halothane-induced hepatotoxicity. Anesthesia and Analgesia 61: 809-811.

Poulton TJ, Ellingson RJ (1984). Seizure associated with induction of anesthesia with isoflurane. Anesthesiology 61: 471-476.

Rasmussen H (1986). The calcium messenger system (first of two parts). New England Journal of Medicine 314: 1094-1101.

Raventós J (1956). The action of fluothane - a new volatile anaesthetic. British Journal of Pharmacology 11: 394-410.

Redick JA, Jakoby WB, Baron J (1982). Immunohistochemical localization of glutathione S-transferases in livers of untreated rats. Journal of Biological Chemistry 257: 15200-15203.

Rehder K, Forbes J, Alter H, Hessler O, Stier A (1967). Halothane biotransformation in man: A quantitative study. Anesthesiology 28: 711-715.

Reichard H (1961). Ornithine carbamyl transferase activity in human serum in diseases of the liver and the biliary system. Journal of Laboratory and Clinical Medicine 57: 78-87.

Reimann IW, Klotz U, Frölich JC (1982). Effects of cimetidine and ranitidine on steady-state propranolol kinetics and dynamics. Clinical Pharmacology and Therapeutics 32: 749-757.

Reitberg DP, Bernhard H, Schentag JJ (1981). Alteration of theophylline clearance and half-life by cimetidine in normal volunteers.
Annals of Internal Medicine 95: 582-585.

Reiz S, Bålfors E, Sørensen MB, Ariola S, Friedman A, Truedsson H (1983). Isoflurane - a powerful coronary vasodilator in patients with coronary artery disease.
Anesthesiology 59: 91-97.

Reves JG, Kissin I, Lell WA, Tosone S (1982). Calcium entry blockers: Uses and implications for anesthesiologists.
Anesthesiology 57: 504-518.

Reves JG, McCracken LE (1976). Failure to induce hepatic pathology in animals sensitized to a halothane metabolite and subsequently challenged with halothane.
Anesthesia and Analgesia 55: 235-242.

Reynolds ES, Moslen MT (1974). Liver injury following halothane anesthesia in phenobarbital-pretreated rats.
Biochemical Pharmacology 23: 189-195.

Reynolds ES, Moslen MT (1977). Halothane hepatotoxicity: Enhancement by polychlorinated biphenyl pretreatment.
Anesthesiology 47: 19-27.

Rice SA, Maze M, Smith CM, Kosek JC, Mazze RI (1987). Halothane hepatotoxicity in Fischer 344 rats pretreated with isoniazid.
Toxicology and Applied Pharmacology 87: 411-419.

Richardson PDI, Withrington PG (1981). Liver blood flow. 1. Intrinsic and nervous control of liver blood flow.
Gastroenterology 81: 159-173.

Riddell JG, McAllister CB, Wilkinson GR, Wood AJJ, Roden DM (1984). A new method for constant plasma drug concentrations: Application to lidocaine.
Annals of Internal Medicine 100: 25-28.

Robson JG, Sheridan CA (1957). Preliminary investigation with fluothane.
Anesthesia and Analgesia 36: 62-74.

Rodriguez M, Paronetto F, Schaffner F, Popper H (1969). Antimitochondrial antibodies in jaundice following drug administration.
Journal of the American Medical Association 208: 148-150.

Rosalki SB (1976). Enzyme tests in diseases of the liver and hepatobiliary tract. In: Wilkinson JH ed. The principles and practice of diagnostic enzymology. London: Edward Arnold (Publishers) Ltd: 303-360.

Rosenberg PH, Wahlström T (1971). Hepatotoxicity of halothane metabolites in vivo and inhibition of fibroblast growth in vitro.
Acta Pharmacologica et Toxicologica 29: 9-19.

Ross G, Cole PV (1973). Cardiovascular actions of sodium nitroprusside in dogs.
Anaesthesia 28: 400-406.

Ross JAS, Monk SJ, Duffy SW (1984). Effect of nitrous oxide on halothane-induced hepatotoxicity in hypoxic, enzyme-induced rats.
British Journal of Anaesthesia 56: 527-533.

Ross WT, Daggy BP, Cardell RR (1979). Hepatic necrosis caused by halothane and hypoxia in phenobarbital-treated rats.
Anesthesiology 51: 327-333.

Satoh H, Fukuda Y, Anderson DK, Ferrans VJ, Gillette JR, Pohl LR (1985a). Immunological studies on the mechanism of halothane-induced hepatotoxicity: Immunohistochemical evidence of trifluoroacetylated hepatocytes.
Journal of Pharmacology and Experimental Therapeutics 233: 857-862.

Satoh H, Gillette JR, Davies HW, Schulick RD, Pohl LR (1985b). Immunochemical evidence of trifluoroacetylated cytochrome P-450 in the liver of halothane-treated rats.
Molecular Pharmacology 28: 468-474.

Satoh K, Kitahara A, Soma Y, Inaba Y, Hatayama I, Sato K (1985c). Purification, induction, and distribution of placental glutathione transferase: A new marker enzyme for preneoplastic cells in the rat chemical hepatocarcinogenesis.
Proceedings of the National Academy of Sciences of the United States of America 82: 3964-3968.

Schatzki PF, Kay S, McGavic JD (1973). Ultrastructural pathology of a case of halothane hepatitis.
American Journal of Digestive Diseases 18: 905-911.

Schemel WH (1976). Unexpected hepatic dysfunction found by multiple laboratory screening.
Anesthesia and Analgesia 55: 810-812.

Schlieble TM, Costa AK, Heffel DF, Trudell JR (1988). Comparative toxicity of halothane, isoflurane, hypoxia, and phenobarbital induction in monolayer cultures of rat hepatocytes. Anesthesiology 68: 485-494.

Schramm M, Towart R (1985). Modulation of calcium channel function by drugs. Life Sciences 37: 1843-1860.

Scott DB, Hibbard BM (1990). Serious non-fatal complications associated with extradural block in obstetric practice. British Journal of Anaesthesia 64: 537-541.

Sear JW, Prys-Roberts C, Dye A (1983). Hepatic function after anaesthesia for major vascular reconstructive surgery. A comparison of four anaesthetic techniques. British Journal of Anaesthesia 55: 603-609.

Serlin MJ, Sibeon RG, Mossman S, Breckenridge AM, Williams JR, Atwood JL, Willoughby JM (1979). Cimetidine: Interaction with oral anticoagulants in man. Lancet 2: 317-319.

Sharp JH, Trudell JR, Cohen EN (1979). Volatile metabolites and decomposition products of halothane in man. Anesthesiology 50: 2-8.

Sharpstone P, Medley DRK, Williams R (1971). Halothane hepatitis - a preventable disease? British Medical Journal 1: 448-450.

Sherlock S (1986). The spectrum of hepatotoxicity due to drugs. Lancet 2: 440-444.

Sherman M, Bass NM, Campbell JAH, Kirsch RE (1983a). Radioimmunoassay of human ligandin. Hepatology 3: 162-169.

Sherman M, Titmuss S, Kirsch RE (1983b). Glutathione S-transferase in human organs. Biochemistry International 6: 109-118.

Shingu K, Eger EI, Johnson BH (1982a). Hypoxia per se can produce hepatic damage without death in rats. Anesthesia and Analgesia 61: 820-823.

Shingu K, Eger EI, Johnson BH (1982b). Hypoxia may be more important than reductive metabolism in halothane-induced hepatic injury. Anesthesia and Analgesia 61: 824-827.

Shingu K, Eger EI, Johnson BH, Van Dyke RA, Lurz FW, Harper MH, Cheng A (1983). Hepatic injury induced by anesthetic agents in rats. Anesthesia and Analgesia 62: 140-145.

Siadat-Pajouh M, Hubbard AK, Roth TP, Gandolfi AJ (1987). Generation of halothane-induced immune response in a guinea pig model of halothane hepatitis. Anesthesia and Analgesia 66: 1209-1214.

Simpson DL, Macrae WR, Wildsmith JAW, Dale BAB (1987). Acute beta-adrenoceptor blockade and induced hypotension. Anaesthesia 42: 243-248.

Sims FH, Rautanen P (1975). Serum arginino-succinate lyase: Observations on the sensitivity and specificity of this test in the detection of minimal hepatocellular damage. Clinical Biochemistry 8: 213-221.

Singh SV, Dao DD, Partridge CA, Theodore C, Srivastava SK, Awasthi YC (1985). Different forms of human liver glutathione S-transferases arise from dimeric combinations of at least four immunologically and functionally distinct subunits. Biochemical Journal 232: 781-790.

Sipes IG, Brown BR (1976). An animal model of hepatotoxicity associated with halothane anesthesia. Anesthesiology 45: 622-628.

Sipes IG, Gandolfi AJ, Pohl LR, Krishna G, Brown BR (1980). Comparison of the biotransformation and hepatotoxicity of halothane and deuterated halothane. Journal of Pharmacology and Experimental Therapeutics 214: 716-720.

Sivarajan M (1986). Hepatic blood flow during controlled hypotension. Anesthesia and Analgesia 65: 424.

Sivarajan M, Amory DW, McKenzie SM (1985). Regional blood flows during induced hypotension produced by nitroprusside or trimethaphan in the rhesus monkey. Anesthesia and Analgesia 64: 759-766.

Skivolocki WP, Pace WG, Thomford NR (1972). Effect of Arfonad on the splanchnic and hepatic circulation. American Journal of Surgery 123: 694-697.

Slater EM, Gibson JM, Dykes MHM, Walzer SG (1964). Postoperative hepatic necrosis. Its incidence and diagnostic value in association with the administration of halothane. New England Journal of Medicine 270: 983-987.

Smith AC, Berman ML, James RC, Harbison RD (1983). Characterization of hyperthyroidism enhancement of halothane-induced hepatotoxicity. Biochemical Pharmacology 32: 3531-3539.

Smith GJ, Ohl VS, Litwack G (1977). Ligandin, the glutathione S-transferases, and chemically induced hepatocarcinogenesis: A review. Cancer Research 37: 8-14.

Snyder SH, Reynolds IJ (1985). Calcium-antagonist drugs. Receptor interactions that clarify therapeutic effects. New England Journal of Medicine 313: 995-1002.

Soma Y, Satoh K, Sato K (1986). Purification and subunit-structural and immunological characterization of five glutathione S-transferases in human liver, and the acidic form as a hepatic tumor marker. Biochimica et Biophysica Acta 869: 247-258.

Somogyi A, Gugler R (1983). Clinical pharmacokinetics of cimetidine. Clinical Pharmacokinetics 8: 463-495.

Sparnins VL, Venegas PL, Wattenberg LW (1982). Glutathione S-transferase activity: Enhancement by compounds inhibiting chemical carcinogenesis and by dietary constituents. Journal of the National Cancer Institute 68: 493-496.

Spence AA (1987a). Halothane in the Doldrums. British Journal of Anaesthesia 59: 529-530.

Spence AA (1987b). Antibody to halothane-induced liver antigen. British Journal of Anaesthesia 59: 1202-1203.

Steen PA, Michenfelder JD (1979). Neurotoxicity of anesthetics. Anesthesiology 50: 437-453.

Steen PA, Newberg LA, Milde JH, Michenfelder JD (1983). Nimodipine improves cerebral blood flow and neurologic recovery after complete cerebral ischemia in the dog. Journal of Cerebral Blood Flow and Metabolism 3: 38-43.

Stephen CR, Margolis G, Fabian LW, Bourgeois-Gavardin M (1958). Laboratory observations with Fluothane. Anesthesiology 19: 770-781.

Stier A, Alter H, Hessler O, Rehder K (1964). Urinary excretion of bromide in halothane anesthesia. Anesthesia and Analgesia 43: 723-728.

Stock JGL, Strunin L (1985). Unexplained hepatitis following halothane. Anesthesiology 63: 424-439.

Stockman PK, Beckett GJ, Hayes JD (1985). Identification of a basic hybrid glutathione S-transferase from human liver. Biochemistry Journal 227: 457-465.

Stoelting RK (1987). Isoflurane and postoperative hepatic dysfunction. Canadian Journal of Anaesthesia 34: 223-226.

Stoelting RK, Blitt CD, Cohen PJ, Merin RG (1987). Hepatic dysfunction after isoflurane anesthesia. Anesthesia and Analgesia 66: 147-153.

Stoelting RK, Dierdorf SF, McCammon RL (1988). Diseases of the liver and biliary tract. In: Anesthesia and co-existing disease. New York: Churchill Livingstone Inc.: 355-392.

Stolz A, Kaplowitz N (1990). Biochemical tests for liver disease. In: Zakim D, Boyer TD eds. Hepatology. A textbook of liver disease. Philadelphia: WB Saunders Company: 637-667.

Stone PH, Antman EM, Muller JE, Braunwald E (1980). Calcium channel blocking agents in the treatment of cardiovascular disorders. Part II: Hemodynamic effects and clinical applications. Annals of Internal Medicine 93: 886-904.

Strum DP, Eger EI, Johnson BH, Steffey EP, Ferrell LD (1987). Toxicity of sevoflurane in rats. Anesthesia and Analgesia 66: 769-773.

Strum DP, Johnson BH, Eger EI (1987). Stability of sevoflurane in soda lime. Anesthesiology 67: 779-781.

Strunin L (1977). Liver function tests. In: The liver and anaesthesia. London, Philadelphia, Toronto: WB Saunders Co Ltd: 24-34.

Strunin L, Davies JM (1983). The liver and anaesthesia. Canadian Anaesthetists Society Journal 30: 208-217.

Sutton TS, Koblin DD, Weiskopf RB, Gruenke LD, Waskell L, Eger EI (1990). Fluoride metabolites following prolonged exposure of volunteers to desflurane. Anesthesia and Analgesia 70: S398.

Suzuki T, Coggan M, Shaw DC, Board PG (1987). Electrophoretic and immunological analysis of human glutathione S-transferase isozymes. Annals of Human Genetics 51: 95-106.

Takahara K, Natelson S (1967). Argininosuccinic acid lyase in human erythrocytes and plasma in health and disease.
American Journal of Clinical Pathology 47: 693-703.

Tegeris AS, Smalley HE, Earl FL, Curtis JM (1969). Ornithine carbamyl transferase as a liver function test. Comparative studies in the dog, swine, and man.
Toxicology and Applied Pharmacology 14: 54-66.

Telerman-Toppet N, Duret ME, Coërs C (1981). Cimetidine interaction with carbamazepine.
Annals of Internal Medicine 94: 544.

Temple RL, Cote RA, Gorens SW (1962). Massive hepatic necrosis following general anesthesia.
Anesthesia and Analgesia 41: 586-592.

Thomas CE, Reed DJ (1989). Current status of calcium in hepatocellular injury.
Hepatology 10: 375-384.

Thompson DS, Friday CD (1978). Changes in liver enzyme values after halothane and enflurane for surgical anesthesia.
Southern Medical Journal 71: 779-782.

Thomson IA, Fitch W, Hughes RL, Campbell D (1983). Effect of increased concentrations of carbon dioxide during halothane anaesthesia on liver blood flow and hepatic oxygen consumption.
British Journal of Anaesthesia 55: 1231-1237.

Touloukian J, Kaplowitz N (1981). Halothane-induced hepatic disease.
Seminars in Liver Disease 1: 134-142.

Trey C, Lipworth L, Chalmers TC, Davidson CS, Gottlieb LS, Popper H, Saunders SJ (1968). Fulminant hepatic failure. Presumable contribution of halothane.
New England Journal of Medicine 279: 798-801.

Trowell J, Peto R, Smith A (1975). Controlled trial of repeated halothane anaesthetics in patients with carcinoma of the uterine cervix treated with radium.
Lancet 1: 821-824.

Tsuru M, Kamisaka K, Hirano M, Kameda H (1978). Quantification of human serum ligandin by radioimmunoassay.
Clinica Chimica Acta 84: 251-253.

Tyden G, Thulin L, Nyberg B (1983). The effect of cimetidine on liver blood flow in anesthetized man.
Acta Chirurgica Scandinavica 149: 303-305.

Uehleke H, Hellmer KH, Tabarelli-Poplawski S (1973). Metabolic activation of halothane and its covalent binding to liver endoplasmic proteins in vitro. Naunyn-Schmiedeberg's Archives of Pharmacology 279: 39-52.

Uetrecht J, Wood AJJ, Phythyon JM, Wood M (1983). Contrasting effects on halothane hepatotoxicity in the phenobarbital-hypoxia and triiodothyronine model: Mechanistic implications. Anesthesiology 59: 196-201.

Usubiaga JE (1975). Neurological complications following epidural anesthesia. International Anesthesiology Clinics 13: 1-153.

Uzunalimoglu B, Yardley JH, Boitnott JK (1970). The liver in mild halothane hepatitis. Light and electron microscopic findings with special reference to the mononuclear cell infiltrate. American Journal of Pathology 61: 457-478.

Vander Jagt DL, Hunsaker LA, Garcia KB, Royer RE (1985). Isolation and characterization of the multiple glutathione S-transferases from human liver. Evidence for unique heme-binding sites. Journal of Biological Chemistry 260: 11603-11610.

Van Dyke RA (1966). Metabolism of volatile anesthetics. III. Induction of microsomal dechlorinating and ether-cleaving enzymes. Journal of Pharmacology and Experimental Therapeutics 154: 364-369.

Van Dyke RA (1973). Biotransformation of volatile anesthetics with special emphasis on the role of metabolism in the toxicity of anaesthetics. Canadian Anaesthetists Society Journal 20: 21-33.

Van Dyke RA (1982). Hepatic centrilobular necrosis in rats after exposure to halothane, enflurane, or isoflurane. Anesthesia and Analgesia 61: 812-819.

Van Dyke RA, Chenoweth MB, Van Poznak A (1964). Metabolism of volatile anesthetics - I. Conversion in vivo of several anesthetics to $^{14}CO_2$ and chloride. Biochemical Pharmacology 13: 1239-1247.

Van Dyke RA, Chenoweth MB (1965). The metabolism of volatile anesthetics - II. In vitro metabolism of methoxyflurane and halothane in rat liver slices and cell fractions. Biochemical Pharmacology 14: 603-609.

Van Dyke RA, Gandolfi AJ (1976). Anaerobic release of fluoride from halothane. Relationship to the binding of halothane metabolites to hepatic cellular constituents.
Drug Metabolism and Disposition 4: 40-44.

Van Dyke RA, Wood CL (1973). Binding of radioactivity from ^{14}C-labeled halothane in isolated perfused rat livers.
Anesthesiology 38: 328-332.

Varma RR, Whitesell RC, Iskandarani MM (1985). Halothane hepatitis without halothane: Role of inapparent circuit contamination and its prevention.
Hepatology 5: 1159-1162.

Vergani D, Mieli-Vergani G, Alberti A, Neuberger J, Eddleston ALWF, Davis M, Williams R (1980). Antibodies to the surface of halothane-altered rabbit hepatocytes in patients with severe halothane-associated hepatitis.
New England Journal of Medicine 303: 66-71.

Vergani D, Tsantoulas D, Eddleston ALWF, Davis M, Williams R (1978). Sensitisation to halothane-altered liver components in severe hepatic necrosis after halothane anaesthesia.
Lancet 2: 801-803.

Virtue RW, Payne KW (1958). Postoperative death after Fluothane.
Anesthesiology 19: 562-563.

Virtue RW, Payne KW, Caranna LJ, Gordon GS, Rember RR (1958). Observations during experimental and clinical use of fluothane.
Anesthesiology 19: 478-487.

Wallin RF, Regan BM, Napoli MD, Stern IJ (1975). Sevoflurane: A new inhalational anesthetic agent.
Anesthesia and Analgesia 54: 758-765.

Walton B (1986). Halothane hepatitis in children.
Anaesthesia 41: 575-578.

Walton B, Dumonde DC, Williams C, Jones D, Strunin JM, Layton JM, Strunin L, Simpson R (1973). Lymphocyte transformation. Absence of increased responses in alleged halothane jaundice.
Journal of the American Medical Association 225: 494-498.

Walton B, Simpson BR, Strunin L, Doniach D, Perrin J, Appleyard AJ (1976). Unexplained hepatitis following halothane.
British Medical Journal 1: 1171-1176.

Wang HH, Liu LMP, Katz RL (1977). A comparison of the cardiovascular effects of sodium nitroprusside and trimethaphan.
Anesthesiology 46: 40-48.

Warholm M, Guthenberg C, Mannervik B (1983). Molecular and catalytic properties of glutathione transferase μ from human liver: An enzyme efficiently conjugating epoxides.
Biochemistry 22: 3610-3617.

Wark HJ (1983). Postoperative jaundice in children. The influence of halothane.
Anaesthesia 38: 237-242.

Wark HJ, Clifton B, Bookallil MJ (1979). Halothane hepatitis revisited in women undergoing treatment of carcinoma of the cervix.
British Journal of Anaesthesia 51: 763-766.

Wark H, O'Halloran M, Overton J (1986). Prospective study of liver function in children following multiple halothane anaesthetics at short intervals.
British Journal of Anaesthesia 58: 1224-1228.

Warner LO, Beach TP, Garvin JP, Warner EJ (1984). Halothane and children: The first quarter century.
Anesthesia and Analgesia 63: 838-840.

Weis K-H, Engelhardt W (1989). Is halothane obsolete? Two standards of judgement.
Anaesthesia 44: 97-100.

West M, Schwartz MA, Cohen J, Zimmerman HJ (1964). Serum enzymes in disease. XV. Glycolytic and oxidative enzymes and transaminases in patients with carcinoma of the kidney, prostate and urinary bladder.
Cancer 17: 432-437.

Whitburn RH, Sumner E (1986). Halothane hepatitis in an 11-month-old child.
Anaesthesia 41: 611-613.

White PF (1989). Clinical uses of intravenous anesthetic and analgesic infusions.
Anesthesia and Analgesia 68: 161-171.

Widger LA, Gandolfi AJ, Van Dyke RA (1976). Hypoxia and halothane metabolism in vivo: Release of inorganic fluoride and halothane metabolite binding to cellular constituents.
Anesthesiology 44: 197-201.

Wildsmith JAW, Sinclair CJ, Thorn J, MacRae WR, Fagan D, Scott DB (1983). Haemodynamic effects of induced hypotension with a nitroprusside-trimetaphan mixture.
British Journal of Anaesthesia 55: 381-389.

Wilhelm K-P, Younes M, Sellin D, Siegers C-P (1987). Halothane hepatotoxicity in glutathione depleted rats.
Journal of Applied Toxicology 7: 105-110.

Wilkinson JH (1976). Chemistry of enzymes of diagnostic interest. I. Oxido-reductases. In: The principles and practice of diagnostic enzymology. London: Edward Arnold (Publishers) Ltd: 43-79.

Wills EJ, Walton B (1978). A morphologic study of unexplained hepatitis following halothane anesthesia.
American Journal of Pathology 91: 11-32.

Wolkoff AW (1980). The glutathione S-transferases: their role in the transport of organic anions from blood to bile.
International Review of Physiology 21: 150-169.

Wood M, Berman ML, Harbison RD, Hoyle P, Phythyon JM, Wood AJJ (1980). Halothane-induced hepatic necrosis in triiodothyronine-pretreated rats.
Anesthesiology 52: 470-476.

Wood M, Uetrecht J, Phythyon JM, Shay S, Sweetman BJ, Shaheen O, Wood AJJ (1986). The effect of cimetidine on anesthetic metabolism and toxicity.
Anesthesia and Analgesia 65: 481-488.

Wouters P, Doursout M-F, Kashimoto S, Lawrence C, Merin RG, Chelly JE (1989). Sevoflurane preserved hepatic arterial blood flow in dogs.
Anesthesiology 71: A306.

Wright PJ, Dundee JW (1982). Attitudes to intravenous infusion anaesthesia.
Anaesthesia 37: 1209-1213.

Wright R, Chisholm M, Lloyd B, Edwards JC, Eade OE, Hawksley M, Moles TM, Gardner MJ (1975). Controlled prospective study of the effect on liver function of multiple exposures to halothane.
Lancet 1: 817-820.

Wróblewski F (1959). The clinical significance of transaminase activities of serum.
American Journal of Medicine 27: 911-923.

Wu AT, Massey IJ, Kushinsky S (1984). A high-performance liquid chromatographic method for the simultaneous determination of nicardipine and its pyridine metabolite II in plasma.
Journal of Pharmaceutical Sciences 73: 1444-1447.

Yeager MP, Coombs DW, Dodge CP, Maloney LL (1986). Effect of cimetidine on biotransformation of enflurane in man.
Canadian Anaesthetists Society Journal 33: 466-470.

Zaric D, Larsen SF, Jacobsen E, Olesen KH, Ranek L (1986). Halothane hepatitis in a prospective study of postoperative complications.
Acta Anaesthesiologica Scandinavica 30: 529-532.

Zimmerman HJ (1978). Experimental hepatotoxicity. In: Hepatotoxicity. New York: Appleton-Century-Crofts: 167-197.

Zimmerman HJ, Seeff LB (1970). Enzymes in hepatic disease. In: Coodley EL, ed. Diagnostic Enzymology. Philadelphia: Lea & Febiger: 1-38.

Zucker JR, Diamond EM, Berman MC (1982). Effect of halothane on calcium transport in isolated hepatic endoplasmic reticulum.
British Journal of Anaesthesia 54: 981-985.

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Several colleagues in the Departments of Anaesthetics and Clinical Chemistry, Royal Infirmary of Edinburgh (named in the papers in Appendix 3), assisted in the performance of the studies. In particular, I would like to thank Dr LM Aldridge with whom I shared the duties of principal investigator in two studies, and who agreed readily to the inclusion of those studies in this thesis. Dr GJ Beckett, who with Dr JD Hayes developed and refined the GST B_1B_1 radioimmunoassay, is also due my special thanks, as are AJ Hussey and AF Howie who performed the analyses of plasma GST concentration.

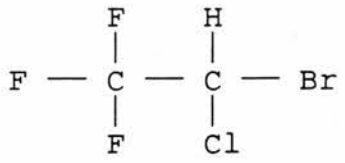
Most of the studies presented in this thesis were performed while I was in receipt of a fellowship from Syntex Pharmaceuticals Ltd, who, in addition, supplied nicardipine for infusion and performed analysis of plasma nicardipine concentrations; Smith, Kline and French Laboratories Ltd provided supplies of cimetidine and placebo tablets.

I am very grateful to the staff of the Erskine Medical Library for their help in obtaining the relevant publications, and to British Journal of Anaesthesia and joint authors for their permission to reproduce some figures and a published paper which is bound in with this thesis.

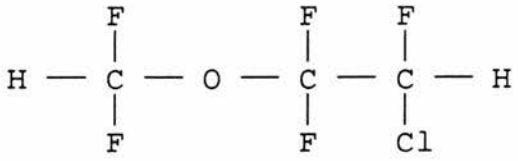
My discussions with Dr GB Drummond, who originally stimulated my interest in GST, have made the completion of this thesis much easier. My advisor, Professor AA Spence has given continued encouragement, support and advice. Finally, I would like to thank my wife for her assistance and understanding of the time I have committed to this project.

APPENDIX 1 - STRUCTURAL FORMULAE OF VOLATILE AGENTS

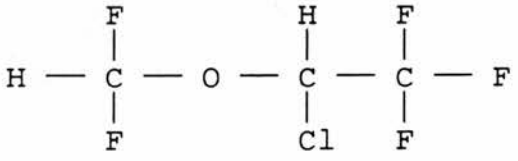
Halothane



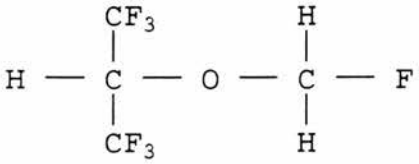
Enflurane



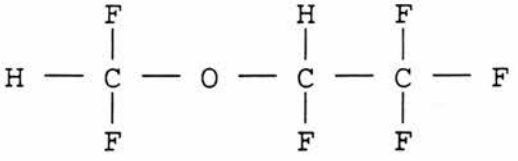
Isoflurane



Sevoflurane



Desflurane



APPENDIX 2 - ABBREVIATIONS

The scientific units, symbols and abbreviations used throughout this thesis conform to those described in:
Units, Symbols, and Abbreviations. A Guide for Biological and Medical Editors and Authors. 4th edition, Baron DN, ed. (1988). Royal Society of Medicine Services: London
Other abbreviations used are listed below:

ALP	alkaline phosphatase
ALT	alanine aminotransferase
ASA	American Society of Anesthesiologists
ASAL	argininosuccinate lyase
AST	aspartate aminotransferase
bil	bilirubin
CCl_4	carbon tetrachloride
CDF	2-chloro-1,1-difluoroethylene
CDNB	1-chloro-2,4-dinitrobenzene
$CHCl_3$	chloroform
CSM	Committee on Safety of Medicines
CTF	2-chloro-1,1,1-trifluoroethane
ENT	ear, nose and throat
F	female
F_IO_2	fractional inspired oxygen concentration
GGT	gamma-glutamyl transferase
GST	glutathione S-transferase
HLA	human lymphocyte antigen
ICDH	isocitrate dehydrogenase
ICG	indocyanine green
IgG	immunoglobulin G
IgM	immunoglobulin M

IPPV	intermittent positive pressure ventilation
LDH	lactate dehydrogenase
M	male
MAC	minimum alveolar concentration
MFOE	mixed-function oxidase enzyme
OCT	ornithine carbamyl transferase
$PaCO_2$	arterial carbon dioxide partial pressure
PaO_2	arterial oxygen partial pressure
PCB	polychlorinated biphenyl
$P_E{}'CO_2$	end-tidal carbon dioxide partial pressure
RIA	radioimmunoassay
SDH	sorbitol dehydrogenase
SGOT	serum glutamic-oxaloacetic transaminase
SGPT	serum glutamic-pyruvic transaminase
SNP	sodium nitroprusside
SV	spontaneous ventilation
T_3	triiodothyronine
TFA	trifluoroacetyl
TFAA	trifluoroacetic acid

APPENDIX 3 - PUBLISHED PAPERS

Hussey AJ, Aldridge LM, Paul D, Ray DC, Beckett GJ, Allan LG (1988). Plasma glutathione S-transferase concentration as a measure of hepatocellular integrity following a single general anaesthetic with halothane, enflurane or isoflurane. British Journal of Anaesthesia 60: 130-135.

Ray DC, Beckett GL, Hayes JD, Drummond GB (1989). Effect of nicardipine infusion on the release of glutathione S-transferase following halothane anaesthesia. British Journal of Anaesthesia 62: 553-559.

Ray DC, Howie AF, Beckett GJ, Drummond GB (1989). Preoperative cimetidine does not prevent subclinical halothane hepatotoxicity in man. British Journal of Anaesthesia 63: 531-535.

(Copies of the above are bound in overleaf)

Br. J. Anaesth. (1988), **60**, 130–135

PLASMA GLUTATHIONE S-TRANSFERASE CONCENTRATION AS A MEASURE OF HEPATOCELLULAR INTEGRITY FOLLOWING A SINGLE GENERAL ANAESTHETIC WITH HALOTHANE, ENFLURANE OR ISOFLURANE

A. J. HUSSEY, L. M. ALDRIDGE, D. PAUL, D. C. RAY, G. J. BECKETT AND L. G. ALLAN

SUMMARY

The plasma concentration of hepatic glutathione S-transferase (GST) was measured in matched groups of patients who received halothane, enflurane or isoflurane anaesthesia for elective minor surgery. The GST concentrations increased significantly at 3 h after anaesthesia in patients who received halothane or enflurane, but not in patients who were given isoflurane. A secondary increase in GST concentration, at 24 h, was seen in a small number of individuals who received halothane or enflurane. Abnormal GST concentrations were found in 50% of patients following halothane anaesthesia, 20% following enflurane and 11% after isoflurane. The small but significant increases in GST concentrations in patients receiving halothane or enflurane suggests an impairment of hepatocellular integrity following the administration of these anaesthetics. In contrast, isoflurane anaesthesia did not appear to be associated with this effect.

Hepatic injury has been associated with the use of volatile halogenated anaesthetic agents [1]. Halothane, a volatile anaesthetic agent still used widely in Great Britain, is the subject of renewed controversy, with acute fulminant hepatitis occurring rarely following its use. The incidence and underlying mechanisms leading to the development of "halothane hepatitis" still remain unknown, but biotransformation [2], a susceptibility factor [3], and hypersensitivity [4] have all been implicated.

With recent reports [5, 6] questioning the future of halothane in clinical anaesthesia, anaesthetists may turn to alternative anaesthetics such as the newer volatile agents, enflurane and isoflurane. However, there have also been reports of hepatic injury associated with enflurane anaesthesia [7, 8]. It has, therefore, been postulated that there is a syndrome of "enflurane hepatitis". Hepatic dysfunction after administration of isoflurane is unlikely [9].

The most widely used method of assessing drug-induced hepatocellular damage in man is the measurement of transaminase activity in plasma [10]. However, these measurements lack sensitivity and may have poor organ specificity. The measurement in plasma of the hepatic isoenzymes of glutathione S-transferase (GST) by radioimmunoassay is a sensitive and specific method for the detection of acute drug-induced hepatocellular damage. In chronic liver disease, the measurement of GST, unlike the aminotransferases, correlates well with liver histology (for a review see [11]). The physicochemical properties of GST offer certain theoretical advantages over measurements of aminotransferase in the investigation of chemically-induced hepatotoxicity. GST are relatively small enzymes (MW ~ 50000) and are present in high concentrations in hepatic

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cytosol. GST are readily and rapidly released in quantity into the circulation following hepatic damage; their short plasma half-life (< 90 min) allows early detection of hepatic damage and its resolution. Unlike the transaminases, which are located periportally, GST are primarily located in the centrilobular hepatocytes [12]. The measurement of plasma GST may, therefore, be of particular relevance in investigating halothane hepatitis, since this syndrome is generally associated with centrilobular necrosis.

We have shown that significant increases in plasma GST concentration occur 3 h after halothane anaesthesia, but not after isoflurane anaesthesia [13, 14]. This suggests that halothane, but not isoflurane, is associated with a mild, transient impairment of hepatocellular integrity. In the present study, we have investigated sequential changes in plasma GST concentration in three matched groups of patients allocated to receive anaesthesia with halothane, enflurane or isoflurane, for surgical procedures that were standard in type and duration.

PATIENTS, MATERIALS AND METHODS

Patients and samples

Only patients undergoing herniorraphy or varicose vein surgery in ASA groups I or II were included in the study. Ethical approval and informed consent were obtained. Patients with pre-existing clinical or biochemical evidence of liver disease or who had received a general anaesthetic in the previous 6 months were excluded. Patients taking regular medication likely to interfere with liver function were also excluded.

Subjects were allocated to three groups: patients in group A received halothane ($n = 30$), group B patients received isoflurane ($n = 30$) and patients in group C received enflurane ($n = 41$). No premedication was given. Anaesthesia was induced with thiopentone 4–6 mg kg^{-1} i.v., and maintained with the volatile anaesthetic agent vaporized in 33 % oxygen and 67 % nitrous oxide. Ventilation was spontaneous in all subjects. During maintenance of anaesthesia, the inhaled fresh gas concentration of halothane varied from 1.0 to 1.5 %, and those of isoflurane and enflurane from 1.0 to 3.0 %. Diamorphine was given i.v. in divided doses at induction and on recovery to a total dose of 0.07 mg kg^{-1}.

Blood samples were taken before the induction of anaesthesia and at 3, 6 and 24 h following the termination of anaesthesia. Plasma concentrations of albumin, alanine aminotransferase (ALT), bilirubin, γ-glutamyltransferase (GGT) and alkaline phosphatase (ALP) were measured in the samples obtained before anaesthesia and at 24 h following anaesthesia. Plasma from all samples was stored at −20 °C before measurement of GST.

Assay procedures

Concentrations of albumin, ALT, bilirubin, GGT and ALP were measured using a Sequential Multiple Analysis with Computer (SMAC) System II (Technicon Instruments Corporation, Basingstoke, U.K.). The concentration of GST B_1 in plasma was measured using a specific radioimmunoassay [15]. The reference range for GST B_1 is 0.7–4.0 μg litre^{-1} (derived from 135 blood donors). The inter-assay coefficient of variation for the GST B_1 RIA was less than 10 %, and the intra-assay coefficient of variation was less than 5 % over the range 2–40 μg litre^{-1}.

Statistical analysis

The Kruskal–Wallis test, for analysis of variance, was used to compare the changes in GST concentrations observed in the three groups, at 3 h following anaesthesia, in order to eliminate multiple testing errors. Changes in GST concentrations within each group were compared using the Friedman test. The Wilcoxon matched-pairs test was used to compare postoperative concentrations of GST and albumin with preoperative values in groups that showed significant changes with the Friedman test. Correlation analysis used the Spearman rank test. A four-fold table chi-squared test was used to compare the incidence of abnormal values in each group. The demographic data from each group were compared using the Kruskal–Wallis test.

RESULTS

Patients with preoperative plasma concentrations of GST, ALT, bilirubin, ALP or GGT that exceeded the upper limit of the reference range were excluded from the study. Statistical analysis of the demographic data for the remaining subjects in group A ($n = 22$), group, B ($n = 18$) and group C ($n = 30$) showed no significant differences (table I). No significant overall changes

in the concentrations of bilirubin, ALT, ALP or GGT occurred as a result of anaesthesia.

Significant differences in the changes in GST concentrations following anaesthesia between the three groups were observed (Kruskal–Wallis: $P < 0.05$), and significant temporal changes in GST concentrations were evident within all three groups (Friedman; group A $P < 0.05$, group B $P < 0.01$ and group C $P < 0.0001$). In groups A and C, that is those patients receiving halothane and enflurane, respectively, these changes were caused by significant increases in GST concentrations at 3 h after anaesthesia (Wilcoxon: group A $P < 0.01$ and group C $P < 0.0001$) and at 6 h following anaesthesia (group C $P < 0.05$). In the patients receiving isoflurane (group B), no significant increase in GST concentrations occurred after anaesthesia. The medians and interquartile ranges of the GST concentrations at times before and after anaesthesia in the three groups are shown in table II. There was no correlation between the changes in GST concentrations and the total dose (duration × maintenance % anaesthetic concentration) of anaesthetic (Spearman rank).

Four patients in group A and three in group C showed secondary increases in GST concentrations, which exceeded the reference range, at

TABLE I. *Demographic data for patients in group A, group B and group C who had normal plasma concentrations of GST, ALT, bilirubin, ALP and GGT before operation*

	Group A Halothane ($n = 22$)	Group B Isoflurane ($n = 18$)	Group C Enflurane ($n = 30$)
Age (yr)			
Mean	42.4	40.2	50.3
SD	17.0	15.8	12.7
Sex (M/F)	11/11	10/8	16/14
Height (m)			
Mean	1.70	1.70	1.71
SD	0.10	0.11	0.12
Weight (kg)			
Mean	70.4	68.6	71.0
SD	13.7	12.2	15.3
Cigarettes (No. day^{-1})			
Median	5	0	0
(1st–3rd quartile)	0–15	0–12	0–10
Alcohol (g week^{-1})			
Median	2.5	6.0	0.1
(1st–3rd quartile)	0.1–15.0	0.7–16.0	0.05–3.0
Duration of anaesthetic (min)			
Median	45	50	45
(1st–3rd quartile)	30–55	33–60	30–55

TABLE II. *Medians (1st–3rd quartile) of GST values (μg litre^{-1}) for groups A, B and C. Significant increases from basal values (Wilcoxon matched-pairs): *P < 0.05; **P < 0.01; ***P < 0.0001*

	Before anaesthesia	After anaesthesia		
		3 h	6 h	24 h
Group A Halothane ($n = 22$)	2.9 (2.3–3.4)	3.4** (2.6–4.1)	3.3 (2.2–4.0)	2.5 (1.8–3.5)
Group B Isoflurane ($n = 18$)	2.9 (2.0–3.3)	2.6 (2.1–3.3)	2.6 (2.1–3.1)	2.0 (1.7–2.4)
Group C Enflurane ($n = 30$)	2.4 (1.9–3.0)	3.0*** (2.3–3.4)	2.9* (2.4–3.3)	2.3 (1.4–2.7)

24 h after anaesthesia. One of the patients who had an increased plasma GST concentration at 24 h after anaesthesia with halothane also had an increased plasma ALT activity (116 u. litre^{-1}) at this time. The changes in GST from preoperative concentrations in the samples obtained after anaesthesia, for all three groups of patients, are shown in figure 1.

Many patients, particularly in groups A and C, showed concentrations of GST which exceeded the upper reference value after anaesthesia (fig. 2). The incidence of abnormal GST concentrations following anaesthesia in all three groups is shown in table III. Patients receiving halothane had a greater incidence of abnormal GST concentrations than those receiving enflurane or isoflurane (four-fold table test: $P < 0.01$ and $P <$

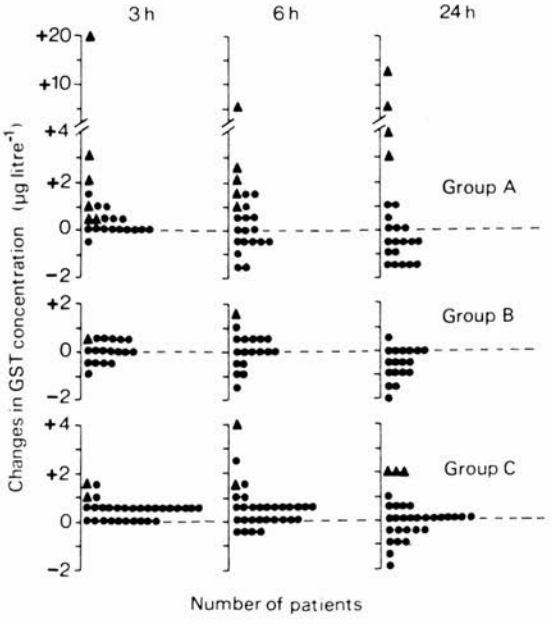

FIG. 1. Individual changes in plasma GST concentrations (µg litre^{-1}) from the preoperative sample. ● = Changes within the reference range; ▲ = increase in GST which exceeded the upper limit of the reference range (4.0 µg litre^{-1}). Group A patients were anaesthetized with halothane, group B patients received isoflurane and group C patients received enflurane.

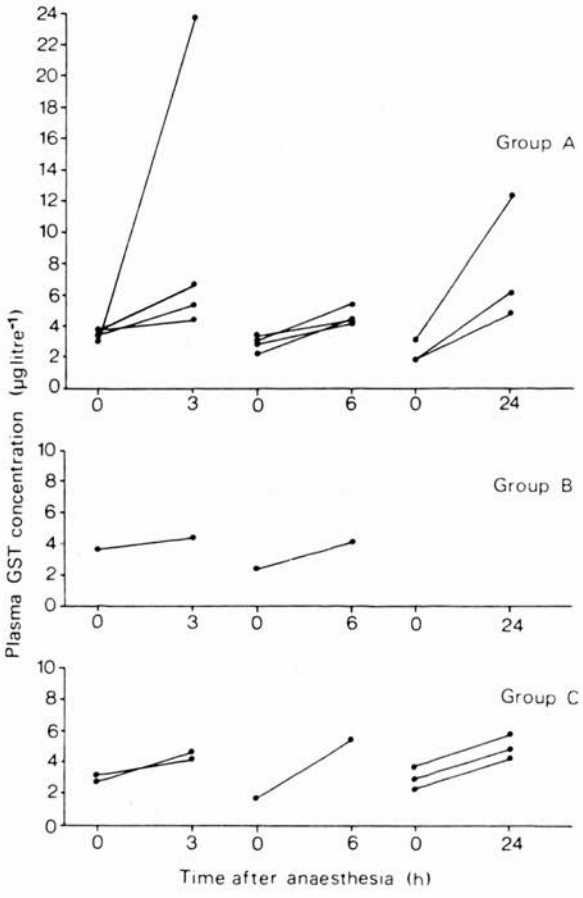

FIG. 2. Abnormal plasma GST concentrations (> 4.0 µg litre^{-1}) following anaesthesia with halothane (group A), isoflurane (group B) or enflurane (group C) compared with concentrations in preoperative samples. The maximum increase in GST concentration for each patient is shown.

TABLE III. *Incidence of abnormal plasma GST concentrations following anaesthesia in patients in groups A, B and C*

GST (µg litre^{-1})	Group A Halothane (n = 22)	Group B Isoflurane (n = 18)	Group C Enflurane (n = 30)
Normal (< 4.0)	11	16	24
Increased (> 4.0)	11	2	6

0.05, respectively). There was no significant difference in the incidence of abnormal GST concentrations between the groups receiving enflurane or isoflurane.

DISCUSSION

We have demonstrated marked differences in the plasma GST response to three halogenated anaesthetic agents. These differences were not attributable to patient selection, since the three patient groups were comparable with respect to factors such as age, sex, alcohol intake and the type and duration of operation. Significant increases in GST concentrations at approximately 3–6 h following anaesthesia were observed with halothane and enflurane, but not isoflurane. These findings are in agreement with the results obtained from a previous study in which only halothane and isoflurane anaesthesia were studied [14].

Changes in GST concentrations result from an impairment in hepatocellular integrity and may be an indication of mild subclinical liver damage of a transient nature. Although the increases in plasma GST were small, a large number of abnormal concentrations were recorded, particularly in the halothane group. In addition, secondary and more marked increases in GST concentrations were seen 24 h after anaesthesia in a few of the patients who had received halothane or enflurane. This secondary phase of impaired hepatocellular integrity was also apparent in previous studies [13, 14]. None of the patients who had received isoflurane showed this secondary increase in concentration.

The relationship between the impairment of hepatocellular integrity that we have observed in the majority of individuals following halothane and enflurane anaesthesia and the rare development of fulminant hepatitis is unclear. Two distinct forms of liver damage, mild (type I) and severe (type II), have been defined following halothane anaesthesia [16]. Hepatic dysfunction of the type I form is probably more common and it is thought to be associated with increased concentrations of serum transaminases [17]. However, not all studies have demonstrated an increased incidence of liver enzyme disturbance following halothane in comparison with other agents, and small, reversible changes in liver function are common following all kinds of anaesthesia [18]. Fulminant hepatic failure following halothane (type II) is a much rarer event which occurs mainly in adults with an incidence of between 1:7000 and 1:30000 [6].

A number of mechanisms have been proposed to account for the hepatotoxic effect of anaesthesia. Biotransformation with the formation of toxic metabolites which may bind to cellular macromolecules and cause necrosis has been suggested [19, 20]. The reductive metabolism of halothane, which is associated with hepatic necrosis in animal models [21], has been demonstrated in man [22]. The degree of biotransformation of the three anaesthetics included in this study differs. Halothane is metabolized to a greater extent (approx. 20 % of an inhaled dose) than enflurane (2 %) and isoflurane (0.2 %) [5]. It is interesting to find that anaesthesia with halothane resulted in a significantly greater incidence of abnormal GST concentrations than did anaesthesia with either enflurane or isoflurane.

The incidence of hepatitis increases with repeated halothane exposure, which would be in keeping with a hypersensitivity reaction. It is of interest that hapten formation involving halothane or a metabolite has been described [4]. There is also evidence to suggest a susceptibility factor responsible for a genetic predisposition to halothane hepatitis in certain individuals [3]. Hepatic hypoxia *per se* has also been implicated in anaesthesia-associated hepatotoxicity [23].

The data presented provide evidence for two phases of mild transient hepatotoxicity after anaesthesia with halothane or enflurane, but not with isoflurane. The first phase occurred in the majority of individuals within 3–6 h of administration of the anaesthetic. In the patients receiving halothane, this is most likely to be caused by the direct specific effect of this agent on hepatic blood flow producing relative tissue hypoxia [24]. However, an increase of GST was also observed in patients receiving enflurane, which is not known to affect hepatic arterial blood flow.

The second phase of impaired hepatocellular integrity, at 24 h, which was indicated by marked changes in GST concentrations in a small number of patients and an increased ALT activity in one patient, may result from the production of toxic metabolites. The changes in GST concentrations seen at this time support evidence for the existence of the mild form of liver damage following halothane (type I) that has been defined by other workers [17]. The proportion of patients

showing this response was greater in the group who received the more extensively metabolized anaesthetic halothane, than in the group who had received enflurane.

Changes in plasma GST concentrations clearly demonstrate the stages of hepatotoxicity which may follow anaesthesia. Their measurement may help in the understanding of the mechanisms involved in the hepatotoxicity related to the inhalation of certain volatile anaesthetic agents.

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REFERENCES

1. Brown BR, Gandolfi AJ. Adverse effects of volatile anaesthetics. *British Journal of Anaesthesia* 1987; **59**: 14–23.
2. Cousins MJ, Gourlay GK, Knights KM, Hall P de la M, Lunam CA, O'Brien P. Randomised prospective controlled study of metabolism and hepatotoxicity of halothane in man. *Anesthesiology* 1982; 57: A223.
3. Farrell G, Prendergast D, Murray M. Halothane hepatitis. Detection of a constitutional susceptibility factor. *New England Journal of Medicine* 1985; **313**: 1310–1314.
4. Vergani D, Mieli-Vergani G, Alberti A, Neuberger J, Eddleston AL, Davis M, Williams R. Antibodies to the surface of halothane altered rabbit hepatocytes in patients with severe halothane-associated hepatitis. *New England Journal of Medicine* 1980; **303**: 66–71.
5. Blogg CE. Halothane and the liver: the problem revisited and made obsolete. *British Medical Journal* 1986; **292**: 1691–1692.
6. Editorial. Halothane-associated liver damage. *Lancet* 1986; **1**: 1251–1252.
7. Lewis JH, Zimmerman HJ, Ishak KG, Mullick FG. Enflurane hepatotoxicity. A clinicopathologic study of 24 cases. *Annals of Internal Medicine* 1983; **98**: 984–992.
8. Eger EI, Shmuckler EA, Ferrell LD, Goldsmith CH, Johnson BH. Is enflurane hepatotoxic? *Anesthesia and Analgesia* 1986; **65**: 21–30.
9. Stoelting RK, Blitt CD, Cohen PJ, Merin RG. Hepatic dysfunction after isoflurane anesthesia. *Anesthesia and Analgesia* 1987; **66**: 147–153.
10. Zimmerman HJ. Experimental hepatotoxicity. In: Zimmerman HJ, ed. *Hepatotoxicity. The Adverse Effects of Drugs and Other Chemicals on the Liver*. New York: Appleton-Century Crofts, 1978; 167.
11. Beckett GJ, and Hayes JD. Plasma glutathione S-transferase measurements and liver disease in man. *Journal of Clinical Biochemistry and Nutrition* 1987; **2**: 1–24.
12. Redick JA, Jakoby WB, Baron J. Immunochemical localisation of glutathione S-transferases in livers of untreated rats. *Journal of Biological Chemistry* 1982; **257**: 15200–15203.
13. Hussey AJ, Howie J, Allan LG, Drummond G, Hayes JD, Beckett GJ. Impaired hepatocellular integrity during general anaesthesia, as assessed by measurements of plasma glutathione S-transferase. *Clinica Chimica Acta* 1986; **161**: 19–28.
14. Allan LG, Hussey AJ, Howie J, Beckett GJ, Smith AF, Hayes JD, Drummond GB. Hepatic glutathione S-transferase release after halothane anaesthesia: Open, randomised comparison with isoflurane. *Lancet* 1987; **1**: 771–773.
15. Beckett GJ, Hayes JD. Development of specific radioimmunoassays for the measurement of human hepatic basic and N/A2b glutathione S-transferase. *Clinica Chimica Acta* 1984; **141**: 267–273.
16. Pohl LR, Gilette JR. A perspective on halothane-induced hepatotoxicity. *Anesthesia and Analgesia* 1982; **61**: 809–811.
17. Neuberger J, Williams R. Halothane anaesthesia and liver damage. *British Medical Journal* 1984; **289**: 1136–1139.
18. Stock JGL, Strunin L. Unexplained hepatitis following halothane. *Anesthesiology* 1985; **63**: 424–439.
19. Widger LA, Gandolfi AJ, Van Dyke RA. Hypoxia and halothane metabolism *in vivo*: Release of inorganic fluoride and halothane metabolite binding to cellular constituents. *Anesthesiology* 1976; **44**: 197–201.
20. Ultrecht J, Wood AJJ, Phythyon JM, Wood M. Contrasting effects on halothane hepatotoxicity in the phenobarbital-hypoxia triiodothyronine model: Mechanism implications. *Anesthesiology* 1983; **59**: 196–201.
21. McLain GE, Sipes IG, Brown BR. An animal model of halothane hepatotoxicity: Roles of enzyme induction and hypoxia. *Anesthesiology* 1979; **51**: 321–326.
22. Cohen, EN, Trudell JR, Edmunds HN, Watson E. Urinary metabolites of halothane in man. *Anesthesiology* 1975; **43**: 392–401.
23. Shingu, R, Eger EI, Johnson BH. Hypoxia may be more important than reductive metabolism in halothane-induced hepatic injury. *Anesthesia and Analgesia* 1982; **61**: 824–827.
24. Gelman S, Rimerman V, Fowler KC, Bishop SP, Bradley EL. The effect of halothane, isoflurane and blood loss on hepatotoxicity and hepatic oxygen availability in phenobarbital pre-treated hypoxic rats. *Anesthesia and Analgesia* 1984; **63**: 965–972.

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EFFECT OF NICARDIPINE INFUSION ON THE RELEASE OF GLUTATHIONE S-TRANSFERASE FOLLOWING HALOTHANE ANAESTHESIA

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SUMMARY

To assess the possible protective effect of calcium channel blockade on hepatic function after halothane anaesthesia, 80 patients were allocated randomly to receive an i.v. infusion of either nicardipine or normal saline. Plasma concentration of glutathione S-transferase B_1 subunits (GST) was measured as a sensitive index of hepatic damage. Data from 53 patients were analysed. Plasma GST concentration increased significantly at 3 and 6 h after induction of anaesthesia in the placebo group ($P < 0.01$), and at 3 h ($P < 0.01$) and 6 h ($P < 0.05$) in the nicardipine group. The administration of nicardipine resulted in a greater increase in plasma GST concentrations at 3 h than did placebo ($P < 0.05$), mainly because of a greater increase in males than in females. The increase in GST at 3 h was related inversely to plasma concentration of nicardipine both at the end of the exponential infusion ($P < 0.01$) and at 2 h after induction ($P < 0.05$), when males had lower plasma nicardipine concentrations than females ($P < 0.05$). Calcium channel blockade with nicardipine in the dose administered was not shown to reduce liver dysfunction after halothane anaesthesia.

Liver dysfunction following exposure to volatile halogenated anaesthetics has long been established [1]. The role of halothane in the development of hepatic injury is the subject of renewed controversy and research. Significant increases in the plasma concentration of the hepatic cytosolic enzyme glutathione S-transferase B_1B_1 (GST) occur after halothane but not isoflurane anaesthesia [2, 3]. This led us to believe that measurement of GST in plasma could be valuable in the investigation of liver dysfunction following anaesthesia as the plasma concentrations of GST allow detection of minor and short-term hepatic injury which is not revealed using conventional hepatic enzymes [2, 3].

The role of calcium in mediating or propagating ischaemic cell injury, and the concept that increased concentrations of intracellular calcium constitute the final common pathway of cell injury have been reviewed [4]. Gelman and Van Dyke have propounded a similar "calciogenic" hypothesis for the development of liver toxicity associated with volatile anaesthetics [5]. Slow calcium channel blockers have a cell protective effect in ischaemic myocardial injury [6] and in ischaemic brain damage in rats [7]. Garay and co-workers [8] found that the calcium antagonist nicardipine might significantly reduce the extent of liver damage in rats after administration of the hepatotoxic agents carbon tetrachloride or d-galactosamine.

We have assessed the possibility that nicardipine given as an infusion before and during halothane anaesthesia in man might reduce the release of GST.

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PATIENTS AND METHODS

Patients and samples

We studied patients aged 18–70 yr, ASA I or II [9], undergoing elective, peripheral orthopaedic surgery expected to last between 30 and 90 min. Ethics Committee approval and written informed consent were obtained. Patients who had undergone halothane anaesthesia within the preceding 3 months were excluded. Those patients receiving

regular oral medication other than benzodiazepines or thiazide diuretics and those who gave a history of parenchymal liver disease, contact with hepatitis or recent blood transfusion were excluded also, as were patients whose average daily intake of alcohol exceeded 3 units and those whose weight was greater than 120 % of that expected for a person of the same age, sex and height [10]. Subjects were allocated, according to a previously prepared, randomized, sex-stratified list, to one of two groups to receive either an i.v. infusion of nicardipine or a comparable infusion of normal saline.

The infusion was designed to achieve a steady state plasma concentration of nicardipine approximately 120 ng ml^{-1} after 1 h of infusion, based on an approximate plasma clearance rate of nicardipine of 600 ml min^{-1} for a 70-kg subject and an elimination rate constant of 0.2 h^{-1} [11]. The contents of a syringe containing nicardipine 5 mg in normal saline 60 ml were infused from a syringe pump at a rate of 1 ml min^{-1} into a closed vial containing nicardipine 20 mg in 20 ml volume, via a fine-bore needle to encourage mixing within the vial. The infusion was led from the vial to the patient. This technique was described by Riddell and co-workers [12]. In the first 1 h of this treatment a patient allocated to receive nicardipine would have been given a combination of a steady infusion of nicardipine 5 mg and an exponentially decreasing infusion with a total of nicardipine 20 mg, 95 % of which would have been administered in that time. Subsequently, an infusion was started of nicardipine 25 mg in normal saline 50 ml at 10 ml h^{-1} for a further 5 h.

The infusion was commenced 1 h before induction of anaesthesia. The electrocardiograph was monitored continuously and systemic arterial pressure measured for the duration of the infusion, which was discontinued if the systolic arterial pressure became less than 75 % of the preinfusion value, or if tachycardia > 150 beat min^{-1} or disorders of cardiac rhythm became apparent.

Premedication comprised temazepam 20 mg by mouth 2 h before operation. Anaesthesia was induced with thiopentone 4–6 mg kg^{-1} and suxamethonium 1 mg kg^{-1} was administered to facilitate tracheal intubation if this was necessary. Anaesthesia was maintained with 66 % nitrous oxide and halothane in oxygen. Ventilation was spontaneous in all patients. End-tidal concentrations of halothane (Penlon halothane meter) and carbon dioxide (Hewlett–Packard 47210A capnometer) were recorded every 5 min for the duration of anaesthesia. The carbon dioxide sensor was attached to the mount of the mask in non-intubated patients. Patients were withdrawn from the study if their end-tidal carbon dioxide partial pressure exceeded 8 kPa. Postoperative analgesia was provided by i.m. diamorphine, oral dihydrocodeine or soluble aspirin as necessary; metoclopramide was prescribed to relieve nausea.

The patient's age, height, weight, alcohol intake, the operation performed and the duration of anaesthesia were recorded, and end-tidal carbon dioxide and halothane concentrations were recorded for each 5-min period. The mean of the end-tidal concentrations of carbon dioxide and halothane was determined for each patient and a time-weighted halothane average calculated (mean end-tidal halothane concentration × duration of anaesthesia (min)). Blood was sampled before commencing the infusion, for GST and conventional liver function tests, full blood count and screening for the following autoantibodies: thyroid cytoplasm, gastric parietal cells, antinuclear factor, smooth muscle, mitochondria and antibrush border. Further samples for measurement of GST were taken 1, 3, 6 and 24 h after induction of anaesthesia. Plasma concentration of nicardipine was assessed at the end of the exponential infusion and 30 min and 1, 2, 3 and 6 h after induction. At 24 h a final sample of blood was taken for repeat blood chemistry and haematological screening.

Assay procedures

Concentrations of bilirubin, and activities of alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) were measured using a Sequential Multiple Analysis with Computer System II (SMAC) (Technicon Instrument Corporation, Basingstoke, U.K.). The plasma concentration of GST B_1B_1 was measured by specific radioimmunoassay [13]. The reference range for GST B_1B_1 is 0.7–4.0 μg $litre^{-1}$. The interassay coefficient of variation for the GST radioimmunoassay was less than 10 %, and the intraassay coefficient of variation was less than 5 % over the range 2–40 μg $litre^{-1}$. Samples for nicardipine assay were separated by centrifugation and the resultant serum was stored at −20 °C until assay was performed by standard high pressure liquid chromatography [14]. Analysis of nicardipine-containing serum was performed

TABLE I. *Median (range) values of conventional liver function tests for 16 patients excluded from analysis because of abnormal values before operation, and median values of GST for these patients*

	Before op.	After operation			
		1 h	3 h	6 h	24 h
Bilirubin (μg litre^{-1})	10 (6–15)	—	—	—	13 (6–26)
ALT (u. litre^{-1})	39 (20–108)	—	—	—	36 (20–85)
GGT (u. litre^{-1})	31 (9–159)	—	—	—	24 (6–151)
GST (μg litre^{-1})	5.9 (4.6–27.6)	5.0 (3.5–24.7)	6.4 (3.3–29.2)	5.0 (3.4–17.4)	3.6 (1.7–8.5)

by Syntex Pharmaceuticals Ltd, Palo Alto, California.

Statistical procedures

The demographic data, the duration of anaesthesia, mean end-tidal halothane and carbon dioxide concentration, and time-weighted halothane average for each group were compared using Student's *t* test. Changes in GST concentrations within each group were compared using the Friedman test. The GST changes from the preoperative values at 1, 3, 6 and 24 h following induction of anaesthesia in each group were compared by the Wilcoxon signed rank test. Differences in GST changes at these times between the two groups were examined by the Wilcoxon rank sum test. The relations between changes in GST concentration and duration of anaesthesia, end-tidal carbon dioxide concentration, and dose of opioid administered in 24 h were examined by Spearman's rank coefficient. This test was also used to investigate the correlation of GST changes and plasma nicardipine concentration for males and females separately.

RESULTS

Eighty patients were entered into the study: 42 received nicardipine (27 males, 15 females) and 38 received placebo (26 males, 12 females). The infusion was discontinued in 11 patients and they were excluded from subsequent analysis. Eight of these had received nicardipine and on each occasion the infusion was stopped because of hypotension. Three patients receiving normal saline also had the infusion discontinued, two because of tachycardia and one whose operation was cancelled for surgical reasons. Data were not analysed in 16 patients who were found to have abnormal liver function tests in the sample taken before operation (table I). All exhibited an increased GST and nine also had abnormal conventional liver function tests. In no patient did end-tidal carbon dioxide partial pressure exceed 8 kPa, and only four patients had values greater than 6 kPa.

The remaining 53 patients from whom data were analysed comprised 29 who received nicardipine (17 males, 12 females) and 24 who were given placebo (13 males, 11 females). Comparisons between the groups revealed no significant difference in age, height, weight, percent of expected weight and daily alcohol consumption (table II). Two patients in the study were taking regular oral medication: one was receiving temazepam and the other bendrofluazide. Both groups received similar doses of thiopentone at induction of anaesthesia, when three patients in the placebo group and two in the nicardipine group received suxamethonium to facilitate tracheal intubation. Mean end-tidal values of halothane and carbon dioxide concentrations did not differ significantly between groups. Patients receiving nicardipine had a significantly longer duration of anaesthesia than those receiving placebo ($P < 0.01$) and, as would be expected, their time-weighted halothane average was correspondingly greater ($P < 0.05$) (table II).

TABLE II. *Demographic data and anaesthetic characteristics (mean (SD))*

	Placebo	Nicardipine
Age (yr)	34 (12)	30 (8)
Height (cm)	171 (10)	174 (11)
Weight (kg)	68 (9)	71 (13)
Percent of expected wt	100 (12)	103 (12)
Alcohol (u. day^{-1})	1.4 (0.8)	1.6 (1.1)
Thiopentone (mg)	414 (63)	428 (86)
Halothane concn (%)	1.1 (0.3)	1.0 (0.2)
$P_{E'_{CO_2}}$ (kPa)	4.9 (0.8)	5.1 (0.7)
Duration of anaesthesia (min)	39 (11)	54 (21)
Time-weighted halothane average	40.3 (13.7)	53.1 (21.5)

TABLE III. *Median values of GST (μg litre^{-1}) (interquartile range).* *P < *0.05;* **P < *0.01 compared with value before operation*

	Before op.	After operation			
		1 h	3 h	6 h	24 h
Placebo	2.2 (1.8–2.8)	2.1 (1.6–2.8)	2.7** (2.2–3.4)	2.8** (2.2–3.3)	2.0 (1.7–2.6)
Nicardipine	2.4 (1.7–2.8)	2.0 (1.7–2.9)	2.9** (2.3–4.2)	2.5* (2.1–3.6)	1.7* (1.4–2.4)

A significant increase in plasma GST concentration above basal values was noted 3 and 6 h following induction in each group and a significant reduction at 24 h in the group receiving nicardipine (table III). The distribution of changes in GST from the preoperative values is shown in figure 1. Nicardipine administration resulted in a greater increase in GST concentration 3 h after induction than did placebo ($P < 0.05$). This difference was almost entirely attributable to males receiving nicardipine, who showed a greater increase in GST value at 3 h ($P < 0.01$) than did females receiving nicardipine (fig. 2). The median plasma concentrations of nicardipine at each time of sampling are shown separately for males and females in table IV. Males receiving nicardipine had significantly lower plasma concentrations of nicardipine than females at the end of the exponential infusion and at 2 h after induction ($P < 0.05$, both times). The plasma concentration of nicardipine at these times was inversely related to the increase in GST at 3 h ($P < 0.01$ at the end of exponential infusion, $P < 0.05$ at 2 h after induction (fig. 3)). There was no correlation between the magnitude of change in GST at 3 and 6 h and duration of anaesthesia, mean end-tidal carbon dioxide concentration or administration of opioid after operation in either group.

The finding of positive autoantibody titres in seven patients (two to gastric parietal cells, two to

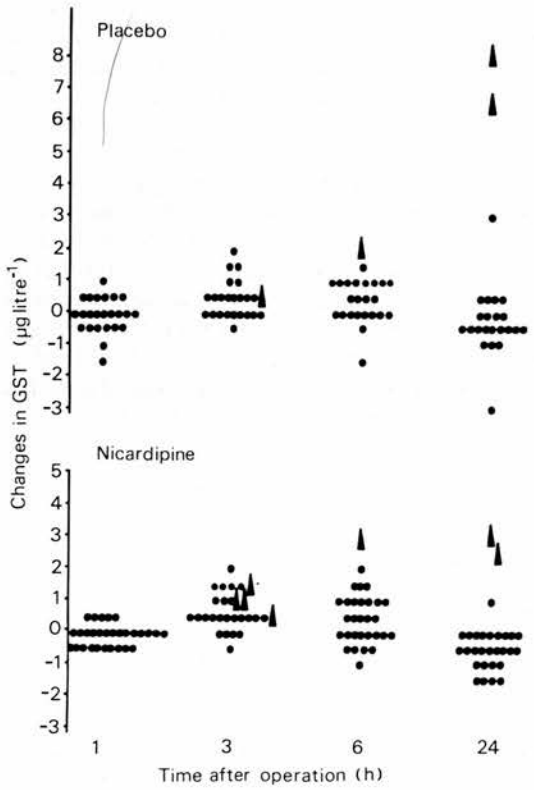

FIG. 1. Individual changes in plasma GST concentrations after operation, relative to the preoperative value, for the patients receiving placebo and nicardipine infusions. Arrowheads = increase in GST that exceeded the upper limit of the reference range.

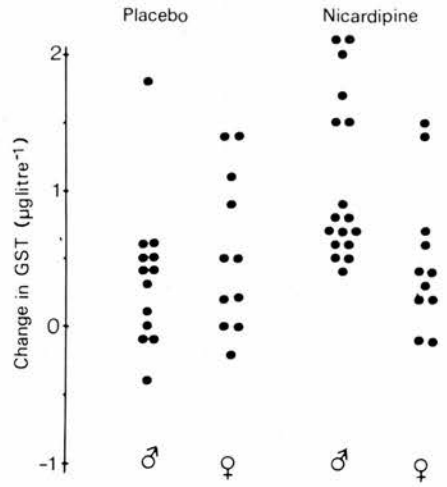

FIG. 2. Individual changes in plasma GST concentrations, relative to the preoperative value, 3 h after operation, showing male and female subjects separately.

TABLE IV. *Plasma nicardipine concentrations (ng ml^{-1}) (median (range)). * $P < 0.05$ between sexes*

	End of exponential infusion	Time after induction				
		30 min	1 h	2 h	3 h	6 h
Males	115.2 (61.0–149.5)	90.8 (67.4–159.3)	90.3 (72.1–117.3)	79.7 (64.9–118.6)	80.6 (63.2–128.3)	35.5 (20.2–58.5)
	*			*		
Females	149.8 (87.5–196.0)	107.7 (52.5–158.2)	99.4 (77.0–166.6)	105.4 (75.7–149.2)	103.8 (59.5–139.6)	34.8 (18.8–54.0)

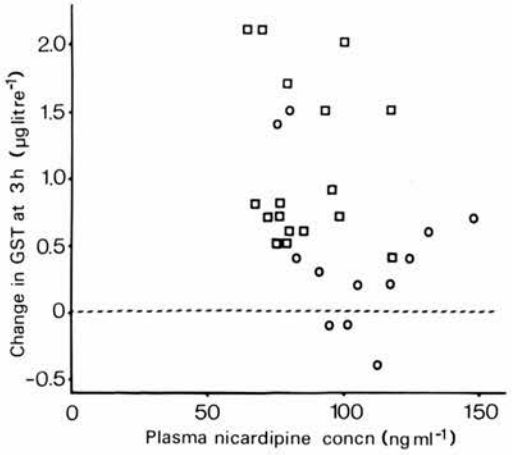

FIG. 3. Relationship between the plasma concentration of nicardipine achieved 2 h after induction of anaesthesia, and the change in GST that occurred at 3 h after induction of anaesthesia. □ = Male patients; ○ = female patients. There is a negative linear correlation ($r = -0.43$, $P < 0.05$) for the patients taken together.

antinuclear factor, and one each to thyroid cytoplasm, smooth muscle and antibrush border) could not be related to an unusual GST response. The concentrations of bilirubin and activities of ALT and GGT at 24 h after induction were not significantly different compared with the values before operation (table V).

TABLE V. *Mean (SD) values of conventional liver function tests*

	Bilirubin (μg litre^{-1})	ALT (u. litre^{-1})	GGT (u. litre^{-1})
Placebo			
Before op.	11 (4)	16 (10)	14 (14)
After op.	11 (4)	14 (8)	12 (13)
Nicardipine			
Before op.	14 (8)	17 (8)	14 (7)
After op.	16 (12)	13 (8)	13 (7)

DISCUSSION

The underlying mechanisms of "halothane hepatitis" remain poorly understood. Hypersensitivity [15, 16], toxic products of metabolism [17–19] particularly non-oxidative metabolism [20], regional hepatic hypoxia [21, 22] and a familial susceptibility factor [23] have all been implicated as contributing factors. Females are reported to be at greater risk than males of developing hepatic damage following halothane anaesthesia [24, 25], although the sex distribution of patients exposed to halothane anaesthesia is not necessarily equal. It was for this reason that we investigated independently the influence of nicardipine infusion on GST release in the two sexes.

It has been suggested that an increase in intracellular calcium acts as the final common event in irreversible cell damage [4]. Such an increase occurs secondary to disruption of the mechanisms that maintain intracellular calcium homeostasis. Recently, it has been postulated that the difference in toxicity between halothane and isoflurane might result from a different ability of anaesthetics to disrupt intracellular calcium homeostasis [5]. The administration of a calcium channel blocker before the initiation of cell injury may therefore prevent a large secondary influx of calcium into the cell and limit the severity of cellular damage. Indeed, studies in the rat suggest that this is the case [7, 8]. We undertook this study to investigate the degree to which calcium channel blockade could reduce liver dysfunction following halothane anaesthesia.

We have confirmed that transient GST release occurs following anaesthesia with halothane in man, as has been demonstrated previously [2, 3]. Although the changes in median concentrations of GST were small, some individual increases were sufficient to increase the plasma concentration of GST above the upper limit of the reference range.

This finding parallels those of earlier studies in which the administration of isoflurane did not result in an increase in plasma concentration of GST [2, 3]. We have also shown that nicardipine is associated with a greater release of GST after operation than is placebo. This difference resulted from an exaggerated GST response in males, but not females, receiving nicardipine. Females had a significantly greater plasma concentration of nicardipine at the end of the exponential infusion and at 2 h after induction than males, and this was related to a lesser increase in GST at 3 h. The larger increase in GST at 3 h in males receiving nicardipine may reflect the combined effects of halothane and nicardipine causing a greater reduction in hepatic perfusion than halothane alone. The greater plasma concentration achieved in females perhaps conferred a degree of hepatoprotection against this combined effect, but possibly were still insufficient to prevent release of GST. Although plasma concentrations of nicardipine throughout the infusion were compatible with those required to give a clinically relevant effect [26] and haemodynamic effects were apparent throughout the infusion (increased heart rate and reduced diastolic arterial pressure), it may be that even greater plasma concentrations are required for hepatoprotection following halothane anaesthesia. Such concentrations may, however, cause undesirable cardiovascular side effects. We were unable demonstrate that nicardipine in the dose administered prevented this type of liver dysfunction.

The finding that nicardipine conferred no hepatoprotection is at variance with animal studies. However, the dose of nicardipine administered to rats [8] was 25 to 50 times greater than the total dose given to patients in the present study. As we have suggested, larger doses of nicardipine given to man might also have the same protective effect. In the same animal studies the agents used to induce hepatic injury were carbon tetrachloride and d-galactosamine. The pattern of hepatotoxicity caused by these agents may not resemble the centrilobular necrosis seen with halothane, so a comparison of the protective effect of nicardipine for different hepatotoxins may not necessarily be appropriate.

In animal studies the severity of hepatic damage caused by halothane is related to halothane concentration, but this has not been demonstrated in humans. Despite the random allocation design of this study, the patients receiving nicardipine had on average a longer duration of anaesthesia and consequently a greater time-weighted halothane average than patients given placebo. This raises the possibility that a greater hepatic insult was imposed in the patients receiving nicardipine. However, no correlation was detected between duration of anaesthesia and magnitude of GST increase, a finding in aggreement with those of Allan [2] and Hussey [3], suggesting that this difference between the groups is unlikely to have influenced our findings.

As in previous studies investigating GST release following halothane anaesthesia, some patients exhibited a large secondary increase in plasma GST activity 24 h after induction (fig. 1). These patients were distributed equally between the two groups and nicardipine seemed of no benefit in preventing this less frequent increase, although many more patients would require study to be certain of this.

In compliance with the study programme, patients with abnormal liver function tests before operation were excluded from analysis. It could be argued that we thus introduced a degree of bias into the results by selecting "normal" patients. However, an examination of GST concentrations after operation in the 16 patients so excluded revealed a pattern of GST change similar to that seen in those patients who were entered into analysis.

GST is a sensitive marker allowing investigation of liver dysfunction following use of volatile anaesthetic agents in man. Further investigations are required to elucidate the mechanisms involved.

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REFERENCES

1. Brown BR, Gandolfi AJ. Adverse effects of volatile anaesthetics. *British Journal of Anaesthesia* 1987; **59**: 14–23.
2. Allan LG, Hussey AJ, Howie J, Beckett GJ, Smith AF, Hayes JD, Drummond GB. Hepatic glutathione S-transferase release after halothane anaesthesia: Open randomised comparison with isoflurane. *Lancet* 1987; **1**: 771–774.
3. Hussey AJ, Aldridge LM, Paul D, Ray DC, Beckett GJ, Allan LG. Plasma glutathione S-transferase concentration as a measure of hepatocellular integrity following a single

general anaesthetic with halothane, enflurane or isoflurane. *British Journal of Anaesthesia* 1988; **60**: 130–135.
4. Cheung JY, Bonventre JV, Malis CD, Leaf A. Calcium and ischemic injury. *New England Journal of Medicine* 1986; **314**: 1670–1676.
5. Gelman S, Van Dyke R. Mechanism of halothane-induced hepatotoxicity: Another step on a long path. *Anesthesiology* 1988; **68**: 479–482.
6. Stone PH, Antman EM, Muller JE, Braunwald E. Calcium channel blocking agents in the treatment of cardiovascular disorders. Part II. Hemodynamic effects and clinical applications. *Annals of Internal Medicine* 1980; **93**: 886–904.
7. Deshpande JK, Wieloch T. Flunarizine, a calcium entry blocker, ameliorates ischemic brain damage in the rat. *Anesthesiology* 1986; **64**: 215–224.
8. Garay GL, Annesley P, Burnette M. Prevention of experimental liver injury in rats by nicardipine, a calcium entry blocker. *Gastroenterology* 1984; **86**: 1319.
9. Schneider AJL. Assessment of risk factors and surgical outcome. *Surgical Clinics of North America* 1983; **63**: 1113–1126.
10. Diem K, Lentner C. *Documenta Geigy Scientific Tables*, 7th Edn. Basle, Switzerland: JR Geigy SA, 1970.
11. Graham DJM, Dow RJ, Hall DJ, Alexander OF, Mroszczak EJ, Freedman D. The metabolism and pharmacokinetics of nicardipine hydrochloride in man. *British Journal of Clinical Pharmacology* 1985; **20**: 23S–28S.
12. Riddell JG, McAllister CB, Wilkinson GR, Wood AJJ, Roden DM. A new method for constant plasma drug concentrations: application to lidocaine. *Annals of Internal Medicine* 1984; **100**: 25–28.
13. Beckett GJ, Hayes JD. Development of specific radioimmunoassays for the measurement of human hepatic basic and NA/2b glutathione S-transferase. *Clinica Chimica Acta* 1984; **141**: 267–273.
14. Wu AT, Massey IJ, Kushinsky S. A high-performance liquid chromatographic method for the simultaneous determination of nicardipine and its pyridine metabolite II in plasma. *Journal of Pharmaceutical Sciences* 1984; **73**: 1444–1447.
15. Vergani D, Mieli-Vergani G, Alberti A, Neuberger J, Eddleston AL, Davis M, Williams R. Antibodies to the surface of halothane altered rabbit hepatocytes in patients with severe halothane-associated hepatitis. *New England Journal of Medicine* 1980; **303**: 66–69.
16. Neuberger J, Gimson AES, Davis M, Williams R. Specific serological markers in the diagnosis of fulminant hepatic failure associated with halothane anaesthesia. *British Journal of Anaesthesia* 1983; **55**: 15–18.
17. Cousins MJ, Gourlay GK, Knights KM, Hall P de la M, Lunam CA, O'Brien P. A randomised prospective controlled study of the metabolism and hepatotoxicity of halothane in humans. *Anesthesia and Analgesia* 1987; **66**: 299–308.
18. Widger LA, Gandolfi AJ, Van Dyke RA. Hypoxia and halothane metabolism *in vivo*: Release of inorganic fluoride and halothane metabolite binding to cellular constituents. *Anesthesiology* 1976; **44**: 197–201.
19. Uetrecht J, Wood AJJ, Phythyon JM, Wood M. Contrasting effects on halothane hepatotoxicity in the phenobarbital-hypoxia and tri-iodothyronine model: Mechanism implications. *Anesthesiology* 1983; **59**: 196–201.
20. McLain GE, Sipes IG, Brown BR. An animal model of halothane hepatotoxicity: Roles of enzyme induction and hypoxia. *Anesthesiology* 1979; **51**: 321–326.
21. Shingu K, Eger EI, Johnson BH. Hypoxia may be more important than reductive metabolism in halothane-induced hepatic injury. *Anesthesia and Analgesia* 1982; **61**: 824–827.
22. Gelman S, Dillard E, Bradley EL. Hepatic circulation during surgical stress and anesthesia with halothane, isoflurane or fentanyl. *Anesthesia and Analgesia* 1987; **66**: 936–943.
23. Farrell G, Prendergast D, Murray M. Halothane hepatitis. Detection of a constitutional susceptibility factor. *New England Journal of Medicine* 1985; **313**: 1310–1314.
24. Walton B, Simpson BR, Strunin L, Doniach D, Perrin J, Appleyard AJ. Unexplained hepatitis following halothane. *British Medical Journal* 1976; **1**: 1171–1176.
25. Neuberger J, Williams R. Halothane anaesthesia and liver damage. *British Medical Journal* 1984; **289**: 1136–1139.
26. Debbas NMG, Jackson SHD, Turner P. A study of the haemodynamic effects of nicardipine infusion in man. *British Journal of Clinical Pharmacology* 1985; **19**: 563P–564P.

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PREOPERATIVE CIMETIDINE DOES NOT PREVENT SUBCLINICAL HALOTHANE HEPATOTOXICITY IN MAN

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SUMMARY

To assess the influence of pretreatment with cimetidine on changes in hepatocellular integrity after halothane anaesthesia, 53 patients were allocated randomly to receive either cimetidine 1600 mg orally or placebo tablets before anaesthesia. Plasma concentrations of glutathione S-transferase (GST) were measured as an index of hepatic damage. Data from 45 patients were available for analysis. Plasma GST concentration increased significantly 3 h after induction of anaesthesia in both groups ($P < 0.01$, both groups) and at 6 h in the cimetidine group ($P < 0.05$). Pretreatment with cimetidine did not influence the magnitude of increase in GST concentration. There was no difference between the groups in the frequency of abnormal GST concentrations at any time. Cimetidine does not appear to prevent release of GST from the liver by halothane anaesthesia in man.

Toxic metabolic products resulting from halothane biotransformation are thought to be a possible mechanism of halothane hepatotoxicity [1, 2]. Metabolism of halothane occurs in man via both oxidative and reductive pathways. Van Dyke has demonstrated that metabolism takes place in liver microsomes [3]; cimetidine has been reported to bind to liver microsomal enzymes, specifically cytochromes P450 and P448 [4]. Consequently, cimetidine can impair the metabolism of drugs that are metabolized by the mixed-function oxidase enzyme system. Drugs known to be affected in man in this way include anticoagulants, benzodiazepines, beta blockers, theophylline, anticonvulsants [5] and lignocaine [6]. Animal experiments suggest that cimetidine reduces halothane metabolism and consequently lessens the severity of hepatic injury. Although two groups of workers have demonstrated this reduction, they differed on whether or not it is the oxidative or reductive pathway of metabolism that is inhibited [7, 8].

Glutathione S-transferase B_1B_1 (GST) concentration in plasma is known to provide a more sensitive index of drug-induced hepatocellular damage [9] and correlates better with hepatic histology than the serum transaminase concentrations [10]. We have demonstrated previously that halothane anaesthesia produced significant increase in plasma GST concentration 3 h after anaesthesia. In some patients a larger secondary increase in GST occurred 24 h after anaesthesia. It has been suggested that the peak in GST occurring at 3 h results from alterations in hepatic blood flow whilst the increase at 24 h may be caused by production of toxic metabolites [11].

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We have assessed the possibility that administration of cimetidine before anaesthesia could prevent the release of GST following halothane anaesthesia in man.

PATIENTS AND METHODS

The study was approved by the Area Ethics Committee. Informed consent was obtained from patients aged 16–70 yr (ASA class I or II) undergoing minor orthopaedic surgery such as arthroscopy. Patients receiving regular oral medication of any type and those who had undergone halothane anaesthesia in the preceding 3 months were not included. Obesity (weight > 120% expected), excessive alcohol consumption (> 3 units day^{-1}) and a history of previous liver disease or recent blood transfusion also resulted in exclu-

sion. Patients were excluded from analysis if their duration of anaesthesia exceeded 90 min. Patients were assigned randomly to one of two groups, to receive cimetidine 800 mg orally the evening before operation with a further 800 mg given at the time of premedication, or equivalent placebo tablets.

Premedication comprised oral temazepam 20 mg 1 h before operation. Anaesthesia was induced with thiopentone 4–6 mg kg^{-1} i.v. and was maintained with 66% nitrous oxide and halothane in oxygen. All patients breathed spontaneously via a Lack Mk II system with a fresh gas flow rate of 6 litre min^{-1}. End-tidal concentrations of halothane (Penlon Halothane meter) and carbon dioxide (Hewlett–Packard 47210A capnometer) were recorded every 5 min for the duration of anaesthesia. Patients were withdrawn if the end-tidal carbon dioxide exceeded 8 kPa. The mean of these end-tidal concentrations was determined for each patient and a time-weighted halothane average calculated (mean end-tidal halothane concentration × duration of anaesthesia in minutes).

Blood samples were taken for GST assay before operation and at 3, 6 and 24 h after induction of anaesthesia. Measurements of conventional liver function tests were made on the day before and the day after anaesthesia.

Concentrations of bilirubin, alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) were measured using a Sequential Multiple Analysis with Computer System II (SMAC) (Technicon Instrument Corporation, Basingstoke, U.K.). Plasma basic GST concentration (B_1 subunits) was measured by specific radioimmunoasay [12], with a reference range for GST of 0.7–4.0 μg $litre^{-1}$. The interassay coefficient of variation was less than 10%, and the intra-assay coefficient of variation was less than 5% over the range 0.2–40 μg $litre^{-1}$.

Statistical analysis

The demographic data and anaesthetic characteristics for each group were compared using Student's *t* test. Changes in GST concentrations within each group were compared using the Friedman test. The GST changes from the preoperative values at 3, 6 and 24 h after induction of anaesthesia were compared using the Wilcoxon signed rank test. Differences in GST changes at these times between the two groups and postoperative conventional liver function tests relative to preoperative values were compared using the Wilcoxon rank sum test. Correlation of GST changes with total dose of halothane adminstered, and with mean end-tidal carbon dioxide concentration was with Spearman's rank coefficient [13]. A four-fold table Chi squared test was used to compare the incidence of abnormal GST values in each group.

RESULTS

Fifty-three patients were studied: 26 received placebo (16 male, 10 female) and 27 cimetidine (18 male, 9 female). Eight patients were subsequently excluded from analysis: three were withdrawn because of excessive duration of anaesthesia (> 90 min), three had abnormal preoperative liver function tests, one refused to have further blood samples taken and surgery was cancelled in one. End-tidal carbon dioxide did not exceed 8 kPa in any patient. Data from the remaining 45 patients were analysed: 24 had received placebo (14 male, 10 female) and 21 cimetidine (14 male, 7 female).

The groups were comparable in age, height, weight, % expected weight and alcohol intake (table I). The mean dose of thiopentone and the mean end-tidal concentrations of halothane and carbon dioxide were similar in the two groups. The total dose of halothane administered, reflected in the time-weighted halothane average, appeared greater in the placebo group, despite the random allocation design of the study, but this failed to achieve statistical significance. Twenty-two patients in the placebo group and 17 in the cimetidine group had received previous anaesthesia.

TABLE I. *Demographic data and anaesthetic characteristics for patients receiving placebo and cimetidine (mean values (SD))*

	Placebo	Cimetidine
Age (yr)	33 (12)	32 (13)
Height (cm)	171 (7)	173 (11)
Weight (kg)	68 (11)	70 (11)
% Expected weight	99 (13)	101 (13)
Alcohol intake (u. day^{-1})	1.5 (1.0)	1.5 (1.2)
Thiopentone (mg)	412 (78)	425 (59)
Halothane (%)	1.2 (0.3)	1.0 (0.3)
End-tidal CO_2 (kPa)	5.1 (0.7)	5.2 (0.8)
Duration of anaesthesia (min)	46 (17)	43 (20)
Time-weighted halothane average	53.6 (24.2)	41.1 (20.1)

TABLE II. *Medians (1st, 3rd quartiles) of GST values (μg litre^{-1}) for both groups.* *P < *0.01;* **P < *0.05 compared with before op.*

	Before op.	3 h	6 h	24 h
Placebo	2.7 (2.1, 3.2)	3.1* (2.6, 3.7)	3.0 (2.6, 3.4)	2.5 (1.9, 2.9)
Cimetidine	2.5 (2.0, 3.7)	3.3* (2.6, 4.4)	3.2** (2.6, 4.2)	2.2 (2.0, 3.0)

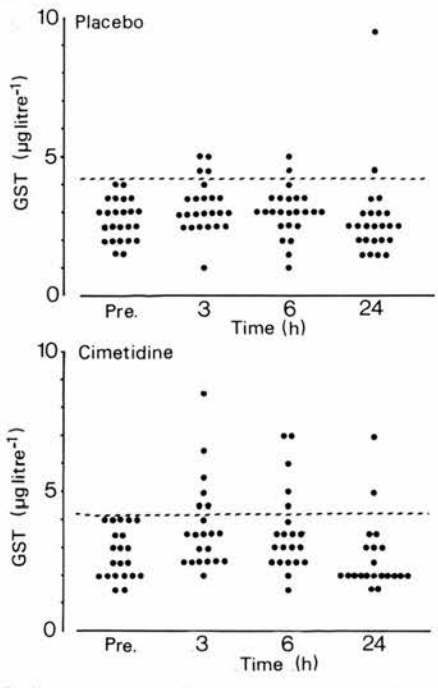

FIG. 1. Individual values for plasma concentration of GST (μg litre^{-1}) at each time of sampling for patients receiving placebo and cimetidine.

Significant changes occurred in postoperative plasma concentrations of GST in both groups ($P < 0.001$ both groups). GST increased significantly 3 h after induction in both groups, and at 6 h in the group receiving cimetidine (table II). Some patients in each group showed an increase in GST concentration which exceeded the upper limit of the reference range (4.0 μg litre^{-1}) (fig. 1). There was no significant difference in the frequency of such abnormal values between the two groups. One patient in the placebo group exhibited a large secondary increase in GST at 24 h, but no patient receiving cimetidine showed a comparable change. Cimetidine did not appear to influence the magnitude of increase in GST at 3 and 6 h. There was no correlation between GST increase and total dose of halothane administered, or mean end-tidal carbon dioxide concentration. Concentrations of bilirubin, ALT and GGT were not significantly different relative to preoperative values in each group (table III).

DISCUSSION

GST has been shown previously to be released following anaesthesia with halothane and enflurane but not isoflurane [14, 15]. The present study has been unable to demonstrate that cimetidine influenced GST release at 3 and 24 h following halothane anaesthesia. Although no patient receiving cimetidine exhibited a large secondary increase in GST concentration at 24 h, it would be necessary to study many more patients to be certain that cimetidine prevented this less frequent increase.

Halothane biotransformation occurs *in vitro* [16] and *in vivo* [17] and this takes place in the

TABLE III. *Median values for pre- and postoperative conventional liver function tests in each group*

	Placebo		Cimetidine		
	Pre.	Post.	Pre.	Post.	Normal range
Bilirubin (μmol litre^{-1})	12	16	14	17	2–17
ALT (u. litre^{-1})	16	15	20	18	10–40
GGT (u. litre^{-1})	16	15	22	20	10–55 (male) 5–35 (female)

microsomal fraction of the liver [3]. The binding of halothane metabolites to rat liver microsomes is enhanced by agents that induce cytochrome P450, such as phenobarbitone [3], and reduced by enzyme inhibitors such as SKF-525-A [18]. In rats exposed to halothane, Jee and colleagues demonstrated increased hepatotoxicity after induction of cytochrome P450 and showed that significant protection was afforded by pretreatment with enzyme inhibitors [19]. More recently, cimetidine has been shown to offer protection against halothane hepatotoxicity in rats [7,8], although the reports differ on the identity of the pathway of metabolism inhibited. In hypoxic rats, Plummer and others [7] suggested that cimetidine reduced reductive metabolism. In contrast, Wood and co-workers [8] showed that cimetidine reduced oxidative metabolism of halothane in normal untreated rats, but failed to demonstrate that cimetidine reduced metabolism by these two pathways in the hypoxia/enzyme-induced or triiodothyronine models.

Possible reasons for the differences in the observations of the influences of cimetidine on metabolism in animals and the negative result of this study in man include other potential effects of cimetidine such as those on hepatic blood flow, and perhaps inadequate dosage of cimetidine.

Original evidence suggested that cimetidine reduced hepatic blood flow. Such a reduction in flow and resultant ischaemia could contribute to the release of GST. Feely and colleagues [20] used clearance of indocyanine green as a measure of hepatic blood flow, to demonstrate a reduction after administration of cimetidine. The limitations of this technique have been stressed by Groszmann, who suggested that the extraction ratio should be measured when utilizing clearance techniques [21]. This was not performed in Feely's study. Further investigation has failed to demonstrate a reduction in liver blood flow by cimetidine using various techniques, such as clearance of galactose [22] and more direct methods including the use of an electromagnetic blood flow meter [23], an ultrasound Doppler system or cineangiography [24]. Thus there is no convincing evidence that cimetidine has any effect on hepatic blood flow. If the increase in GST concentration at 3 h results from changes in liver blood flow caused by halothane, data from the present study suggest that cimetidine causes no additional alteration in hepatic perfusion.

The doses of cimetidine given in the rat studies were enormous (120 and 180 mg kg^{-1}) and it is obviously not possible to administer such large doses to man. Most human studies which have shown that cimetidine impairs metabolism of other drugs have involved giving a larger total dose of cimetidine than that given in this study. This usually involved pretreatment with cimetidine for 2–14 days [25–27]. Klotz and co-workers have shown that a single oral dose of cimetidine 800 mg is sufficient to interfere with the pharmacokinetics of midazolam administered concurrently [28]. This single dose also achieved plasma concentrations of cimetidine within the therapeutic range as evaluated by Somogyi [29]. However, when cimetidine was given in a total dose of 600 mg to patients undergoing enflurane anaesthesia, the metabolism of enflurane was not altered significantly as assessed by serum fluoride concentrations [30].

If it can be assumed that the dose of cimetidine used was sufficient to reduce halothane metabolism, our data suggest that products of halothane metabolism are not responsible for the early release of GST described previously [11, 14, 15]. Several other theories should be investigated before the true nature of liver dysfunction associated with halothane (as indicated by GST release) is fully understood.

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REFERENCES

1. Cousins MJ, Gourlay GK, Knights KM, Hall P de la M, Lunam CA, O'Brien P. A randomised prospective controlled study of the metabolism and hepatotoxicity of halothane in humans. *Anesthesia and Analgesia* 1987; **66**: 299–308.
2. Widger LA, Gandolfi AJ, Van Dyke RA. Hypoxia and halothane metabolism *in vivo*: Release of inorganic fluoride and halothane metabolite binding to cellular constituents. *Anesthesiology* 1976; **44**: 197–201.
3. Van Dyke RA. Biotransformation of volatile anaesthetics with special emphasis on the role of metabolism in the toxicity of anaesthetics. *Canadian Anaesthetists Society Journal* 1973; **20**: 21–33.
4. Desmond PV, Patwardhan R, Parker R, Schenker S, Speeg KV. Effect of cimetidine and other antihistaminics on the elimination of aminopyrine, phenacetin and caffeine. *Life Sciences* 1980; **26**: 1261–1268.

5. Bauman JH, Kimelblatt BJ. Cimetidine as an inhibitor of drug metabolism: Therapeutic implications and review of the literature. *Drug Intelligence and Clinical Pharmacy* 1982; **16**: 380–386.
6. Feely J, Wilkinson GR, McAllister CB, Wood AJJ. Increased toxicity and reduced clearance of lidocaine by cimetidine. *Annals of Internal Medicine* 1982; **96**: 592–594.
7. Plummer JL, Wanwimolruk S, Jenner MA, Hall P de la M, Cousins MJ. Effects of cimetidine and ranitidine on halothane metabolism and hepatotoxicity in an animal model. *Drug Metabolism and Disposition* 1984; **12**: 106–110.
8. Wood M, Uetrecht J, Phythyon JM, Shay S, Sweetman BJ, Shaheen O, Wood AJJ. The effect of cimetidine on anesthetic metabolism and toxicity. *Anesthesia and Analgesia* 1986; **65**: 481–488.
9. Beckett GJ, Chapman BJ, Dyson EH, Hayes JD. Plasma glutathione S-transferase measurements following paracetamol overdose: Evidence for early hepatocellular damage. *Gut* 1985; **26**: 26–31.
10. Sherman M, Bass NM, Campbell JAH, Kirsch RE. Radioimmunoassay of human ligandin. *Hepatology* 1983; **3**: 162–169.
11. Hussey AJ, Howie J, Allan LG, Drummond G, Hayes JD, Beckett GJ. Impaired hepatocellular integrity during general anaesthesia, as assessed by measurements of plasma glutathione S-transferase. *Clinical Chimica Acta* 1986; **161**: 19–28.
12. Beckett GJ, Hayes JD. Development of specific radioimmunoassays for the measurement of human hepatic basic and N/A2b glutathione S-transferase. *Clinica Chimica Acta* 1984; **141**: 267–273.
13. Lentner C. *Geigy Scientific Tables*, Vol. 2, 8th Edn. Basle, Switzerland: Ciba-Geigy, 1982.
14. Allan LG, Hussey AJ, Howie J, Beckett GJ, Smith AF, Hayes JD, Drummond GB. Hepatic glutathione S-transferase release after halothane anaesthesia: Open, randomised comparison with isoflurane. *Lancet* 1987; **1**: 771–773.
15. Hussey AJ, Aldridge LM, Paul D, Ray DC, Beckett GJ, Allan LG. Plasma glutathione S-transferase concentration as a measure of hepatocellular integrity following a single general anaesthetic with halothane, enflurane or isoflurane. *British Journal of Anaesthesia* 1988; **60**: 130–135.
16. Van Dyke RA, Chenoweth MB. The metabolism of volatile anesthetics—II. *In vitro* metabolism of methoxyflurane and halothane in rat liver slices and cell fractions. *Biochemical Pharmacology* 1965; **14**: 603–609.
17. Van Dyke RA, Chenoweth MB, Van Poznak A. Metabolism of volatile anesthetics—I. Conversion *in vivo* of several anesthetics to $14CO_2$ and chloride. *Biochemical Pharmacology* **1964; 13**: 1239–1247.
18. Van Dyke RA, Wood CL. Binding of radioactivity from ^{14}C labeled halothane in isolated perfused rat livers. *Anesthesiology* **1973**; **38**: 328–332.
19. Jee RC, Sipes IG, Gandolfi, AJ, Brown BR. Factors influencing halothane hepatotoxicity in the rat hypoxic model. *Toxicology and Applied Pharmacology* 1980; **52**: 267–277.
20. Feely J, Wilkinson GR, Wood AJJ. Reduction of liver blood flow and propranolol metabolism by cimetidine. *New England Journal of Medicine* 1981; **304**: 692–695.
21. Groszmann RJ. The measurement of liver blood flow using clearance techniques. *Hepatology* 1983; **3**: 1039–1040.
22. Henderson JM, Ibrahim SZ, Millikan WJ, Santi M, Warren WD. Cimetidine does not reduce liver blood flow in cirrhosis. *Hepatology* 1983; **3**: 919–922.
23. Tyden G, Thulin L, Nyberg B. The effect of cimetidine on liver blood flow in anesthetized man. *Acta Chirurgica Scandinavica* 1983; **149**: 303–305.
24. Onishi K, Nakayama T, Saito M, Hatano H, Nomura F, Iida S, Okuda K. Effect of cimetidine and ranitidine on splanchnic hemodynamics in patients with chronic liver disease. *American Journal of Gastroenterology* 1985; **80**: 290–293.
25. Jackson JE, Powell JR, Wandell M, Bentley J, Dorr R. Cimetidine decreases theophylline clearance. *American Review of Respiratory Disease* 1981; **123**: 615–617.
26. Desmond PV, Patwardhan RV, Schenker S, Speeg KV. Cimetidine impairs elimination of chlordiazepoxide (Librium) in man. *Annals of Internal Medicine* 1980; **93**: 266–268.
27. Heagerty AM, Donovan MA, Castleden CM, Pohl JF, Patel L. Influence of cimetidine on pharmacokinetics of propranolol. *British Medical Journal* 1981; **1**: 1917–1919.
28. Klotz U, Arvela P, Rosenkranz B. Effect of single doses of cimetidine and ranitidine on the steady-state plasma levels of midazolam. *Clinical Pharmacology and Therapeutics* 1985; **38**: 652–655.
29. Somogyi A, Gugler R. Clinical pharmacokinetics of cimetidine. *Clinical Pharmacokinetics* 1983; **8**: 463–495.
30. Yeager MP, Coombs DW, Dodge CP, Maloney LL. Effect of cimetidine on biotransformation of enflurane in man. *Canadian Anaesthetists Society Journal* 1986; **33**: 466–470.