THE ASSESSMENT OF LIVER DISEASE BY THE CLEARANCE OF TIN COLLOID AND
OTHER CLEARANCE STUDIES

Eugene Philip PERRY

M B Ch B, F R C S Ed.

Submitted for the degree of Doctor of Medicine,
University of Leicester
1998

This work was carried out in the Departments of Surgery and Medical Physics, Freeman Hospital,
Newcastle-Upon-Tyne and the Departments of Surgery and Medical Physics, Walsgrave Hospital,
Coventry.
Acknowledgments

My prime acknowledgment is to the patients who gave their time and consent for experimental studies and without them nothing would have been possible.

A big thank you goes to both Mr Peter Wright and Mr Ian Fraser for their friendship and advice throughout these studies. I was fortunate to have the guidance of Mr. Peter Wright when this study was started in Newcastle-Upon-Tyne and equally fortunate to receive encouragement from Mr Ian Fraser to complete the work at Coventry.

The Staff of two Medical Physics departments greatly assisted in the testing of the patients and Mr Terry Hawkins, Mrs. Pauline Keevey, Mr. David Taylor and Mr. John Barham deserve special thanks along with their admirably skilled radiographers.

I acknowledge the following invaluable assistance:

Dr. Elwyn Elias, Dr. Chris Vickers and Dr Steve Smith with caffeine assays and metabolisms.

Dr. Paul Emery, Dr John Wyke and Dr Mike Robinson with fibronectin assays and metabolisms.

Dr. Jan Freeman with indocyanine green assays

Dr. Paul Davies on medical statistics

Mrs. Julia Haddington for typing

Similarly none of the work in this thesis would have been performed without the support and cooperation of the nursing staff and junior medical staff in the two hospitals.

Finally I thank my wife, Kath, and my 3 sons, James, Matthew and Jack who endured many evenings of solitude and still encouraged me throughout the writing of this thesis.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALD</td>
<td>Alcoholic Liver Disease</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine aminotransferase</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate aminotransferase</td>
</tr>
<tr>
<td>AP</td>
<td>Alkaline Phosphatase</td>
</tr>
<tr>
<td>Disida</td>
<td>Disofenin</td>
</tr>
<tr>
<td>FSC</td>
<td>Fasting Serum Caffeine</td>
</tr>
<tr>
<td>ICG</td>
<td>Indocyanine Green</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>IS</td>
<td>Injection Sclerotherapy</td>
</tr>
<tr>
<td>ISDN</td>
<td>Isosorbide Dinitrate</td>
</tr>
<tr>
<td>Kicg</td>
<td>Blood Clearance of Indocyanine Green</td>
</tr>
<tr>
<td>K1u</td>
<td>Liver Uptake Constant of Tin Colloid</td>
</tr>
<tr>
<td>Ktc</td>
<td>Blood Clearance of Tin Colloid</td>
</tr>
<tr>
<td>K1d</td>
<td>Liver Uptake Constant of Disida</td>
</tr>
<tr>
<td>K2d</td>
<td>Liver Elimination Constant of Disida</td>
</tr>
<tr>
<td>K1c</td>
<td>Initial Rate Constant of Caffeine Clearance</td>
</tr>
<tr>
<td>K2c</td>
<td>Second, metabolic rate constant of caffeine clearance</td>
</tr>
<tr>
<td>LFT</td>
<td>Liver function test</td>
</tr>
<tr>
<td>NS</td>
<td>No statistical significance</td>
</tr>
<tr>
<td>RES</td>
<td>Reticuloendothelial system</td>
</tr>
<tr>
<td>U &amp; E's</td>
<td>Serum urea and electrolyes</td>
</tr>
<tr>
<td>Chapter</td>
<td>Title</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>Chapter 1</td>
<td>Aims of Study</td>
</tr>
<tr>
<td>Chapter 2</td>
<td>Introduction</td>
</tr>
<tr>
<td>Chapter 3</td>
<td>Clinical Studies 1</td>
</tr>
<tr>
<td>Chapter 4</td>
<td>Clinical Studies 2</td>
</tr>
<tr>
<td>Chapter 5</td>
<td>Clinical Studies 3(a)</td>
</tr>
<tr>
<td>Chapter 6</td>
<td>Clinical Studies 3(b)</td>
</tr>
<tr>
<td>Chapter 7</td>
<td>Conclusions</td>
</tr>
<tr>
<td>Appendices</td>
<td></td>
</tr>
<tr>
<td>References</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 1

AIMS OF STUDY
AIM OF STUDY

Over the past fifty years we have learnt a great deal about the pathogenesis and pathophysiology of hepatic disorders but it is disconcerting that the prognosis of patients with cirrhosis has changed little. Such patients are routinely assessed by tests collectively referred to as 'liver function tests', the name given to a group of static biochemical tests used in assessment of liver disease even though none of the tests directly measures liver function.

Compared to the clinical routine of quantitatively measuring functional impairment in renal or pulmonary disease, the estimation of severity of liver disease, except in the case of decompensation, remains largely guesswork. It would be helpful to have quantitative measures of liver function which could enable selection of patients for certain treatments and measure clinical changes. A number of procedures have been designed to obtain quantitative information on specific hepatic functions based on the clearance principle. In general they are more complex, take longer to perform and are more expensive.

The work described in this thesis assesses the place of the clearance of radiolabelled tin colloid both individually and in combination with other hepatic clearances in the diagnosis of liver disease and as a measure of liver function. Initially simple nuclear medicine bedside tests were used before proceeding to more sophisticated tests which required specialist nuclear medicine equipment. The results are presented chronologically as the study developed.
The first part of the study investigated the use of radiolabelled tin colloid to measure colloidal clearance which has previously been described using sulphur colloid and to distinguish between controls and patients with liver disease.

Conventional serum biochemical liver function tests do not show abnormalities characteristic of cirrhosis and may be normal inspite of significant disease. In the second part of this section the clearance of tin colloid, clearance of indocyanine green and biochemical liver function tests have been used both individually and in combination to assess liver function and prognosis in a heterogenous group of patients with liver disease.

Biochemical liver function tests usually confirm that the liver is abnormal and can be used to follow the progress of the disease and give some idea of prognosis. A better general prognostic guide in cirrhotic liver disease is given by Child’s classification which grades severity by taking into account jaundice, ascites, encephalopathy, serum albumin concentration and nutrition. More recently this has been modified by Pugh to include the prothrombin time referred to in this work as Child-Pugh grading.

In the third part of the study tin colloid has been used individually and in combination with indocyanine green, caffeine and disida in clearance studies to assess the severity of disease in alcoholic cirrhotic patients and compared with Child-Pugh clinical grading. The use of the clearance of Disida has not been reported previously in this clinical condition and the first, fast component of the clearance of caffeine has not previously been described.
The patients in this part of the study were randomised to treatment of their oesophageal varices by injection sclerotherapy with or without long term Isosorbide Dinitrate therapy. By comparing their liver function tests the effects of Isosorbide Dinitrate on liver function has been assessed,

Central to the assessment of severity of liver disease the clinical outcome of the patients in terms of survival over a follow-up of at least 12 months was carefully monitored.

STUDY DECLARATION

The thesis is divided into three sections as outlined below:-

(1) **Clearance of Radiolabelled Tin Colloid**

The principle clearance of tin colloid is characterised including methods, results and conclusions and presented in Chapter 3. All measurements were performed by myself; the radiopharmaceutical was prepared by Mr. T. Hawkins of the Medical Physics Department.

(2) **Clinical Studies of Tin Colloid and Indocyanine Green**

The results of comparing the clearance of tin colloid and indocyanine green are given in Chapter 4. This section includes a two year follow up on the patients. All measurements were performed by myself and the radiopharmaceutical prepared by Mr. T. Hawkins of the Medical Physics Department.
Clinical Studies of the Clearance of Tin Colloid, Disida, Indocyanine Green and Caffeine

The results of combinations of clearance tests of tin colloid, disida, indocyanine green and caffeine are presented. This section includes the findings of the affects of Isosorbide Dinitrate on these clearance tests in patients with liver disease. All measurements were performed by myself and the radiopharmaceuticals were prepared by Mr. J. Barham of the Medical Physics Department.
CHAPTER 2

INTRODUCTION

The history and use of both biochemical and clearance tests of liver function are reviewed before the newer substances used in the clearance tests of this study are introduced.
<table>
<thead>
<tr>
<th>Contents</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1. <strong>Historical Background</strong></td>
<td>8</td>
</tr>
<tr>
<td>2.2 <strong>Liver Structural</strong></td>
<td>11</td>
</tr>
<tr>
<td>a - Hepatocytes</td>
<td></td>
</tr>
<tr>
<td>b - Kupffer Cells</td>
<td></td>
</tr>
<tr>
<td>2.3 <strong>Liver - Disease</strong></td>
<td>15</td>
</tr>
<tr>
<td>a - Alcoholic Liver Disease</td>
<td></td>
</tr>
<tr>
<td>b - Cirrhotic Liver Disease</td>
<td></td>
</tr>
<tr>
<td>c - Metastatic Liver Disease</td>
<td></td>
</tr>
<tr>
<td>2.4 <strong>Assessment of Liver Disease</strong></td>
<td>18</td>
</tr>
<tr>
<td>2.5 <strong>Current Clinical Measurement of Liver Function</strong></td>
<td>20</td>
</tr>
<tr>
<td>2.6 <strong>Use of Liver Function Tests</strong></td>
<td>21</td>
</tr>
<tr>
<td>a - tests that detect injury to hepatocytes</td>
<td></td>
</tr>
<tr>
<td>(i) Enzymes that detect hepatocellular necrosis</td>
<td></td>
</tr>
<tr>
<td>(ii) Enzymes that detect cholestasis</td>
<td></td>
</tr>
<tr>
<td>b - Tests of the Liver’s biosynthetic capacity</td>
<td></td>
</tr>
<tr>
<td>c - Tests that detect chronic inflammation or altered immunoregulation</td>
<td></td>
</tr>
<tr>
<td>d - Tests of the capacity of the liver to transport organic anions and metabolise drugs</td>
<td></td>
</tr>
<tr>
<td>2.7 <strong>Clearance Tests of Liver Function</strong></td>
<td>31</td>
</tr>
<tr>
<td>2.8 <strong>Tests of Liver Function Used in this Study</strong></td>
<td>38</td>
</tr>
<tr>
<td>a - Tin colloid</td>
<td></td>
</tr>
<tr>
<td>b - Indocyanine Green</td>
<td></td>
</tr>
<tr>
<td>c - Disida</td>
<td></td>
</tr>
<tr>
<td>d - Caffeine</td>
<td></td>
</tr>
</tbody>
</table>
2.1 HISTORICAL BACKGROUND

The liver holds a central position in the functions of the individual human body but before these functions were as accurately delineated as they are in the twentieth century the liver was sometimes used to control the functions of many human beings. In the first and second millennium B.C. the Babylonians and Assyrians attached great importance to "inspecting the liver" before important decisions were undertaken. This external inspection of mostly bull and sheeps livers was undertaken by Priests who from the characteristic of the liver could determine which decision was to be made and was continued by both Greek and Roman statesmen and generals despite the development of schools of hepatology in both empires.

Of the Greeks, Hippocrates (460-370 B.C) in the fourth and fifth centuries B.C. recognised infectious jaundice, ascites caused by liver disease and liver cysts and abscesses. He also wrote about the symptoms associated with liver disease. Those who are mad on account of phlegm are quiet, but those on account of bile are vociferous, vicious and do not keep quiet. The most renowned Roman physician was Celcius (30 A.D) who as a practical hepatologist even undertook operations on the liver.

Roman hepatology reaches its peak with Galen (120 - 200 A.D). Galen experimented on animals and distinguished obstructive icterus, concomitant icterus and haemolytic icterus. His anatomical, physiological and pathological conceptions continued to be accepted without criticism until the fifteenth century.

The enlightenment from Galen's views were illuminated by the distinguished works of Leonard da Vinci (1452-1519) who studied the anatomy of the human liver and
described various disease states including cirrhosis. Andreas Vesalius (1514 - 1564), another distinguished anatomist, recognised the causal relationship of excessive alcohol consumption to cirrhosis of the liver.

The functional capacity of the liver parenchyma was described by Glisson (1597-1677) who described the capsule and named the hepatic lobules. Marcello Malpighi (1628-1694) deduced that bile was manufactured by the liver and not by the gallbladder.

In the eighteenth century Morgagni observed patients with cirrhosis and described an initial phase in which patients became stupid and forgetful with the later development of violent delirium leading to terminal coma.

After 1848 hepatology made great strides when Claude Bernard (1813-1878) reported the discovery of glycogen and that the liver produces sugar and Carl von Liebermaster (1892) described the division of cirrhosis into 2 forms, portal and biliary. The course of cirrhotic liver disease was documented by Copland (1858) who ascribed the coma in cirrhotics to alcohol whilst Budd (1845) considered that in cirrhotics ‘the intellect and senses were free from disorder to the last’. The poor prognosis of cirrhotic liver disease was recognised by Frerichs (1860) in a study of 36 fatal cases.

Not all jaundiced patients had dark urine and in 1866 van Leyden suggested that urine could be classified as acholuric or obstructive. Therefore the jaundice associated with bile duct obstruction was accounted for by the regurgitation of bile, but there was no good explanation for the jaundice of patients with liver disease in the absence of biliary obstruction.
Despite treatments that included venesection, application of leeches over the liver or at the anus, injection of whole liver extracts and assorted mixtures, all hepatologists recognised that alcohol abuse causes chronic liver disease and so the fundamental treatment of liver disorders was alcohol restriction.

In the twentieth century the early discoveries of the reticuloendothelial system by Aschoff\(^1\) in 1922 and the serum bilirubin test of van den Bergh\(^2\) laid the foundation for the more recent rapid strides. As knowledge of hepatic physiology increased the serum bilirubin estimation was followed by a whole series of useful liver function tests.

Ribbert\(^3\) (1904) demonstrated the selective storage of intravenously injected particles in certain cells of the liver, spleen and bone marrow. Nagoa\(^4\) emphasised the localisation of the intravenously administered Indian ink in phagocytic cells lining blood sinuses of the liver, spleen and bone marrow. In 1924 Aschoff\(^1\) introduced the term 'reticulo-endothelial system' to describe the monocuclear macrophages which had the property of ingesting and accumulating foreign colloidal and particulate matter.

The major advance observed by van den Bergh and Muller\(^2\) was that some bilirubin in sera reacted directly with Erlich's diazo reagent whereas some required alcohol for the development of colour (indirect bilirubin). The direct reacting bilirubin was found regularly with mechanical obstruction of the bile ducts but not with haemolytic jaundice. The van den Bergh test was qualitative although it was claimed initially that variations in the observed colour allowed distinction between obstructive jaundice and that due to catarrhal or toxic jaundice.
The biochemical basis of the van den Bergh test was still not clear and until 1954 it was generally assumed that it depended on the proportion of bilirubin bound to serum protein. In 1954 Cole identified bilirubin as the indirect reacting material and Billing and Lathe showed that direct-reacting bilirubin was a mixture of bilirubin mono and di-glucuronides.

Roberts studied serum alkaline phosphatase activity in patients with a variety of conditions. He studied 52 jaundiced patients and found high levels in toxic, infective and catarrhal jaundice. The increase was attributed to the regurgitation of bile phosphatase. In 1934 King and Armstrong described their method for the measurement of serum alkaline phosphatase and this was widely used.

There was a major advance in the clinical biochemistry of liver disease when de Ritis found marked elevation of serum glutamic-oxaloacetic transaminases (SGOT or AST) in patients with viral hepatitis. In the same year Wroblewski and LaDue reported a high level of SGOT in various hepatic disorders and considered it to be an index of liver cell damage since it is rarely difficult to decide that an elevation of AST is due to hepatic change. The year 1955 marked the birth date of modern clinical enzymology.

2.2. LIVER (STRUCTURAL)

The liver is the largest organ in the body weighing 1200 to 1500 gms and comprises one fiftieth of the total body weight. The main structural unit of the liver is the acinus (or lobule). The simple liver acinus is defined as a microscopic parenchymal mass, irregular in shape and size, arranged round an axis consisting of the terminal hepatic
arteriole, portal venule and bile ductule, lymph vessels and nerves which grow out together from similar pre-terminal structures in a small triangular portal space. The acinus lies between 2 or more terminal hepatic venules (the central veins) from which the acinar vascular and biliary axis interdigitates.

The liver is an exceptionally complex and diverse organ that functions as an exocrine gland. It secretes bile, which contains many constituents in addition to bile salts. It synthesises and releases many substances including prohormones, albumin, clotting factors, glucose, fatty acids and various lipoproteins. It has a dual blood supply providing a rich mixture of nutrients and other absorbed substances via the portal vein and oxygen rich blood via the hepatic artery.

Since the liver receives blood from the intestinal tract and also produces bile the organisation of each cell must meet each of these functions. This functional heterogeneity is accompanied by cellular heterogeneity with the liver containing many cells types including hepatic parenchymal cells (hepatocytes), Kupffer cells, Ito cells and endothelial cells. The most abundant cell type, the hepatocytes, are biochemically and structurally heterogenous.

The main groups of cells in the liver are the hepatocytes which comprise 80% of the cells by volume, the reticulo-endothelial cells (Kupffer cells) which comprises 5% of the cells by volume, the endothelial cells and the fat storing cells or Ito cells.
2.2a. **Hepatocytes**

The special ultrastructure of the liver with efficient transport systems allows for the removal and excretion of many compounds that cannot be excreted by the kidney because of extensive protein binding.

The first biological barrier for substances taken up by the liver is the cell membrane of the hepatocytes. The uptake of materials has the characteristics of a membrane carrier transport process which includes saturation kinetics, competitive inhibition and isotope counter transport.

After uptake by the hepatocyte substances may remain unchanged or undergo biotransformation or biosynthesis, intracellular storage or transport and finally excretion into blood, lymph or bile.

Biotransformation of drugs by the liver has been described as biphasic. The first phase consists of oxidative, reductive or hydrolytic alterations of the drug molecule: the second phase consists of conjugation of the altered molecule with one or more organic acids such as glucuronidation.

Transport of compounds from the hepatocyte to the bile depends greatly on their structure and a molecular weight greater than 850, the presence of a strongly polar group and lipid solubility enhances biliary excretion\textsuperscript{13}.
2.2b. **Kupffer Cells**

About 5% of the cells of the liver are fixed macrophages or Kupffer cells. They are stellate cells with a fuzzy coat and numerous irregular pseudopodia that hang in the flowing blood of the liver sinusoids with some of their processes inserted between the endothelial cells. Lying as they do within the sinusoids, adjacent to the fenestrated endothelium that gives access to the hepatocytes. Kupffer cells are clearly in a strategic position to clear the bloodstream of unwanted material.

Phagocytosis is the most important physiological feature of Kupffer cells. Kupffer cell phagocytosis is impaired by hypoglycaemia\(^1\), hypothyroidism\(^2\), general anaesthesia\(^3\), alcohol\(^4\) and infusion of artificial plasma expanders\(^5\). Characterisation of phagocytic activity is by the measurement of colloidal clearance.

Kupffer cells are known to behave as typical macrophages in that they become activated to enhance their defensive role. The glycoprotein, fibonection, is known to play an important part as an opsonin in this activation\(^6\). The importance of opsonins for RES clearance is shown by the increased susceptibility to infection and poor RES clearances after extensive surgery or major trauma\(^7\), after severe burns\(^8\), in the course of septicaemia\(^9\), after high dose corticosteroid therapy\(^10\) after protein starvation\(^11\) and cytotoxic therapy\(^12\).

The importance of the Kupffer cell system is evident whenever its function is depressed and excess endotoxins or immune complexes appear in the circulation. This results in disseminated intravascular coagulopathy (DIC), respiratory insufficiency (shock
lungs) and renal tubular necrosis (shock kidneys). In baboons, RES blockade with hepatic ischaemia results in 100% mortality.

Moreover the action of Kupffer cells is not independent of hepatocyte function. They both produce interferon to combat viral infections and by the formation of glycoprotein, lymphokines and growth factor, Kupffer cells can direct the production of proteins by hepatocytes and induce hepatocytes to proliferate. Many substances that are ingested by Kupffer cells are partially degraded by them and the products passed on to hepatocytes.

In animal models of hepatitis, Kupffer cells are involved before the hepatocytes can be invaded and it is likely that Kupffer cells protect hepatocytes and that hepatitis is a reflection of the inadequacy of the RES defences. Acute alcoholic intoxication also depresses Kupffer cell phagocytosis and endotoxin, that enters the portal vein, will be able to damage hepatocytes and by by-passing the paralysed Kupffer cell is able to inflict its adjuvant and mitogenic potential on the immune system.

2.3. LIVER DISEASE

The liver subserves a very large number of important physiological functions and accordingly is liable to damage or injury from a wide variety of causes. The liver cell is especially liable to injury because of its function of taking up and dealing with many metabolites, toxic substances, drugs and poisons. Biliary tract obstruction if sufficiently prolonged results in secondary biliary cirrhosis and, although primary liver tumours are uncommon in this country, the liver is a common site of metastatic carcinoma particularly from tumours of the gastrointestinal tract.
Disturbances of function resulting from lesions of the liver and biliary tract are varied and complex in their effects. They are often considered under three broad headings: hepatocellular failure, portal hypertension and biliary obstruction. Hepatocellular failure arises when total liver cell function falls below the minimum required to maintain a physiological state.

In the main section of this study patients with alcoholic cirrhotic liver disease have been investigated and in the earlier studies patients with metastatic liver disease and a miscellaneous group which were mainly cirrhotic liver disease of other aetiology were also investigated.

2.3a. Alcoholic Liver Disease

Alcohol is a source of pleasure, profit, employment and tax revenue on a large scale but problems with disease from alcohol intake are increasing. The association of alcohol with cirrhosis of the liver is well recognised and the quantity of alcohol consumed correlates with the mortality of the condition. In France the mortality from cirrhosis was reduced by 60% after 1 year of wine rationing.

However, not all those who abuse alcohol develop liver damage and the incidence of cirrhosis amongst alcoholics at autopsy is about 10%. The prognosis of patients with alcoholic liver disease is generally considered to be associated with the presence or absence of cirrhosis. Patients with alcoholic cirrhosis present a clinical spectrum that extends from patients who are completely asymptomatic to those who present with severe
liver failure\textsuperscript{36,37,38,39}. This wide spectrum reflects the effect on the functional mass of the liver.

Alcohol cannot be stored in the body and obligatory oxidation takes place predominantly in the liver. The healthy individual cannot metabolise more than 160 to 180 grams per day. Alcohol induces enzymes used in oxidation and the alcoholic, at least while the liver is relatively unaffected, may be able to metabolise more.

The changes in the liver brought about by high alcohol consumption include fatty liver, alcoholic hepatitis, hepatic fibrosis and cirrhosis. The histology of alcoholic cirrhosis is of a micronodular type and has no formal architecture.

In most patients with alcoholic liver disease abstention from alcohol results in improving liver function\textsuperscript{40} but some patients develop progressive hepatic impairment despite abstaining\textsuperscript{41}. The explanation of the apparent predisposition of certain people to develop alcoholic cirrhosis is unknown. In the United Kingdom alcohol is the most important cause of cirrhosis.

2.3b Cirrhotic Liver Disease

Cirrhosis is a condition in which the hepatic parenchyma is changed into a large number of nodules separated from one another by irregular branching and anastamosing sheets of fibrous tissue\textsuperscript{42}. It results from long continued loss of liver cells, with a persistent inflammatory reaction accompanied by fibrosis and compensatory hyperplasia. The condition is irreversible and the fibrosis and distortion of lobular architecture interfere with the flow of blood through the liver. Death usually results from heptocellular failure,
portal hypertension or a combination or both. Cirrhosis can be classified on an aetiological basis or on the morphological appearances of the liver.\textsuperscript{43}

2.3c **Metastatic Liver Disease**

The liver is a very common site of secondary carcinomas of all kinds, notably from the gastrointestinal tract, lung, melanoma and breast. Secondary carcinoma may develop as one or two main masses but often the whole organ is permeated by tumour nodules. The liver becomes enlarged and intrahepatic cholestasis and jaundice may develop from pressure on bile radicles.\textsuperscript{44} As the liver has the highest incidence of secondary cancer of any organ in the body, it might imply a contribution of Kupffer cells to the regulation and control of malignant cell populations.

2.4. **THE ASSESSMENT OF LIVER DISEASE**

The ideal liver function test must be safe, easy to perform (both for the patient and the doctor) and easy to analyse. The test substance must have minimal interactions with concomitant medications and environmental factors (such as nutritional status) and its kinetics must be simple. For widespread use it must provide diagnostic information, be cheap and quick results should be obtainable.\textsuperscript{45}

Compared with the clinical routine of quantitatively measuring functional impairment in renal or pulmonary disease, estimation or severity of liver disease except in the case of decompensation remains largely guesswork.\textsuperscript{46} The presence of liver disease is established on clinical and/or biochemical grounds. A precise diagnosis to establish the nature and cause of the underlying condition is necessary to allow correct treatment and to assess prognosis.
The ‘gold standard’ investigation for diagnosis is liver biopsy. This is an invasive procedure which carries a small but definite risk of serious complications. Whilst histology is often important in establishing a diagnosis, there are problems in using morphological criteria to assess severity and prognosis in liver disease. Firstly it requires a patient population in which biopsies can be performed safely and therefore it selects the patients. If the scoring of severity is required at frequent intervals than it becomes ethically impossible to carry out. In addition the possibility of sampling error must be considered.

Other investigations which have similar problems are percutaneous or endoscopic cholangiography. It is hardly surprising that hepatologists have sought diagnostic assistance by other simpler and less hazardous methods.

One major problem is to define an absolute yardstick of liver function. The functions of the liver are numerous and no particular current single test has been shown to be better than any other. In cirrhotic liver disease the clinical grading of Child which depends on jaundice, ascites, encephalopathy, serum albumin concentration and nutrition and the modification of Pugh, adding the prothrombin time, have a good correlation with short term clinical outcome as measured by death or the complications of liver disease such as bleeding from oesophageal varices and encephalopathy. This classification has problems including inter observer interpretation of the variables.
Significant indicators of death risk have been advanced age, male sex, encephalopathy, haemorrhage, varices, prothrombin time, hepatitis B antigen positive and hepatocellular carcinoma. Using Cox's regression model, the following variables had a significant prognostic effect: sex, age, prothrombin time, acetylcholinesterase, eosinophil leucocytes in liver parenchyma, liver cell necrosis, inflammation in liver connective tissue and efferent veins in parenchymal nodules.

All of these multivariate analyses are complex and unlikely to find routine clinical use. Within this study the clearance of both older and newer substances has been used to assess liver function. I have chosen to use death as my yardstick to measure the usefulness of these liver function tests as death is a definitive endpoint.

2.5. CURRENT CLINICAL MEASUREMENTS OF LIVER FUNCTION

The measurement of liver function is conventionally by a group of tests known as 'liver function tests'. The term liver function tests is usually given to a group of static biochemical investigations used in the management of liver disease. Many tests have been proposed but each clinical laboratory selects its own battery of tests. The combination which is used in our laboratories is the measurement of serum bilirubin, aspartate aminotransaminase and alkaline phosphatase. These tests are simple to perform and relatively inexpensive.

Despite the name, liver function tests are not useful for assessing liver function because they lack sensitivity and precision. Once in serum, enzymes behave like...
other serum proteins in that they are distributed in plasma and interstitial fluids and have characteristic half-lives of disappearance, usually measured in days. This means that a single point estimation of these enzymes does not reflect what is happening in the liver at that time but may represent liver cell damage incurred days previously. They can reflect both Kupffer as well as hepatocyte function in that the aminotransaminases may be cleared by the RES.

Conventional liver function tests are of value when used to screen for liver disease, to confirm its presence, to estimate severity, assess prognosis and evaluate therapy. They will usually differentiate between hepatocellular and cholestatic liver disease. However, their use is gradually declining due to more specific methods such as viral hepatitis markers, immunological tests for primary biliary cirrhosis, modern imaging techniques and clearance studies$^{45,56,57}$.

2.6. **USE OF CONVENTIONAL LIVER FUNCTION TESTS**

2.6a **Tests that Detect Injury to Hepatocytes**

The liver contains thousands of enzymes, some of which are also present in serum in very low concentrations. These enzymes have no known function in serum and behave like other serum proteins. The elevation of a given enzyme activity in serum is thought primarily to reflect its increased rate of entrance into serum from damaged liver cells; serum enzymes can be grouped into two categories: (i) enzymes whose elevation reflects general damage to hepatocytes and (ii) enzymes whose elevation reflects cholestasis.
2.6(i) **Enzymes that Detect Hepatocellular Necrosis**

**Aminotransferases** (AST, ALT)

The greatest diagnostic value of AST measurements is in acute hepatitis due to either viral infection or drugs when the serum level may be extremely high. However, AST levels are only helpful in the early stages of the illness as the blood levels tend to drop very rapidly. A marked elevation is also seen with shock and acute heart failure. Diagnostic problems may result because similar high elevations of plasma AST are occasionally seen in extra-hepatic obstruction, chronic active hepatitis and ascending cholangitis.

Aminotransferases are usually elevated in all liver disorders and attempts have been made to use the degree of abnormality of serum levels of AST and ALT but heptocellular injury and necrosis invariably leads to discharge of cell contents into the bloodstream and the serum level of various enzymes including AST and ALT are increased. Since the kinetics of leakage from and disposition within the body of these enzymes are largely unknown, the extent of change in serum concentrations is more influenced by the timing of the blood collection than the state of the liver disease.

In addition changes in AST relative to changes in bilirubin, alkaline phosphatase and ALT have been used in order to differentiate different hepatobiliary disorders but the results have been equally disappointing with one exception, the recognition of alcoholic liver disease. If the ALT is less than 300, an AST and ALT ratio of more than 2 is suggestive of alcoholic liver disease\textsuperscript{58}. 
Elevated serum aminotransferase values are not specific for hepatobiliary disorders and they are also found in patients with severe cardiac and skeletal muscle damage$^{59,60}$.  

**Other Tests**

There are a number of other serum enzyme tests of hepatocellular necrosis which will be mentioned but none of these have found widespread use.

**Glutamate Dehydrogenase**

The serum values of glutamate dehydrogenase were investigated as a specific marker for liver disorders that affect centrilobular hepatocytes such as alcoholic hepatitis$^{61,62,63}$. This initial report was not confirmed by other workers$^{62,63}$ and glutamate dehydrogenase is rarely used as a liver function test.

**Lactate Dehydrogenase**

Lactate dehydrogenase is raised in hepatobiliary disease but is not as sensitive for hepatocellular damage as the aminotransferases and is more useful as a marker of myocardial infarction and haemolysis$^{64}$

**Cholinesterase**

Cholinesterase is a non-specific esterase synthesised by the liver which decreases in hepatocellular disease and has found some use in monitoring hepatocellular damage due to chemicals.
2.6a(ii) Enzymes that Detect Cholestasis

Alkaline Phosphatase

Measurement of total serum alkaline phosphatase has remained a standard clinical test, although it has been recognised that an elevation of alkaline phosphatase occurs in a wide variety of pathological conditions. There are several alkaline phosphatase isoenzymes which can be identified by electrophoresis but this is not routinely carried out. These isoenzymes are found in varying concentrations depending on the underlying pathological or physiological circumstances and originate in liver, bone, intestine, placenta and tumours.

The function of alkaline phosphatase is unknown but serum levels of alkaline phosphatase rise in cholestasis and to a lesser extent when liver cells are damaged. The mechanism of this rise are complex and include increase in hepatic synthesis of alkaline phosphatase and increase in biliary secretion because of leaky tight junctions and increased hepatocyte plasma membrane secretion.

The major value of the serum alkaline phosphatase is the diagnosis of liver disorders is in the recognition of cholestasis. About three quarters of the patients with prolonged cholestasis will have serum alkaline phosphatase values increased fourfold or greater. Such elevations occur in both extrahepatic and intrahepatic obstruction and there is no difference between the values found in obstructive jaundice due to malignancy, bile duct stones, sclerosing cholangitis or bile duct stricture.
Other Enzymes that Detect Cholestasis

5' Nucleotidase

Serum values of this enzyme are elevated primarily in hepatobiliary disease with a spectrum of abnormality similar to that found for alkaline phosphatase and both are of equal value in differentiating obstructive from parenchymal liver disease\textsuperscript{62}.

\[\text{\gamma- Glutamyl Transpeptidase}\]

The major clinical value of \(\gamma\)-glutamyl transpeptidase (\(\gamma\)GT) lies in its use in conferring organ specificity to an elevated value for alkaline phosphatase, since \(\gamma\)GT activity is not elevated in patients with diseases of bone\textsuperscript{66}. In addition, high \(\gamma\)GT levels are found in people who take medicines such as barbiturates or ingest large quantities of alcohol even when values for other serum enzyme tests and serum bilirubin are normal\textsuperscript{67}. \(\gamma\)GT offers no advantage over aminotransferase and alkaline phosphatase as up to 68% of patients with an elevated \(\gamma\)GT have disease not involving the liver\textsuperscript{68}.

2.6b Tests of the Biosynthetic Capacity of the Liver

Serum Proteins

The liver is the major site for synthesis of many of the proteins circulating in the body and is the sole site of formation of \(\alpha\) and \(\beta\) globulin. Changes in serum proteins in liver disease reflect a multifactorial reaction which includes a response to foreign agents and the effects of decreased protein synthesis.

There are electrophoretic patterns of serum proteins associated with certain liver diseases. In acute viral hepatitis there may be a fall in the \(\alpha\)-1 globulin with an increase in the \(\beta\) globulin. In cirrhosis the albumin is reduced with a generalised increase in the \(\gamma\)
globulin fraction. This may be accompanied by a merging of the β and γ globulin bands due to an increase in IgA\(^5\).

**Albumin**

Albumin is quantitatively the most important plasma protein and is synthesised exclusively by the liver. The serum level at any time reflects the rate of synthesis, the degradation rate and the volume of distribution. Serum albumin levels tend to be normal in diseases such as acute viral hepatitis, drug-induced hepatotoxicity and obstructive jaundice. Hypoalbuminaemia is more common in chronic liver disorders such as cirrhosis and chronic hepatitis and usually reflects severe liver damage and decreased albumin synthesis. Low serum albumin is not specific for liver disease and may occur in protein malnutrition of any cause including protein-losing enteropathies, chronic infection and nephrotic syndrome.

**Prothrombin Time**

The liver is the major site of synthesis of many blood coagulation proteins\(^6\) and the liver is involved in clearing some of the clotting factors from serum\(^7\). The prothrombin time measures the rate at which available prothrombin is converted to thrombin in the presence of thromboplastin, calcium ions and coagulation factors.

The prothrombin test is not a sensitive index of liver disease because, even in severe cirrhosis, results may be normal or prolonged only slightly. A prolonged prothrombin time is not specific for liver disease and is observed in various congenital deficiencies of coagulation factors\(^8\) and in acquired conditions including consumption of clotting factors and ingestion of drugs\(^9\).
A prolonged prothrombin time, if the above are excluded, is due to either hypovitaminosis K, as observed with prolonged jaundice, steatorrhoea or dietary deficiency, or poor utilisation of vitamin K due to parenchymal liver disease. In patients with liver disease, the prothrombin time permits an assessment of the tendency to bleed before attempting any invasive procedure and the test has high prognostic value in acute hepatocellular disease.

**Other Tests of Biosynthetic Capacity of the Liver**

**Lipoproteins**

Cholesterol, phospholipids and triglycerides are insoluble in water and transport of these lipids in plasma involves lipoproteins. A number of different protein subunits, apoproteins, are present in lipoproteins. In hepatocellular disease and in obstructive jaundice the plasma triglycerides tend to be increased and in cholestasis an abnormal lipoprotein, lipoprotein X, very rich in cholesterol and lecithin is found. The serum cholesterol esters, lipoproteins and lipoproteins X are not estimated routinely.

**Caeruloplasmin**

Caeruloplasmin is the major copper containing protein in plasma and a low concentration is found in 95% of those who are homozygous and about 10% of those heterozygous for Wilson’s disease. Low values are also found in very severe decompensated cirrhosis which is not due to Wilson’s disease and high values are found in pregnancy, following oestrogen therapy and with bile duct obstruction.
Transferrin

In the intestinal cell iron links to a glycoprotein, transferrin, by which it is carried in serum. Transferrin is largely synthesised by the hepatocyte and this process is reduced in alcoholic cirrhosis.\textsuperscript{77}

\(\alpha1\)-Antitrypsin

\(\alpha1\)-Antitrypsin deficiency is associated with cirrhosis in childhood and is specifically diagnosed by abnormally low values of \(\alpha1\) globulin on protein electrophoresis and intracellular inclusions in the parenchymal cells of the hepatocyte.

2.6c Tests that Detect Chronic Inflammation or Altered Immunoregulation

Immunoglobulins

Studies of the changes in the immunoglobulins in patients with liver disease have been concerned with measurements of IgG, IgA and IgM. IgA is markedly increased in alcoholic cirrhosis and also in primary biliary cirrhosis and cryptogenic cirrhosis. IgM is greatly increased in primary biliary cirrhosis and to a lesser extent in viral hepatitis and cirrhosis. IgG has elevated levels in chronic active hepatitis and cryptogenic cirrhosis.\textsuperscript{78}

The measurement of immunoglobulins in acute liver disease is of little value although a persistent elevation is suggestive of the development of chronic liver disease.\textsuperscript{79} Patterns of changes in the immunoglobulins are not diagnostic of any one disease and only give suggestive evidence.
2.6d Tests of the Capacity of the Liver to Transport Organic Anions and Metabolise Drugs

Bilirubin

Bilirubin, a tetrapyrrole pigment, is a breakdown of ferroprotoporphyrin IX (heme) which is an integral part of heme-containing proteins. It is esterified almost exclusively in the liver and subsequently is excreted in bile mainly as glucuronide derivatives. The bilirubin present in serum represents a balance between input from production and hepatic removal of the pigment.

This is the most widely used liver function test and the measurement of serum bilirubin levels serves to confirm the presence or absence of jaundice. Due to the complexities of bilirubin metabolism, increased serum levels cannot be assigned to a single pathological process. The finding that unconjugated bilirubin is the predominant pigment in plasma is of diagnostic importance. Haemolytic disorders must be excluded as a cause but the largest group of patients falling into this category are those with a benign hyperbilirubinaemia. If conjugated bilirubin is the predominant pigment in plasma, it is generally higher in patients with bile duct obstruction than in parenchymal liver disease, but the overlap between the groups is considerable.

Total serum bilirubin is not a sensitive indicator of hepatic dysfunction and may not accurately reflect the degree of liver damage. Hyperbilirubinaemia may not be detected in instances of moderate or severe hepatic parenchymal damage or of a partially or briefly obstructed bile duct.
The greatest problem is the diagnostic classification of patients with jaundice where the classes are collectively exhaustive and mutually exclusive. The most useful classification is into unconjugated hyperbilirubinaemia, extrahepatic biliary obstruction and parenchymal liver disease.

The presence of conjugated bilirubin in the urine indicates hepatobiliary disease. Unconjugated bilirubin is tightly bound to albumin, is not filtered by the glomerulus, and is not present in urine.

**Other Tests of the Capacity of the Liver to Transport Anions and Metabolise Drugs**

**Bile Acid Tests**

The primary bile acids, trihydroxycholic acid and dihydroxychenodeoxycholic acid, are synthesised from cholesterol in hepatocytes, conjugated to glycine or taurine and then secreted into bile. Bile acids move rapidly down the intestinal tract, where some are absorbed throughout the whole intestine by nonionic passive diffusion and most are actively reabsorbed by carrier mediated transport in the terminal ileum and carried back to the liver via the portal vein⁸¹.

Maintenance of normal serum bile acid concentrations is dependent upon hepatic blood flow, hepatic uptake, secretion of bile acids and intestinal motility. Diseases that affect any of these functions affect serum bile acid levels and serum bile acid levels are very sensitive but non-specific indicators of hepatic dysfunction. Sensitive tests to measure serum bile acid concentration are available but they have yet to find a role in the evaluation of patients with suspected liver disease⁸².
2.7. **CLEARANCE TESTS - INTRODUCTION**

The measurement of exogenously administered substances has been widely used to measure liver function\(^8\). The clearance is dependent on both hepatic blood flow and extraction efficiency of the substance and linked by the equation:-

\[
\text{Clearance (K)} = \text{EF} \times \text{BF}
\]

Where \(\text{EF}\) = Extraction efficiency and \(\text{BF}\) = Hepatic blood flow

Hepatic blood flow is composed of 2 components, portal venous flow and hepatic arterial flow. Portal flow is not controlled by the liver but represents the sum of outflows of several organs. Portal flow fluctuates widely but the hepatic artery compensates for these fluctuations to keep total hepatic perfusion constant\(^8\). There is a tendency for hepatic blood flow to remain constant for a wide range of hepatic pathologies and prevent changes in the rates of hepatic clearance of endogenous substances such as hormones\(^8\).

Extraction efficiency is defined as the percentage of inflow concentration removed in one circulation and substances may have high or low extraction efficiencies. The hepatic clearance of a substance depends on hepatic intrinsic clearance, which is an index of hepatocellular enzyme activity, and on hepatic blood flow. If intrinsic clearance is very low, it becomes rate limiting, whereas if intrinsic clearance is very high, blood flow becomes rate limiting\(^8\). Which of these processes is rate limiting will depend on the particular substance under study. The intrinsic clearance of any compound can only be measured from plotting its hepatic venous concentration against time and doing so requires an invasive procedure to cannulate the hepatic vein and is not routinely justified\(^8\).
For compounds with high extraction rates such as indocyanine green systemic clearance is limited mainly by the delivery to the liver and the clearance constant should be considered more dependent on blood flow than extraction efficiency. Uptake of these compounds requires hepatocyte membrane carrier transport and this transport system facilitates both entry into and exit from liver cells.

For substances with low extraction rates such as caffeine, systemic clearance is limited by hepatic metabolism, and its measurement reflects hepatic intrinsic clearance.

The clearance of other substances have been used. These include galactose, caffeine, antipyrine and lignocaine in blood; aminopyrine, caffeine and galactose in breath, caffeine and antipyrine in saliva and debrisoquine, mephenytoin and dextromethorphan in urine. It is reasonable to suggest that clearance tests assess liver cell mass according to enzymatic (aminopyrine, caffeine, galactose) and non-enzymatic (ICG) functions when providing an estimation of functional reserve capacity in subjects with liver disease.

Bromosulphophthalein, another dye similar to indocyanine green, was used previously to measure hepatic functional reserve but its use has been discontinued because of a significant incidence of severe allergic reactions. Hoffman assessed the plasma disappearance of a labelled, conjugated bile acid, cholyil-14C-glycine, but found a marked loss of extraction efficiency in severe liver disease.

Lignocaine is metabolised to mono-ethyl-glycine-xylidide (MEG-X) by a cytochrome P-450 dependent process. It has been used as a peri-operative marker of liver function to assess prognosis in patients with liver dysfunction undergoing surgery.
There are doubts about the safety of this drug and the interactions and complex kinetics make use of this test questionable\textsuperscript{45}.

Aminopyrine and antipyrine are both almost exclusively metabolised by the liver. Hepatic uptake is slow and therefore independent of blood flow allowing these compounds to be used extensively to assess liver function\textsuperscript{93}. Aminopyrine was measured by a breath test and like all breath tests is costly. In general breath tests have failed to find use outside of research centres because of their complex nature\textsuperscript{90}. In addition for the aminopyrine breath test there are variations in activity of the mixed function oxidase system with time and from patient to patient\textsuperscript{94}.

Galactose elimination capacity has also been used as a test of liver function but corrections are necessary as a considerable proportion is processed by extrahepatic non-renal metabolism and this has limited its clinical use\textsuperscript{95}.

2.7a. **Clearance Calculations**

Clearance of a drug is defined as the volume of biological fluid cleared of the drug per unit time and clearance from the bloodstream can be defined by one or more exponential functions.

(i) The clearance in a one compartmental model is a simple exponential and may be represented by:-

\[ Pt = P_0 \times e^{-kt} \]

where \( Pt \) = the plasma concentration at time \( t \) minutes

\( P_0 \) = the initial plasma concentration
\( K \) = the clearance constant

On plotting the natural logarithm of the plasma concentration as a function of time, the straight line of the slope represents the clearance rate constant. The clearance constant is related to the half time of disappearance by the expression:-

\[ K = \frac{0.693}{t\frac{1}{2}} \]

(ii) If after intravenous administration the clearance is biexponential than a two compartmental model is needed and is represented by the function:

\[ Pt = Ae^{-at} + Be^{-bt} \]

where \( Pt \) = the plasma concentration at time \( t \) minutes

A and B are constants and \( A + B = Po \)

Where \( Po \) = the plasma concentration at time 0

a and b are the rapid and slow exponential constants with appropriate half-lives.

2.7b. **Use of Clearance Test to Measure Hepatic Function**

Quantitative tests of hepatic function have not so far been part of the routine clinical work-up of patients with liver diseases. Unlike other biochemical markers, clearance tests quantitate the functional capacity of the liver and are capable of detecting subclinical hepatic disease. They can also be used to monitor the progress of healing after damage has occurred. Clearance tests have been shown to be the procedures of choice to determine the severity and prognosis of liver disease but are of little use in diagnosis\(^{45, 83, 96}\). They have been shown to be an accurate indicator of prognosis in alcoholic hepatitis\(^{97}\), paracetamol poisoning\(^{98}\), fulminant liver failure\(^{99}\) and graft function after liver transplantation\(^{100, 101}\). In addition they have been shown to provide a reliable assessment
of severity in cirrhosis\textsuperscript{88, 102, 103}, allow an estimation of survival in non-alcoholic cirrhosis\textsuperscript{104} and predict deterioration in primary biliary cirrhosis\textsuperscript{105}.

The majority of these studies have measured liver function using breath tests involving aminopyrine, caffeine, galactose or phenacetin. These breath tests are complex, costly and have not achieved general popularity\textsuperscript{90, 93}. They are largely used for research purposes. It would be more convenient, if a test is to achieve popularity, to measure the clearance of substances already used routinely to investigate other aspects of liver disease such as biliary imaging agents.

During the clinical course of chronic liver disease the longest period of time is the stage of compensated or clinically non-specific liver disease. Once decompensation occurs, further fatal deterioration occurs rapidly. According to this concept, tests of functional capacity of the liver have potential to stage the disease long before clinical decompensation occurs. Therefore quantitative liver function tests should have a prognostic potential to guide clinical management.

2.7c \textbf{Colloidal Clearance - Measurement of RES Function of Kupffer Cells}

Phagocytosis is the most overtly physiological feature of the RES and studies of the function have proved most useful in the assessment of the RES. The most important factors which influence phagocytosis are blood flow, metabolic activity, status of the RES, opsonins, specific antibodies, particle dose and particle size.

Impairment of phagocytic activity results in changes in antigen distribution and large immune complexes that fix complement are avidly cleared by the liver. In cirrhotic
liver disease these functions are impaired and complexes accumulate in the plasma\textsuperscript{106, 107}. In some cases this causes significant activation of C3 and causes tissue damage.

Phagocytosis is a metabolically dependant event requiring an expenditure of energy for ingestion of the particle but adherence of the particle to the membrane of the phagocytic cell does not require energy. Impaired RES activity may be related to oxygen consumption. In most cases phagocytosis of foreign material by macrophages requires the presence of certain serum factors called opsonins or recognition factors\textsuperscript{108}. Opsonins are protein substances in normal serum or plasma which stimulate the RES and are similar to natural antibodies. Well known opsonins are immunoglobulins and complement factor which binds to the substrate prior to the attachment of the latter to phagocytic cells.

Fibronectin which was initially called cold-insoluble globulin has been identified by electrophoresis of the A2 globulins as the main non-specific component of serum which stimulates phagocytosis.

As the phagocytic activity of the RES is its most important characteristic, the clearance of colloid removed from the bloodstream by the RES can be used as a measure of RES function. The disappearance of an intravenously injected colloid has an exponential decay and the rate constant of this process can be calculated (K). The rate constant is defined as the fraction reduction of a substrate per unit time and its units are min\textsuperscript{−1}. If the substance is cleared by more than one body compartment its elimination rates will have more than one rate constant.
The function of Kupffer cells of the liver is of particular importance in hepatocellular damage. Approximately 90% of a test dose will become localised in the hepatic and splenic macrophages while the remainder becomes localised primarily in the lungs and bone marrow. With increase in the dose injected there is a tendency for greater spleen, lung or bone marrow localisation on a percentage basis. Therefore the clearance of small doses of injected colloid will provide a good estimate of Kupffer cell function but not of overall RES function.

This measurement of the clearance rate of an intravenously injected, radioactively labelled colloid has been widely used to obtain an index of liver blood flow. As colloidal clearance depends on both hepatic flow and hepatic extraction efficiency and on the blood flows and extraction efficiencies of other sites of reticulo-endothelial function the value of K only provides an index of liver blood flow.

Generally the colloidal constant (K) is reduced in severe liver disease. By observing the fractional clearance of heat denatured albumin labelled with 131 I Chiandussi showed that colloidal clearance is only modestly reduced in cirrhosis.

Taplin noted that K values in patients with cirrhotic liver disease were significantly lower than that observed in normals but this difference was not observed between non-cirrhotic alcoholic liver disease and normals. De Nardo used 99 m Tc labelled sulphur colloid to measure K, and in 7 normal patients the clearance rate was significantly lower than the clearance rate in 7 patients with cirrhotic liver disease.
Horisawa\textsuperscript{115} in 1976 found a reduction of both K and extraction efficiency of sulphur colloid. His results were consistent with either reduced numbers of RES cells in the liver, or with intrahepatic shunts bypassing RES cells. The previous observations of only a modest reduction of RES cells in cirrhosis by Chiandussi\textsuperscript{112} suggests that bypassing of RES cells may be important.

The K value of the liver uptake of sulphur colloid was shown by Miller\textsuperscript{116} to be significantly less in cirrhotics compared with normal patients. These observations were confirmed in a repeated set of tests performed by Houston\textsuperscript{117} in 1980.

The importance of Kupffer cell function during the course of fulminant hepatic failure is reflected in the significantly worse RES function found in patients who also develop renal failure\textsuperscript{118}. Renal failure in fulminant hepatic failure has been attributed to systemic endotoxaemia\textsuperscript{119} which results in reduced renal perfusion\textsuperscript{120} and this is likely to be present only when RES function is no longer adequate to prevent the spread of endotoxins from the portal to the systemic circulation.

Despite the ease of measurement of colloidal clearance it has never gained popularity as a liver function test.

2.8. **SUBSTANCES USED IN CLEARANCE TESTS OF LIVER FUNCTION IN THIS STUDY**

2.8a. **Tin Colloid**

Tin colloid labelled with technetium-99m has become recognised as a convenient alternative for liver scintigraphy to the previously widely used 99m Tc-sulphur colloid.
The preparation of tin colloid is much simpler and more convenient than the preparation of sulphur colloid in that no additional reagents or heating are required.

In clinical comparisons of its use for liver scintigraphy, tin colloid has been shown to be superior to both sulphur and phytate colloids\textsuperscript{121,122}.

The particle size of liver, imaging colloidal radiopharmaceuticals is probably the most important single parameter which determines their organ distribution\textsuperscript{123}. Whateley\textsuperscript{124} in 1985 suggested that the particle size of tin colloid may increase when injected due to flocculation within the syringe. However Frier\textsuperscript{125} has shown that in clinical measurements of hepatic perfusion index the changes in particle size induced by aging of the colloid within the syringe were clinically insignificant.

Chadwick\textsuperscript{126} showed a reduction in Kupffer cell clearance of technetium labelled tin colloid in rats in both reticuloendothelial blockade and severe sepsis.

As far as the author is aware tin colloid has not previously been used in dynamic studies of liver function in man.

2.8b. **Indocyanine Green**

Indocyanine green is a tricarbocyanine (anhydro-3,3,3,3-tetramethyl-1,1-di-(4-sulfobutyl)-4,5,4,5-dibenzoindotricarbocyanine hydroxide sodium salt) first developed for use in colour emulsion and used as an indicator in dye solution techniques for the measurement of cardiac output\textsuperscript{127}. Wheeler\textsuperscript{128} in 1958 showed that following intravenous injection in dogs, the dye was bound to albumin and was rapidly distributed in the
circulating plasma. The rapid plasma clearance by the liver suggested that the dye might be used in the evaluation of hepatic function.

Caesar\textsuperscript{129} showed that it is removed from the circulation specifically by the liver, it is not conjugated and there is no evidence of extrahepatic removal or of an enterohepatic circulation. Since this time it has been used extensively both as a liver function test and for the measurement of liver blood flow.

After intravenous injection, ICG is rapidly and completely bound to globulins, probably $\alpha_1$-lipoproteins, in the plasma protein\textsuperscript{130}. For its removal from the blood, ICG has to be taken up into the hepatocyte through the sinusoidal liver cell membrane at specific binding sites\textsuperscript{131} which it shares with BSP and to a lesser extent with bilirubin\textsuperscript{132}. Within the hepatocyte, ICG is bound to acceptor proteins\textsuperscript{133}. This binding permits the hepatocyte to store ICG within the liver cell prior to its excretion into the bile. The storage phenomenon is evident from the delay between plasma removal and biliary excretion of ICG observed after rapid single injection of ICG\textsuperscript{134}. ICG is not chemically altered during its passage through the liver and may be recovered in the bile unchanged\textsuperscript{135}. Further evidence of the exclusive role of the liver in removing ICG from the blood stream is the negligible (less than 0.16\% per minute) disappearance rate of ICG from the plasma of hepatectomised dogs\textsuperscript{136}.

The ICG removal rate from the blood and its hepatic uptake are dose dependant and exhibit a saturation phenomenon. Since the excretory apparatus of the liver under physiological conditions operates far below its functional capacity, slight impairment of organ function can only be detected if the transport system is saturated. At doses that are

40
safe to administer, the excretory capacity of the liver for ICG is far below its maximum\textsuperscript{137} and therefore mild liver damage may not be detected. This problem may be overcome by extrapolation from several submaximal transport rates according to the Michaelis-Menten equation\textsuperscript{138} but this approach is extremely cumbersome and rarely needed.

The clearance of ICG is a relatively simple technique which assesses indirectly the functional hepatic blood flow\textsuperscript{139}. The use of the peripheral plasma clearance of ICG to act as an indicator of liver blood flow is restricted to normal patients\textsuperscript{140} as the calculation of extraction ratio (and hence liver blood flow) from the peripheral plasma clearance of ICG has been shown to be an inaccurate estimate of true anatomical liver blood flow in patients with severe liver disease but the reasons for this failure are not yet known\textsuperscript{141}.

Cohn\textsuperscript{142} demonstrated in 15 patients with alcoholic liver disease that the clearance of ICG is markedly reduced with increased severity of the disease. The clearance of antipyrine and ICG was compared by Branch\textsuperscript{143} in 1976 and the clearance of ICG as a test of liver cell mass gave the best correlation with clinical outcome.

Gottlieb\textsuperscript{144} used serial measurements of the clearance of ICG to investigate the clinical course after hepatic trauma in 7 patients and showed the values obtained to be an early and sensitive indicator of impaired hepatic function.

In evaluating the post-operative course in 50 patients who underwent hepatic resection Matsumata\textsuperscript{145} proved that the clearance of ICG was a good marker for predicting the post-operative course. Like Gottlieb, he found that measurements made early in the post-operative course were the best indicator of outcome.
Similar results were found in a study of hepatic function following hepatic transplantation using indocyanine green clearance. In these studies clearance tests performed in the early post-operative periods were the best predictors of graft function\textsuperscript{101}.

The clearance of ICG has a defined normal range from 0.14 to 0.28 min\textsuperscript{-1}\textsuperscript{45}.

2.8c Disida

Clinicians have accepted 99m-Tc IDA scintigraphy as a reliable and cost effective test in acute cholecystitis but it has failed to make the expected clinical impact in assessment of other hepatobiliary diseases.

The initial IDA agents gave poor results unless the liver function was near to normal and these agents appeared on the market at the same time as CT scanning and ultrasound and were unfavorably compared in clinical capabilities. In addition work on normal patients in this initial phase of study was wrongly interpreted resulting in erroneous criteria in diagnosis for patients\textsuperscript{146}. The quantitative interpretation of the scans was also neglected. In 1982 diisopropyl IDA (Disida) was approved for use and since then the potential to disapprove the initial criticisms has existed.

The Tc-99m labelled N-acetonilido-iminodiacetic acids are a family of hepatobiliary imaging agents in which a chelating group (Iminodiacetic acid, IDA), capable of binding a gamma-emitting radiometal, are attached to an analogue of lidocaine. Following intravenous injection, IDA derivatives bind to plasma albumin and are rapidly taken up by the hepatocytes by a non-sodium dependant organic anion pathway. It is
secreted into the bile without conjugation and once it enters the bile canaliculi, it follows the path taken by hepatic bile and enables the study of major ducts much like a contrast cholangiogram\textsuperscript{147}.

Disida is commercially known as disofenin (tradename Hepatolite) and is present in bile in the highest concentration during the first 60 minutes after injection when compared with other IDA-derivatives. It has been found to be superior in its ability to visualise the biliary system in the presence of abnormal bilirubin concentrations\textsuperscript{148, 149}. Studies with baboons\textsuperscript{150} and humans\textsuperscript{147, 151} have shown that Disida is cleared rapidly from the circulation under normal conditions with about 10\% of the injected activity remaining in the bloodstream 30 minutes post injection. It has a low urinary excretion rate (10\% of the dose in urine at 120 minutes) and is almost exclusively cleared by the liver. Intestinal reabsorption has not been reported. These properties potentially make Disida a useful agent for investigating hepatic function.

Empirical observations have suggested that there is abnormally slow uptake of IDA hepatobiliary imaging agents in various states of liver disease\textsuperscript{152, 153}.

Brown\textsuperscript{154} in 1988 using Disida measured hepatic extraction fraction by deconvolutional analysis and liver excretion by a non linear least squares method in 13 normal patients, 14 with common bile duct obstruction, 13 with sclerosing cholangitis (SC) and 14 with alcoholic cirrhosis. Hepatic extraction fraction was significantly reduced in the alcoholics, but only those in Childs Group C, compared with the other groups tested. The liver excretion was prolonged in all 3 liver disease categories compared with the normals suggesting that this is a non-specific method of distinguishing
hepatobiliary disease from normality. The 13 patients with SC were all in the early stages of the disease as they lacked signs of cirrhosis or evidence of portal hypertension whereas the alcoholic cirrhotics all had severe disease with only 4 out of 14 patients in Childs group A and the rest in B or C. This suggests that whilst both groups had liver disease the severity of the disease was not comparable between the groups and no other quantitative assessment of liver function was performed.

The whole blood activity following the intravenous injection of Disida was shown to exhibit a clear biexponential behaviour with a slow and fast component of disappearance.

2.8d Caffeine

Caffeine (1,3,7 trimethylxanthine) is present in tea, coffee, cocoa and cold type soft drinks and is a widely used and socially acceptable drug. It is present in both prescription and nonprescription medications including many “over the counter” stimulant preparations. Recently it has received particular interest as a drug whose metabolism may reflect liver function.

Peak plasma levels occur about 40 minutes after oral administration and after almost complete absorption, caffeine is metabolised in the liver by the microsomal enzymes through demethylation and oxidation to 1-methyluric acid and 1-methylxanthine. The metabolites and about 5% unmetabolised caffeine are excreted by the kidneys into the urine. Therefore metabolism of caffeine is modified by conditions that will affect liver and kidney function such as age, disease and malnutrition.
It has been reported that cigarette smoking increases caffeine clearance due to induction of hepatic aryl hydrocarbon hydroxylase activity\textsuperscript{159, 160} which represents the main initial step in caffeine metabolism. On the other hand, cimetidine and disulfuram have been shown to inhibit caffeine clearance\textsuperscript{160, 161, 162} as does the oral contraceptive pill\textsuperscript{163}.

Importantly, single doses of caffeine (approximately 1 to 2 cups of coffee or 150 mgs caffeine) may be considered innocuous and caffeine costs only a few pence per dose\textsuperscript{164}.

It was initially observed that there is a prolonged plasma half-life of caffeine in alcoholic liver disease\textsuperscript{165} and Desmond\textsuperscript{166} in 1980 showed that the plasma disappearance of caffeine in patients with cirrhosis is delayed compared to controls.

Renner\textsuperscript{167} showed impaired elimination of caffeine in patients with liver disease when studying 15 cirrhotics, 11 patients with miscellaneous liver diseases and 10 normal volunteers. These changes in hepatic caffeine metabolism parallel alterations in functioning hepatic cell mass as determined by the clearance of bromsulphalein (BSP).

Jost\textsuperscript{168} in 1987 demonstrated a close correlation between plasma and salivary levels of caffeine and concluded that an overnight caffeine clearance measured in saliva may represent a less invasive but equally accurate test. Significant differences were shown between caffeine clearance for controls and both cirrhotic and non cirrhotic alcoholic liver disease. Correlations with caffeine clearance were also observed with ICG fractional clearance, galactose elimination capacity and aminopyrine breath test. The closest
relationship with caffeine clearance was the aminopyrine breath test confirming that caffeine clearance probably quantifies hepatic microsomal enzyme function although aminopyrine and caffeine are processed by different isoenzymes (Cytochrome P450 and P448 respectively).

Jost also deduced that the fasting morning concentration of caffeine was highly correlated with caffeine clearance and suggested further simplification of the test to a single point measurement. Further confirmation of the value of fasting plasma caffeine levels was described by Hasegawa\textsuperscript{169} in 1989 who showed a significant difference between 34 controls and 46 patients with cirrhotic liver disease.
CHAPTER 3

CLINICAL STUDIES 1

In this chapter the blood clearance of tin colloid is evaluated for both controls and patients with liver disease. Confirmation of previous results is obtained and the reproducibility of the method is measured.

CONTENTS

3.1. Introduction 48

3.2. Patients, Materials, Methods and Calculations 49

3.3. Results 54

3.4 Discussion 62
3.1 INTRODUCTION

The clearance of a radiolabelled colloid is a well described technique of measuring reticulo-endothelial function\textsuperscript{113, 116}. Tin colloid has been shown to have superior handling and imaging properties to the previously used sulphur colloid and has been used to measure the colloidal clearance rate by gamma camera and computer assisted analysis. A bedside technique was used employing a mobile detector over the forehead as external counting is a satisfactory and reproducible method of determining colloidal clearance\textsuperscript{170}. Positioning over the forehead, using the eyebrows as the lower limit of the circular detector, allows the detector to be accurately positioned and repositioned, if necessary, instead of using other peripheral areas of interest such as the limbs.

Conventional static liver imaging, using peripherally injected intravenous radiocolloid, reflects hepatic reticuloendothelial cell function and has been used for many years in the detection of local and diffuse liver disease\textsuperscript{171, 172, 173}. Patchy, reduced hepatic uptake, with increased splenic and later bone marrow uptake occurs in part as a result of altered haemodynamics through the development of intrahepatic shunts\textsuperscript{174} which lead to a redistribution of extractable colloid\textsuperscript{115, 175}. Using a variety of scoring systems based on such static images, correlations with wedged hepatic vein pressures can be obtained as a non-invasive index of portal hypertension\textsuperscript{176, 177} and may indicate the presence of oesophageal varices\textsuperscript{178}. In the patients in this chapter the relationship between scan score and the colloidal clearance rate of tin colloid has been investigated. This concept is very attractive because if the static liver scan could be adapted to give a quantitative measure of liver function then no additional testing would be necessary.
Since this work was completed the static assessment of the liver for diagnosis has largely been undertaken by ultrasound, CT scanning and MRI scanning. These developments have caused a marked decline in the use of isotope liver scanning, such that it is now rarely used routinely in clinical practice.

In addition in this part of the study a check has been made that the blood clearance of tin colloid is being measured as well as checks on the reproducibility of the external head counting method.

3.2. PATIENTS, MATERIALS, METHODS, CALCULATIONS

3.2a Patients

Forty one patients were studied. 12 controls (7 male) mean age 55 years and 29 patients (20 male) mean age 54 years with liver disease. All patients gave written consent and all studies were approved by the hospital ethical committee.

(i) Controls

Controls were taken from patients admitted for routine surgery for vascular disease (n=2), hernia repair, drainage of ischiorectal abscess, resection for Crohn’s disease (n=2) cholecystectomy (n=2), division of adhesions (n=2) and haemorrhoids (n=2). All had normal biochemical liver function tests and no evidence either from their history and examination or operative findings that they had liver disease. All were tested after an overnight fast and patients admitted for routine surgery on an afternoon list were invited to take part as they could be studied on the morning of the day of surgery to minimise inconvenience. No patient was tested within 7 days of a general anaesthetic.
(ii) **Liver Disease**

Patients with liver disease were selected from patients admitted for investigation of their liver disease under the care of Prof. O. James. Only inpatients were studied and they were chosen from patients on the ward on the days of testing. All had their diagnosis of liver disease confirmed on histological grounds. No patient was studied within 2 weeks of an acute bleeding episode.

The patients studied had cirrhotic alcoholic liver disease (n=10), metastases in the liver (n=9) and a further miscellaneous group (n=10) with cases of primary biliary cirrhosis (n=3), sclerosing cholangitis (n=2), hepatoma (n=2) and individual cases of obstructive jaundice, liver trauma and chronic active hepatitis.

### 3.2b Material

**Tin Colloid**

The preparation of 99m Tc tin colloid involves the addition of isotonic 99m Technetium sodium pertechnetate generator eluate to the freeze dried preparation (Amersham International, Amersham, United Kingdom).

### 3.2c Methods

(i) **Forehead Counting**

All patients were fasted overnight, positioned supine and their head supported to fix the placement. A collimated sodium iodide detector (5 cms x 2.5 cms thickness) was placed over the forehead. Eighty MBq in 0.5 mls of Tc 99m stannous colloid was injected intravenously as a bolus with saline follow through. Disappearance of colloid from the circulation was recorded for 30 minutes and stored in a microcomputer (Apple IIe). A typical time-activity is shown in figure 3.1.
Figure 3.1 A typical time-activity curve of the disappearance of radiolabelled tin colloid from the bloodstream measured by head counting.
(ii) **Blood Counting**

Five mls of blood was withdrawn as a blank. After infusion of the tin colloid into one cubital fossa, blood was withdrawn from the other cubital fossa at 3 minute intervals for 30 minutes. One millimetre samples were pipetted into cuvettes and their activity measured in an automatic gamma camera.

Eighteen of the patients (5 controls) had these blood samples taken whilst undergoing forehead counting.

### 3.2d Calculations

#### (i) **Head Counting**

The disappearance from the bloodstream was biexponential and is represented by the equation:

\[ C_t = Ae^{-at} + Be^{-bt} \]

Where \( C_t \) = count at time \( t \)

\( C_0 \) = count at time \( 0 \)

\( C_0 = A + B \)

\( a \) and \( b \) are the respective rate constants of fast and slow disappearance of tin colloid.

Analysis of information from the first 2 minutes was omitted as contributions from non-uniform mixing could be ignored. The disappearance rate constant of tin colloid (Ktc) was calculated from the data between 2 and 5 minutes. The fast rate constant, \( a \), was greater than 0.06 min\(^{-1}\) and the slow rate constant, \( b \), was less than 0.005 min\(^{-1}\) (Figure 3.1) The extrapolated amplitude of the second exponential at the time of injection is only 2% of the amplitude of the first. Therefore the use of a single function to fit the observed
data between 2 and 5 minutes introduces a negligible error into the estimation of the disappearance rate constant of tin colloid (Ktc)\textsuperscript{116}.

Using the equation (from above):-

\[ C_t = Ae^{-at} + Be^{-bt} \]

B=0 and therefore

\[ C_t = Ae^{-at} \]

Where A = Co and a = Ktc (disappearance rate constant of tin colloid)

For comparison with blood counting a second rate constant (Ktc-A) was derived from analysis of the curve from 2 to 15 minutes with correction by a single point at 30 minutes (assuming the second exponential to be a constant)

(ii) **Blood Samples**

The counts per minute of samples from 3 to 15 minutes inclusively, after correction for a single point at 30 minutes (assuming the second exponential is a constant), were plotted against time on a semilogarithmic scale and an exponential type of slope gave the half life t\textsuperscript{1/2}. From this the disappearance rate constant Kbc was derived from the formula:-

\[ Kbc = \frac{0.693}{t^{1/2}} \]

Where Kbc = disappearance rate constant of tin colloid measured from the blood samples.

(iii) **Reproducibility**

Eight of the patients (2 controls) consented to have the same procedure with both forehead counting and blood samples performed on the next day under identical conditions.
(iv) **Radionuclide Liver Imaging**

The liver scan was scored on a 0 to 9 basis \(^{115,171}\) as follows: for liver mottling, 0 = none, +1 = mild, +2 = more marked than scattered defects, and +3 = liver faintly visualised. For splenic uptake, 0 = none, +1 = less than liver uptake, +2 = equal to liver uptake, and +3 = greater than liver uptake. For bone marrow uptake, 0 = none, +1 = faint visualisation, +2 = more marked but less than liver uptake, and +3 = equal to liver uptake. All scans were scored by an independent observer who did not have prior knowledge of the patient.

**Statistics**

Comparison between groups of patients was made using the Mann Whitney test. For paired and repeat tests the average differences from the line of identity was calculated.

3.3. **RESULTS**

3.3a **Head Counting**

The scatter of Kt_c for different categories of liver disease is shown in Figure 3.2. The initial clearance rate of tin colloid (Kt_c) in the controls has a median value of 0.18 and a normal range of 0.12 to 0.24 (from our results). For the patients with cirrhotic alcoholic liver disease the median value of the clearance of tin colloid at 0.08 is significantly lower than the controls (p<0.01, Mann Whitney) and of the patients studied only 3 out of the 10 were in the normal range.

The median of the clearance for the patients with metastases was not significantly different from the median of the clearance for controls (NS, Mann Whitney) and 5 out of the 9 patients studied were in the normal range.
The miscellaneous group of patients with liver disease had a significantly lower median clearance compared with the controls (p<0.05, Mann Whitney) with only 3 out of 10 patients studied in the normal range.
SPECIAL NOTE

THIS ITEM IS BOUND IN SUCH A MANNER AND WHILE EVERY EFFORT HAS BEEN MADE TO REPRODUCE THE CENTRES, FORCE WOULD RESULT IN DAMAGE
Figure 3.2 The clearance of tin colloid by head counting (Ktc) for each disease category and controls.

<table>
<thead>
<tr>
<th>Category</th>
<th>Controls (n=12)</th>
<th>Liver Disease (n=10)</th>
<th>Liver Disease (n=9)</th>
<th>Liver Disease (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>0.18</td>
<td>0.08*</td>
<td>0.17</td>
<td>0.08**</td>
</tr>
<tr>
<td>IQR</td>
<td>0.09</td>
<td>0.09</td>
<td>0.10</td>
<td>0.09</td>
</tr>
</tbody>
</table>

* p<0.01 for differences to control group (Mann Whitney)

** p<0.05 for differences to control group (Mann Whitney)
3.3b Blood Clearance

Figure 3.3 shows the relationship between the blood clearance of tin colloid (Kbc) and the clearances measured by head counting over the same timescale (Ktc-A) for the 21 patients studied. The average difference from the line of identity is 0.20 showing that the two methods of measuring blood clearances are similar.

The median value for Kbc at 0.19 was 27% higher than for Ktc.

3.3c Reproducibility

Figure 3.4 shows the relationships between the respective paired measurements for Ktc, Ktc-A and Kbc. The respective average differences from the line of identity are 0.11, 0.06 and 0.07.

3.3d Scan Scores

Scan scores were only evaluated for those patients with diffuse liver disease. All patients with metastases (n=9) had these diagnosed by a radiolabelled image and are not included in this analysis. Figure 3.5 shows the relationship between scan score and Ktc. There was no relationship demonstrated between these tests of liver function.

3.3e Bladder

Ten consecutive patients with liver disease had the detector positioned over the bladder on completion of measuring blood clearance and no activity was detected. No patient voided during the tests.
Figure 3.3 The corrected head count clearance of tin colloid ($K_{tc-A}$) compared with the blood clearance ($K_{bc}$). (In 21 patients)
Figure 3.4 The reproducibility of all 3 clearance measurements (Ktc, Ktc-A and Kbc) in 8 paired measurements (except for Kbc where only 7 results were measured).

3. 4a Ktc

Ktc (initial) min⁻¹

0.20

0.10

0.10 0.20 0.30

Ktc (repeat) min⁻¹

3. 4b Ktc-A

Ktc-A (initial) min⁻¹

0.30

0.20

0.10

0.10 0.20 0.30 0.40

Ktc-A (repeat) min⁻¹
3. 4c Kbc

Kbc (initial)
min$^{-1}$

Kbc (repeat) min$^{-1}$
Figure 3.5 The clearance of tin colloid (Ktc) compared with the scan score

colloid clearance od

SCAN SCORE

0.30
0.20
0.10
0
DISCUSSION

From figure 3.4 the reproducibility of measuring the disappearance rate constant of radiolabelled tin colloid by forehead counting is confirmed and is essential for its clinical application.

Similar results (Figure 3.3) have been achieved by comparing the disappearance rate constant directly from the blood stream (Kbc) with a corrected rate constant measured from head counting over a similar time scale (Ktc-A). This confirms that the disappearance rate constant measured over the forehead does represent blood clearance. The difference between these rate constants is accounted for by the inability of head counting to allow for background radiation activity as a very small percentage of tin colloid is taken up by the surrounding tissues.

The disappearance of tin colloid from the blood stream is a biexponential process. The first rate constant is rapid with a mean half-life of about 2 to 5 minutes in normal patients and this mean may be extended in severe liver disease. The second rate constant is much slower with a half life of greater than 3 hours and therefore has a very small rate constant.

It has been shown that the normal liver clears over 90% of colloid from the blood flowing through it\textsuperscript{179}. With hepatic disease it is possible that the clearance of colloid by the liver is reduced, thus increasing the amount of colloid available for uptake by the spleen and other sites of reticuloendothelial cells such as the bone marrow. However, the uptake of colloid by the liver is maximal in the first 5 minutes after injection and it is only after this time that the other sites of reticuloendothelial function take a more active role in
colloid uptake. The study of ten patients in which there was no activity over the bladder suggests that neither colloid nor free Technetium are excreted in the urine. In this analysis the uptake over the period 2 to 5 minutes is represented by Ktc and over this period the influence of the slower second exponential component is insignificant\textsuperscript{116}.

All of the patients studied with liver disease were severe enough to need hospital admission rather than outpatient investigation. For Ktc, 18 (62\%) had an abnormal clearance and if the patients with metastatic disease are excluded the abnormals rise to 70\% which makes the initial fast rate constant suitable as a liver function test.

A decrease in colloidal clearance in metastatic liver disease would not be expected unless a large percentage of functional hepatic mass was replaced by tumour and this probably only occurs when death is imminent. Kupffer cells normally mount a cytotoxic reaction when activated\textsuperscript{180, 181} and only when there is a local deficiency of Kupffer cells can cancer cells establish themselves within the liver but overall Kupffer cell numbers are rarely depleted in metastatic liver disease\textsuperscript{182}.

There was no significant relationship demonstrated between scan score and Ktc (Figure 3.5) suggesting that using the static liver image to derive a functional, quantitative measure is not clinically useful as a liver function test.

A reduced clearance of colloid by the liver is unlikely to be due to reduction in the number of Kupffer cells since the amount of colloid used in scanning is less than 2\% of that required to saturate the RES\textsuperscript{112}. Reduction of the phagocytic ability of the Kupffer
cells is another possibility but in cirrhosis Chiandussi\textsuperscript{112} found evidence that the phagocytic ability of the RES, as a whole, was increased rather than decreased.

Clearance of colloid by the liver would be decreased by the presence of intrahepatic shunts which bypass the sinusoids. Such shunts are commonly present in cirrhosis and Shaldon\textsuperscript{179} showed that this was the explanation for the reduced clearance of colloidal albumin in this condition. Extrahepatic portosystemic collaterals further reduce the amount of colloid reaching the liver and Castell\textsuperscript{171} has shown a close relationship between the extent of the increased activity in the spleen on liver scans from patients with cirrhosis and the degree of abnormality of the ammonia tolerance test, a known index of intra and extrahepatic collateral circulation. Eddleston\textsuperscript{172} showed a similar relationship between increases in splenic activity in cirrhotics and the presence of oesophageal varices on barium swallow. Significant portosystemic collateral circulation is rarely seen in patients with diseases involving the RES\textsuperscript{172}.

Previous authors\textsuperscript{114,116,117} have suggested that the colloidal clearance rate may be a useful adjunct to measure at the time of performing a static liver scan to assist in the categorisation of liver disease.

In this study we have shown a significant difference between controls and patients with liver disease. This confirms the usefulness of the colloidal clearance rate as a means of classifying liver disease and functional scintigraphy has the advantage of being convenient, non-invasive and quantitative. Using tin colloid and measuring blood disappearance, results similar to those found for the liver uptake of sulphur colloid have been obtained, but direct comparisons of the 2 colloids has not been made.
Although the disease categories only have small numbers, the presence of widespread liver disease produced more clearcut results than the focal lesions found in metastatic disease.

The failure of colloidal clearance rate to diagnose patients with metastatic liver disease is not unexpected and the use of hepatic perfusion indices produces good diagnostic discrimination in metastatic disease\textsuperscript{183,184}.

Colloidal clearance is a good measure of reticuloendothelial cell function and the relationship between colloidal clearance and conventional methods of assessing liver disease by liver function tests and or Child-Pugh grading needs to be investigated.

The importance of measurement of RES function is emphasised by Canalese\textsuperscript{118} in 1982 who showed that loss of RES function is a grave prognostic sign in acute fulminant hepatitis. Although patients with this condition were not available in this study, the loss of RES function in patients with severe liver disease may be an important prognostic indicator.
CHAPTER 4

CLINICAL STUDIES 2

A comparison of the clearance of tin colloid with biochemical liver function tests and the clearance of indocyanine green. There was a 2 year follow up of all patients.

Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1. Introduction</td>
<td>67</td>
</tr>
<tr>
<td>4.2. Patients, Methods, Materials and Calculations</td>
<td>68</td>
</tr>
<tr>
<td>4.3. Results</td>
<td>72</td>
</tr>
<tr>
<td>4.4. Discussion</td>
<td>81</td>
</tr>
</tbody>
</table>
4.1. Introduction

In Chapter 3 the disappearance rate constant of tin colloid was shown to be able to classify liver disease in a similar way to that reported for sulphur colloid. The initial rate constant of tin colloid over the period of 2 to 5 minutes after injection provided a good discrimination of liver function.

Single biochemical liver function tests fail to give a quantitative estimate of functioning hepato-cellular mass\textsuperscript{46, 56, 96}. It may be that alterations of a single enzyme system is unlikely to predict outcome accurately. The potential of use of more than one liver function test in patients with severe liver disease has been investigated. Severe liver disease is associated with varying degrees of hepatic functional impairment and it is important to have an accurate measure of hepatic reserve. The clearance of indocyanine green is recognised as giving an accurate measure of hepatic functional reserve\textsuperscript{83} and has been used in this chapter.

Kupffer cell function is not independent of hepatocyte function. Opsonisation of particulate matter in the blood stream facilitates phagocytosis and involves specific antibodies and non-specific opsonins such as fibronectin. All of these proteins are manufactured by the hepatocytes. The interdependence of both Kupffer and hepatocyte cell function is illustrated by studies on patients with fulminant acute hepatitis by Canalese\textsuperscript{118} who found in 32 patients that those with worse reticuloendothelial function in addition to their hepatocyte dysfunction had a greater incidence of complications.
In this chapter the combination of an hepatocyte and a Kupffer cell function test is investigated as a method of predicting outcome in these patients with significant liver disease and compared with conventional biochemical tests of liver function.

4.2. **Patients, Materials, Methods and Calculations**

4.2.1 **Patients**

**Liver Disease**

Twenty one patients with liver disease were divided between alcoholic liver disease (n=8), metastatic disease (n=6) and other liver diseases (n=7). This latter group consisted of 3 cases of primary biliary cirrhosis, 3 cases of sclerosing cholangitis and a single case of hepatoma. The diagnosis was arrived at by a combination of clinical and laboratory findings and all patients had a liver biopsy.

Patients with significant liver disease who were admitted to hospital were selected for investigation. They were recruited on the day of testing to try to minimise any bias in the selection of patients. No patient was studied within 2 weeks of an acute bleeding episode and both newly diagnosed and old patients were included.

**Follow up:** All patients were followed up in the out-patients clinic at 1 to 6 monthly intervals.

**Controls**

Controls were selected from patients admitted for routine surgery of hernia repair (n=3), haemorrhoidectomy (n=2) and varicose vein surgery (n=1). All had normal biochemical liver function tests and no evidence from their history, examination or
operative findings of liver disease. All were tested after an overnight fast and patients admitted for routine surgery on an afternoon list were invited to take part as they could be studied on the morning of the day of surgery to minimise inconvenience. These patients were selected from in-patients on the ward and no in-patient was tested within 7 days of a general anaesthetic.

4.2.2 Methods

All patients were starved overnight and positioned supine on a bed. The detector was carefully placed over the forehead (as in chapter 3) and 19 gauge butterfly intravenous cannulae inserted into both cubital fossae. After withdrawing 20 mls of blood as a blank an intravenous bolus of tin colloid (80 MBq in 0.2. mls) and indocyanine green (0.5 mgs/Kg) was rapidly injected with a saline wash through. Blood was withdrawn from the other cubital fossa at 3 minute intervals for up to 30 minutes.

From the initial 20 mls sample, 10 mls was sent for determination of biochemical liver function tests, full blood count and prothrombin time. The other 10 mls was centrifuged and used as a serum blank for the indocyanine green determination.

All other samples were centrifuged for 20 minutes at 5,000 r.p.m. and the serum extracted. This was pipetted into cuvettes and the absorbance at 805 µm was measured using a Spectrocam 1092 spectrophotometer\textsuperscript{112}. 

69
4.2.3 Materials and Calculations

1. Tin Colloid

Tin colloid was prepared as in Chapter 3.

The peripheral disappearance of tin colloid was calculated as in Chapter 3 with the initial rate constant, $K_{tc}$, used as the measure of Kupffer cell function.

2. Indocyanine Green

Under sterile conditions the Cardio Green was dissolved with aqueous solvent and the solution used within 4 hours of preparation (Becton Dickinson, Baltimore, Maryland, USA).

In the single injection technique ICG kinetics were analysed according to a one compartmental open model. The 3 minute peripheral plasma ICG concentrations were plotted against time on a semi-logarithmic scale and an exponential type of slope determined by the method of least squares. A typical plot of absorbance against time is shown in figure 4.1. The slope of this plot gave a measure of the half-life $t_{1/2}$. The clearance $K_{icg}$ was determined from the formula:

$$K_{icg} = \frac{0.693}{t_{1/2}}$$

where $K_{icg} =$ disappearance rate constant of indocyanine green.

Statistics

Predictions of outcome were assessed by the Fisher’s exact test. Comparisons between groups of patients were made using the Mann Whitney test.
Figure 4.1 A typical plot of the plasma disappearance of indocyanine green against time.

ICG plasma concentration
mgs/1 x 10^{-2}

Time (minutes)
4.3. Results

Over a 2 year follow up 12 (57%) of the patients died. Three of the 8 patients with alcoholic liver disease died, 2 of them with progressive liver failure and 1 following haemorrhage from oesophageal varices. Of the patients with liver metastases 5 out of the 6 died within 2 years from carcinomatosis. Within the third category of miscellaneous liver diseases, there were 4 deaths out of 7 patients from liver failure. In 2 of these patients, the terminal illness was complicated by bleeding from their oesophageal varices.

In table 4.1. the results of all liver function tests are summarised. All patients had biopsy proven advanced liver disease and in this table the sensitivity of each of the tests is given. As a predictor of liver disease, bilirubin, AST and Ktc and Kicg were very similar in their ability to predict liver disease with a success rate of 62% to 67%. The 2 synthetic measurements of liver function were the least successful at predicting liver disease with albumin only having a rate of 48% and prothrombin the lowest success rate of 19%. Alkalkine phosphatase estimations were the most accurate with a success rate of 95%. Only 1 patient had all biochemical liver function tests in the normal range.
**Table 4.1.** The ability of each liver function test to predict abnormal liver function. For all 21 patients with biopsy proven liver disease.

<table>
<thead>
<tr>
<th>TEST</th>
<th>Patients with abnormal result</th>
<th>NUMBER</th>
<th>PERCENTAGE</th>
<th>NORMAL RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td></td>
<td>14</td>
<td>67%</td>
<td>&lt;15 μmols/L</td>
</tr>
<tr>
<td>AST</td>
<td></td>
<td>13</td>
<td>62%</td>
<td>&lt;37 iu/L</td>
</tr>
<tr>
<td>Alk Phos</td>
<td></td>
<td>20</td>
<td>95%</td>
<td>28 - 92 iu/L</td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td>10</td>
<td>48%</td>
<td>34 - 50 gms/L</td>
</tr>
<tr>
<td>Prothrombin Ratio</td>
<td></td>
<td>4</td>
<td>19%</td>
<td>&lt;1.5</td>
</tr>
<tr>
<td>Ktc</td>
<td></td>
<td>13</td>
<td>62%</td>
<td>0.12 - 0.24 mins⁻¹</td>
</tr>
<tr>
<td>Kicg</td>
<td></td>
<td>14</td>
<td>67%</td>
<td>0.14 - 0.28 mins⁻¹</td>
</tr>
</tbody>
</table>
In table 4.2, the mean values of each liver function test are shown for the patients with liver disease, subdivided into, alive (those who survived 2 years), dead (those who died within two years of follow up) and for the control patients tested. Overall there were significant differences between the total liver disease group and the controls for all liver function tests except albumin and prothrombin time. When the liver function tests are compared between those who died and those who survived bilirubin, alkaline phosphatase and Ktc are significantly different.

The results have been subdivided for the 3 categories of liver disease in Table 4.3. Although the numbers are small, only Ktc and Kicg retain the differences across all three categories of liver disease and these differences are most marked in the patients with intrinsic liver disease as against those with metastatic liver disease.

The clearance of tin colloid (Ktc) is compared with the biochemical measures of liver function bilirubin, aspartate transferase (AST), alkaline phosphatase, albumin and prothrombin time. These results are presented in Appendix 1 and no relationship was demonstrated between the respective pairs of measurements.

In Table 4.4, the ability of individual liver function tests to predict outcome (death) is summarised. Only serum albumin determination was significant in predicting death (p<0.05, Fisher’s exact).
**Table 4.2.** Mean value of each liver function test for both controls and patients with liver disease. In addition, the patients with liver disease are divided by death of survival. (Results from the 21 patients with liver disease and 6 controls).

<table>
<thead>
<tr>
<th>Liver Function Test</th>
<th>Mean Value of Liver Function Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (n = 6)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>AST</td>
<td>28</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>58</td>
</tr>
<tr>
<td>Albumin</td>
<td>41</td>
</tr>
<tr>
<td>Prothrombin Time</td>
<td>12</td>
</tr>
<tr>
<td>Ktc</td>
<td>0.20</td>
</tr>
<tr>
<td>Kicg</td>
<td>0.22</td>
</tr>
</tbody>
</table>

* p<0.01 for differences to control group (Mann Whitney)

** p<0.005 for differences to control group (Mann Whitney)

*** p<0.01 for differences to dead group (Mann Whitney)
Table 4.3. Liver function tests for each category of liver disease divided by death and survival for the 21 patients with liver disease tested.

<table>
<thead>
<tr>
<th></th>
<th>Alcoholic Liver Disease</th>
<th>Metastatic Liver Disease</th>
<th>Other Liver Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dead (n=3)</td>
<td>Alive (n=5)</td>
<td>Dead (n=5)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>55</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>AST</td>
<td>56</td>
<td>29</td>
<td>54</td>
</tr>
<tr>
<td>AP</td>
<td>111</td>
<td>110</td>
<td>167</td>
</tr>
<tr>
<td>Albumin</td>
<td>33</td>
<td>39</td>
<td>36</td>
</tr>
<tr>
<td>Prothrombin Time</td>
<td>15</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Ktc</td>
<td>0.07</td>
<td>0.13</td>
<td>0.16</td>
</tr>
<tr>
<td>Kicg</td>
<td>0.04</td>
<td>0.11</td>
<td>0.16</td>
</tr>
</tbody>
</table>
Table 4.4  Ability of each liver function test (whether in the normal range) to predict death, subdivided by the category of liver disease, for the total of 12 deaths from the 21 patients tested. Results expressed as a fraction of the deaths in the patients in each group.

<table>
<thead>
<tr>
<th>Liver Function Test</th>
<th>Alcoholic Liver Disease (n=8)</th>
<th>Metastatic Liver Disease (n=6)</th>
<th>Miscellaneous Liver Disease (n=7)</th>
<th>Total (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
<td>Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0/2</td>
<td>3/6</td>
<td>4/5</td>
<td>1/1</td>
</tr>
<tr>
<td>AST</td>
<td>0/5</td>
<td>3/3</td>
<td>2/3</td>
<td>3/3</td>
</tr>
<tr>
<td>AP</td>
<td>0</td>
<td>3/8</td>
<td>1/1</td>
<td>4/5</td>
</tr>
<tr>
<td>Albumin</td>
<td>1/6</td>
<td>2/2</td>
<td>2/3</td>
<td>3/3</td>
</tr>
<tr>
<td>PT</td>
<td>2/6</td>
<td>1/2</td>
<td>5/6</td>
<td>0</td>
</tr>
<tr>
<td>Ktc</td>
<td>0/3</td>
<td>3/5</td>
<td>2/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Kicg</td>
<td>0/2</td>
<td>3/6</td>
<td>3/4</td>
<td>2/2</td>
</tr>
</tbody>
</table>
Table 4.5 summarises how many of the patients were abnormal for both of the tests and the number of deaths in these patients. The best predictability of death occurred with the combinations serum albumin and clearance of tin colloid, serum albumin and clearance of indocyanine green and serum albumin and serum bilirubin (p<0.01, Fisher’s exact). The combinations of clearance of tin colloid and serum AST, and clearance of ICG and serum AST were also significant (p<0.05, Fisher’s exact).

The combination of clearance of indocyanine green and clearance of tin colloid was not significant in its ability to predict death. This combination is presented in figure 4.2 with the deaths marked and distinguished as being from progressive liver disease or from metastatic disease. It is clearly shown that if only death from intrinsic liver disease is considered then the predictability of death in this subgroup is greatly improved by combining the tests as 4 out of the 5 patients with metastatic liver disease who died had either Ktc or Kicg in the normal range.
Table 4.5  Combination of liver function tests showing the numbers of deaths expressed as a fraction of the patients in each group who have both of the liver function tests abnormal. (There were a total of 12 deaths out of 21 patients).

<table>
<thead>
<tr>
<th>Liver Function Tests</th>
<th>Ktc</th>
<th>Bilirubin</th>
<th>AST</th>
<th>AP</th>
<th>Albumin</th>
<th>Prothrombin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8/11</td>
<td>8/11</td>
<td>8/10**</td>
<td>9/12</td>
<td>8/8*</td>
<td>3/4</td>
</tr>
<tr>
<td>Ktc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kieg</td>
<td>--</td>
<td>7/14</td>
<td>8/10**</td>
<td>9/14</td>
<td>7/7*</td>
<td>3/4</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>--</td>
<td>--</td>
<td>8/11</td>
<td>7/7*</td>
<td>7/8**</td>
<td>3/4</td>
</tr>
<tr>
<td>AST</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>10/13</td>
<td>10/13</td>
<td>3/3</td>
</tr>
<tr>
<td>AP</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>9/10**</td>
<td>3/4</td>
</tr>
<tr>
<td>Albumin</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>3/3</td>
</tr>
</tbody>
</table>

* p<0.01 (Fisher's exact)

** p<0.05 (Fisher's exact)
Figure 4.2 Comparison of the clearance of tin colloid (Ktc) with the clearance of indocyanine green (Kicg) with all deaths marked by triangles. (Those from intrinsic liver disease marked by △ and those from metastatic disease by ▲).
4.4 Discussion

The clearance of tin colloid and indocyanine green were only successful in predicting liver disease in about 60% of patients. They were both similar to the individual biochemical liver function tests of bilirubin and AST but worse than the serum alkaline phosphatase which was the most specific. Similarly the combination of biochemical liver function tests was just as accurate, as this combination includes the serum alkaline phosphatase, only failing to give a reliable diagnosis of liver disease in 1 case. As all of the patients with liver disease had significant disease such that they were admitted to hospital for investigation, it is disappointing that the clearance tests did not fare better than the biochemical tests. The good result with the battery of tests known as biochemical liver function tests illustrates why they have stood the test of time and been used to screen for the diagnosis of liver disease.

One of the main difficulties in studying liver function tests is to be able to compare any new test with a recognised 'gold standard' test of liver function. The liver has numerous functions and each liver function test only measures one of these. Therefore no single test has found favour but the Child-Pugh clinical grading has been recognised as a useful prognostic indicator in cirrhotic liver disease. As these tests have found most use as a predictor of outcome (death), I have used death as my yardstick to measure the usefulness of newer liver function tests. In this particular study only 15 patients had intrinsic liver disease (by excluding the patients with metastatic liver disease) and the relationship with Child-Pugh grading was not explored.

Of the patients studied with liver disease, 12 (56%) died over the 2 years of follow-up which emphasises that this group of patients had significant liver disease.
single reading the finding of an abnormal serum albumin was the only test which was significantly able to predict outcome (p<0.05, Fisher's exact) but the mean value of serum albumin was not significantly different between either controls and liver disease, and within liver disease by death or survival (NS, Mann Whitney). The serum albumin is known to be low in patients with chronic liver disease as the rate of synthesis falls from about 10 grams per day to 4 grams per day. As the half-life of albumin is about 22 days, this fall in serum concentration is slow\textsuperscript{185}. Therefore a patient with fulminant hepatic failure may die with a normal serum albumin. In this study no patient had acute liver failure but many were newly diagnosed with liver disease which may explain why they all had a low serum albumin such that there was no significant difference (NS, Mann Whitney) between deaths and survivors and not all had yet fallen out of the normal range.

This indicates another difficulty in defining a normal range for each test or more importantly a threshold level below which the test has clinical relevance because clearly the serum albumin is a useful test of liver function when used to predict outcome but did not show significant differences between death and survival.

These results confirm the usefulness of serum albumin estimations which has long been recognised\textsuperscript{53, 186} with the incorporation of serum albumin in the clinical gradings of Child\textsuperscript{48} and Pugh\textsuperscript{49} probably the most widely used in cirrhotic liver disease as they convey better prognostic information than individual serum values.

In this work, the prognostic value of measuring the clearance of tin colloid as a measure of Kupffer cell function and the clearance of indocyanine green as a measure of hepatocyte function has been particularly investigated. Individually and in combinations,
they were not significant in predicting outcome (NS, Fisher's exact). As individually both
of the clearance tests of liver function were comparable to the individual biochemical tests
of liver function (except for serum alkaline phosphatase), would it be possible to improve
these results? There is a clear suggestion from figure 4.2 that if deaths from intrinsic liver
disease alone are considered (excluding deaths from metastatic liver disease) then the
combination of a low clearance of indocyanine green and a low clearance of tin colloid
carries a very poor prognosis as 7 out of the 10 patients died.

Although the numbers were small in Table 4.3, the conventional liver function tests
were less discriminatory for metastatic liver disease than the other two categories. Even
the clearance of tin colloid and indocyanine green, which were different for all categories,
were less so for the metastatic liver disease than the other groups. This emphasises that
heterogeneity exists in liver disease. Even a disease process such as cirrhosis has a
multitude of aetiological factors. To improve the interpretation of results it will be
necessary to use a tightly defined group of patients with liver disease such as patients with
cirrhotic alcoholic liver disease.

Bircher\textsuperscript{46} had stated the major disappointment of quantitative liver function tests
was they have not been useful in differential diagnosis and their strength lay in their
prognostic capabilities which were largely independent of aetiological categories. This
work in Chapters 3 and 4 suggests otherwise. In Chapter 3 the clearance of tin colloid is
helpful in the differential diagnosis of liver disease and in this Chapter there is the
suggestion that the prognostic capabilities of clearance tests are dependent on the aetiology
of the liver disease.
The clearance of tin colloid and the clearance of indocyanine green are both rapid with a half life of between 2 and at the most 20 minutes. As such they are both dependent on liver blood flow as clearance is the product of liver blood flow and extraction efficiency. The clearance of high extraction compounds is blood flow dependent and itself depends on the processes of membrane carrier transport which subserves both entry into and exit from liver cells. As intravenous disappearance curves are calculated from data obtained over a period of many circulation times, they contain the results of not only influx but also efflux from the cells and must represent an amalgam arising from the influx and efflux processes. Would a low extraction test of liver function such as caffeine or Disida work better as these sorts of tests are less dependent on liver blood flow?

In this work, indocyanine green was evaluated using an intravenous dose of 0.5 mgs per kilogram on patients with advanced liver disease. Paumgartner has shown that at these concentrations the clearance of indocyanine green is only sensitive in patients with severe liver disease and in patients with less severe disease repeat studies may be necessary at varying concentrations of indocyanine green. These sorts of studies would be extremely cumbersome. Theoretically indocyanine green disappearance curves can be measured without repeated blood sampling by dichromatic ear lobe densitometry but this method is not always reliable and the ear lobe readings do not correlate closely with simultaneously obtained plasma values.

In the work of Canalese, the second set of tests gave greater prognostic value. In this part of the study only a single set of tests were evaluated but possibly repeat tests or changes between tests could be more important in predicting outcome.
There is the clear suggestion from figure 4.2 that the combination of a low clearance of tin colloid and a low clearance of indocyanine green carries a poor prognosis. Why should Kupffer cell function affect outcome in chronic liver disease and is the combination of hepatocyte and Kupffer cell malfunction more lethal? Previous studies have looked at similar combinations. Hofer in 1955 and Taplin in 1961 both combined the clearance of colloid and dye to derive a fractional dye extraction, F, which is the ratio of the dye clearance and the colloid clearance. Hofer found that only when hepatocyte cell function begins to fail does F decrease to values below normal. Taplin used this ratio to monitor the recovery from obstructive jaundice. In most patients with cirrhosis values for F do not differ significantly from those found in normal subjects. This suggests that the reduced blood clearance or increased dye retention seen in these patients is brought about by a reduction of liver blood flow rather than by an impairment of parenchymal cell function.

The ratio failed to find favour clinically and on theoretical grounds is unlikely to work. Clearance is the product of liver blood flow and extraction efficiency. If liver blood flow is unchanged than F is the ratio of the extraction efficiencies. Consequently F may remain normal even in the presence of severe liver disease if the extraction efficiencies decline at the same rate.

A major contribution to the demise of these patients may occur when the RES is no longer adequate to prevent the spread of endotoxins from the portal to the systemic circulation. Whether this is primary damage to the Kupffer cell or secondary damage is not known. Phagocytosis by the Kupffer cells is facilitated by both specific and non-specific opsonins. All of these non-specific proteins are manufactured by hepatocytes and
the most important of them is fibronectin\textsuperscript{16,190}. Possibly reduced quantities of circulating fibronectin contributes to the loss of clearance by the Kupffer cells. In addition low circulating levels of complement factors C3 and C4 have been reported and may contribute to loss of phagocytic activity\textsuperscript{107,191}. These factors which are influential in the interdependence of Kupffer cells and hepatocytes should also be investigated.
CHAPTER 5

Clinical Studies 3 (a)

In this chapter the clearance of tin colloid, indocyanine green, Disida and caffeine are measured and the correlation of these tests with both liver disease and prognosis is studied over a year follow up.

CONTENTS

5.1. Introduction 88
5.2a Patients 91
   (i) Liver Disease
   (ii) Controls

5.2b Materials and Calculations 92
   (i) Tin Colloid
   (ii) Indocyanine Green
   (iii) Disida
   (iv) Caffeine
   (v) Fibronectin
   (vi) Complement

5.2c Methods
5.2d Statistics

5.3 Results 99
5.4 Discussion 133
5.1 INTRODUCTION

In Chapters 3 and 4 tin colloid clearance was a valuable measure to classify liver
disease and the results achieved were similar to those by sulphur colloid clearance.
Secondly the combination of clearance tests of ICG and tin colloid was investigated but
were not found to be a better indicator of prognosis than the serum albumin alone.

In Chapters 3 and 4 a bedside technique utilising a portable gamma camera was
used to measure tin colloid clearance and in this part of the study a fixed gamma camera
has been used. With only a single portable detector it is not possible to investigate more
than one region of interest but the fixed gamma camera has enabled measurement over
both the heart, spleen and liver with calculation of the plasma disappearance rate constant,
the liver uptake constant and the spleen to liver ratio by analysis of the static pictures.

Wasnich\textsuperscript{192} compared average splenic activity to average right lobe liver activity
and formulated the spleen to liver activity ratio (S:L ratio). In 100 control patients the S:L
ratio had a mean of 0.74 whereas patients with cirrhosis (n=9) and fatty metamorphosis
(n=15) of the liver were found to have raised S:L ratios. McLaren\textsuperscript{193} measured the liver to
spleen (L:S) ratio in 33 controls and 46 cirrhotics. The L:S ratio was significantly
decreased in patients with cirrhosis (sensitivity of 87\%). Both raised portal pressures and
decreased hepatic extraction of colloid lead to an increase in functional splenic activity and
quantification of the L:S ratio provided an index of distribution. There was a close
relationship between increased splenic activity and the presence of oesophageal varices.
In a similar way that the scan score may be achieved, the L:S ratio offers a further possible
quantification of hepatic function which as it is derived from static pictures is an attractive
proposition.
In Chapter 4 it was noted that there is a possible interdependence between the hepatocytes and the Kupffer cells. Opsonins, some of which are manufactured by the hepatocyte, are important for phagocytosis by the Kupffer cell and to investigate this further the serum levels of fibronectin and the complement fragments C3 and C4 have been measured.

Fibronectin describes a family of structurally and immunologically related high molecular weight glycoproteins that are present on many cell surfaces, in extracellular fluid, in connective tissue and in most basement membranes. It plays an important part in opsonising cell debris, serving the attachment of collagen fibrils and proteoglycans to microparticles. Low plasma values are found in fulminant hepatic failure, probably due to failure of hepatic synthesis and to consumption by the phagocytic process\textsuperscript{194}. Reduced concentrations of fibronectin in patients with sepsis and trauma may be responsible for the impairment of RES function and low serum levels were associated with a poor outcome in hepatic failure\textsuperscript{195}.

In hepatic cirrhosis, the removal of immune complexes by the reticuloendothelial cells of the liver is impaired and these complexes accumulate in the plasma\textsuperscript{196}. In most cases these complexes do not result in activation of C3 but if activated, significant tissue damage would result. Complement components are produced by either the mononuclear phagocytes or hepatocytes and are often reduced in acute and chronic liver disease\textsuperscript{107, 197} but they maybe normal or even raised\textsuperscript{198}. Faced with this conflict of opinion, the serum levels of total C3 and C4 levels have been measured.
Both the clearances of tin colloid and indocyanine green are blood flow dependent. In this chapter the use of low extraction clearance tests of hepatocyte function will be investigated so that the effect of blood flow may not dominate and the results of a greater variety of tests are available for comparison. Therefore clearances of two newer compounds, caffeine and Disida will be evaluated to assess whether alone or in combination these tests can improve the predictability of outcome.

Caffeine and Disida have been investigated because both have the potential to provide a fast and slow component of clearance as each offers a blood flow and a metabolic rate constant. The kinetics of the disappearance of caffeine following an intravenous bolus have been investigated and the fast rate constant described which has not previously been defined. Previous studies have used oral doses and only described the second, slow clearance constant. The liver uptake and hepatic elimination of Disida have also been described in alcoholic cirrhotic liver disease for the first time to my knowledge.

These tests were conducted in a homogeneous group of patients with severe alcoholic liver disease chosen in order to minimise discrepancies between liver disease of varying aetiology. The prognosis in alcoholics is much better than in other forms of cirrhosis but this depends on whether the alcoholic can overcome his addiction. Continued heavy drinking is associated with poor survival and the highest mortality with alcoholic cirrhosis is in the first year of follow up. In this study, death has been used as the yardstick by which the severity of the liver disease is measured over a one year follow up.
5.2 Patients, Materials, Methods and Calculations

5.2a Patients

(i) Liver Disease

Twenty five patients (19 males and 6 females), aged 36 to 82 years, were entered into the study with alcoholic liver disease either diagnosed by biopsy or a clinical problem compatible with a strong history of alcohol intake. Fourteen of the patients had had a liver biopsy confirming the diagnosis of cirrhosis (the decision to perform a liver biopsy was dependent on the consultant in charge of each case). All had objective evidence of portal hypertension manifested by oesophageal varices which had previously bled, and were currently undergoing injection sclerotherapy. No patient was tested within 2 weeks of an acute bleeding episode.

(ii) Controls

a) Caffeine

Ten controls (2 females) were studied. Five were patients admitted for routine surgery for varicose veins (n=2), circumcision (n=2) and hernia repair (n=1). Five were volunteers from the research laboratory workers.

b) Disida

Ten controls (4 female) were studied and all were patients admitted for routine surgery of hernia repair (n=5), varicose veins (n=2), haemorrhoidectomy (n=2) and circumcision (n=1).

All 20 controls had normal liver function tests and no evidence from their history or examination of liver disease. They were all non-smokers with a mean age of 45 years.
and a range of 25 to 75. All were tested after an overnight fast and patients admitted for routine surgery on an afternoon list were invited to take part as they could be studied on the day of surgery to minimise inconvenience. No patient was tested within 7 days of a general anaesthetic.

5.2b Materials and Calculations

(i) Tin Colloid

Tin colloid was prepared and administered as in Chapter 3.

a) Blood clearance and liver uptake

Digital images were recorded as 128 x 128 matrices in a 2 stage dynamic acquisition and stored using an on-line computer facility. For blood clearance a region of interest, avoiding other organs, was constructed over the heart and the clearance calculated from counts between 2 and 5 minutes.

The liver uptake constant was calculated by subtracting each point on the curve from the plateau value which was taken as the mean amplitude of the liver uptake curve between 12 and 15 minutes. The value of K was obtained by a least squares linear regression on the natural logarithm of the subtracted curve.\textsuperscript{116}

b) Liver to Spleen Ratio

A liver to spleen activity ratio (L:S ratio) was measured by calculating the geometric mean of counts from the anterior and posterior images.\textsuperscript{193}

(ii) Indocyanine Green

Indocyanine green was prepared, administered and calculations performed as in Chapter 4.
(iii) **Disida**

Tc-99m disofenin was prepared to immediate requirements from kits using sodium pertechnetate from a generator with 110 to 140 MBq of 99m Tc labelled Diisopropyl iminodiacetic acid (Disofenin) injected intravenously.

A. **Liver Uptake and Elimination of Disida**

Images were acquired in a 64 by 64 matrix by a large field of view Anger camera into a dedicated Nuclear Medicine computer system. The framing rate was 1 image per second for 80 seconds followed by an image every 30 seconds for the next 90 minutes.

At the conclusion of the study a region of interest was drawn over the right lobe of the liver, care being taken to avoid the gall bladder and the major bile ducts. A time activity curve was then generated from the pixels in this region and a typical time activity curve is shown in figure 5.1.

The liver uptake and elimination of Disida is a biexponential process represented by:-

\[ C = Ae^{at} + Be^{bt} \]

Where \( C \) = counts over the liver

A and B = y intercept constants

a and b = fast and slow rate constants

t = time in minutes

\( a = K1d \) = liver uptake constant of Disida

\( b = K2d \) = liver elimination constant of Disida
Figure 5.1 A typical time activity curve for the uptake and elimination of Disida measured over the liver.
(iv) **Caffeine**

Caffeine was prepared under sterile conditions in the hospital pharmacy as a sterile sodium benzoate solution in water for injection containing 100 mgs of caffeine and 100 mgs of sodium benzoate per 10 ml ampoule.

Caffeine was measured by the enzyme immunoassay supplied by EMIT\(^{201}\).

**A) Intravenous Clearance**

The plasma activity curves exhibited a biexponential behavior (Figure 5.2) according to the equation:

\[
\text{Concentration (c) at time (t)} = Ae^{at} + Be^{bt}
\]

Where \(A\) and \(B\) are y intercept constants

\(a\) and \(b\) are rate constants for rapid and slow exponentials

\(t\) is the time in minutes

\(K_{1c} = a\) was measured over the first 5 to 20 minutes

\(K_{2c} = b\) was measured over the period 60 minutes to 360 minutes.

In addition the fasting serum caffeine (FSC) level was recorded.

**B) Salivary Clearance**

Assuming first orders kinetics, the caffeine elimination constant was calculated as follows:-

\[
K_{cs} = \frac{\ln C_1 - \ln C_2}{\delta t}
\]

Where \(C_1\) = the caffeine saliva concentration at 60 minutes

\(C_2\) = the caffeine saliva concentration at 360 minutes

and \(\delta t\) = the time elapsed between collections
Figure 5.2 A typical plot of the intravenous clearance of a bolus of caffeine with time.
(v) **Fibronectin**

Plasma fibronectin was measured by radial immunodiffusion against monospecific antiserum (Cappel laboratories) using dilutions of purified fibronectin and standard plasma (Boehringer, Mannheim).²⁰⁰

(vi) **Complement**

Quantification of C3 and C4 levels in serum was performed by means of immunoelectrophoresis employing monospecific antisera (Boehringer, Mannheim).

5.2c **Methods**

The liver disease patients were tested on 2 consecutive days. On both occasions they were fasted for at least 8 hours prior to the start of the investigation. All had blood taken for FBC, LFT’s and clotting studies. On day 1 the patients were rested supine on a bed and had intravenous access in a cubital fossa with a 3 way tap arrangement to allow blood samples to be taken. Initially both blood and saliva samples were taken.

An intravenous bolus of 110 to 140 MBqs of Disida was injected with saline flush and the activity over both the heart and liver recorded on a gamma camera. Planar data was recorded in a 64 x 64 byte mode computer matrix at 1 second frames for 80 seconds and then 90 minutes of thirty second frames.

Simultaneously an intravenous bolus of caffeine (0.5 mgs/kg) was administered and heparinised blood samples taken at 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, 240, 300 and 360 minutes respectively. Saliva samples were aspirated by pipette at 0, 60, 120, 180, 240 and 360 minutes respectively (9 patients and 5 controls).
In 5 patients the clearance of caffeine had to be repeated by the same technique because they imbibed caffeine containing drinks or foodstuffs during the course of the study.

On day 2 in a similar manner the patients were placed supine on a bed and had intravenous access in a cubital fossa with a 3 way tap arrangement to allow blood samples to be taken. An initial blood sample was taken and then an intravenous bolus of 30 to 35 MBqs of Technetium 99m tin colloid with saline flush was given. Activity over the heart and liver was recorded by a gamma camera at 1 frame every 20 seconds. Simultaneously an intravenous bolus of indocyanine green (0.5 mgs/kg) was injected and heparinised blood samples taken at 0, 3, 6, 9, 12, 15, 21 and 30 minutes respectively.

The patients were then randomised by drawing sealed envelopes to either no treatment or to treatment with isosorbide dinitrate 20 mgs qds (See Chapter 6).

All patients then had repeat clearance studies over 2 days as above 20 to 28 weeks after the initial study.

Clinical progress was reviewed at regular attendance for sclerotherapy at intervals of between 1 week and 6 months depending on their clinical requirements which were determined by the Gastroenterologist.
All control patients were tested after an overnight fast for either caffeine clearance or Disida uptake and clearance in a manner similar to the liver disease patients.

5.2d Statistics

Comparison between groups was made using the Mann Whitney test. Predictions of outcome were assessed by Fisher’s exact test. Paired results were analysed using the paired Wilcoxon test.

5.3. Results

Eight patients died during the 12 months of follow up. All eight died of progressive liver failure although the final illness in two patients was complicated by significant gastrointestinal bleeding from their oesophageal varices. After initial testing 4 patients died 1, 3, 4 and 23 weeks after the first tests and 3 of these were in Child-Pugh grade C and 1 in Child-Pugh grade B. After the second set of liver function tests 4 further patients died 8, 14, 15 and 20 weeks after testing and 3 of these patients were in Child-Pugh grade B and 1 in Child-Pugh grade A (Figure 5.3)

Tin Colloid - Clearance

The liver uptake (Klu) and the blood clearance (Ktc) of tin colloid are compared in figure 5.4 for all 46 results in both sets of tests. The median of Ktc is about 60% of the median of Klu and only 3 points in figure 5.4 are lower than the line of identity.

Blood Clearance and Liver Uptake

In figure 5.5, the blood clearance of tin colloid (Ktc) is shown in a scatter diagram for both all results and when divided by survival or not and in figure 5.6, similar results
are shown for the liver uptake constant (Klu). There is a significant difference between
deaths and survivors (p<0.01, Mann Whitney) for Ktc but not for Klu (NS, Mann
Whitney). The changes between the repeat tests for Ktc and Klu are shown in Appendix 2
and summarised in Tables 5.1 and 5.2 with respect to the 4 deaths that occurred after the
second set of tests.
Graph detailing survival with time over the 1 year of the study.
Comparison of the blood clearance of tin colloid ($K_{tc}$) with the uptake of tin colloid ($K_{lu}$). (All 46 results from both sets of tests)
Figure 5.5 Scatter diagram of the blood clearance of tin colloid ($K_{tc}$) with respect to liver disease and within liver disease by death or survival. (First set of tests only)

Liver disease | Liver disease | Liver disease
---|---|---
Total (n=25) | Alive (n=17) | Dead (n=8)
MEDIAN (IQR) | 0.10 (0.07) | 0.13 (0.08) | 0.08* (0.06)

* p<0.01 for differences to alive group (Mann Whitney)
Figure 5.6 Scatter diagram of the liver uptake of tin colloid (Klu) with respect to liver disease and within liver disease by death or survival. (First set of tests only)

Liver Disease

<table>
<thead>
<tr>
<th></th>
<th>Total (n=25)</th>
<th>Alive (n=17)</th>
<th>Dead (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (IQR)</td>
<td>0.18 (0.09)</td>
<td>0.18 (0.07)</td>
<td>0.14* (0.12)</td>
</tr>
</tbody>
</table>

* no differences to alive group (Mann Whitney)
Table 5.1 The ability of changes in the blood clearance of tin colloid (Ktc) and the liver uptake of tin colloid (Klu) to predict death between initial testing and repeat testing at 6 months. (The 4 deaths are expressed as a fraction of those patients in each group from the total of 21 patients) - By changes between the 2 tests.

<table>
<thead>
<tr>
<th>Changes between 2 sets of tests</th>
<th>Clearance Constant of Tin Colloid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ktc</td>
</tr>
<tr>
<td>Increased</td>
<td>1/7</td>
</tr>
<tr>
<td>Decreased</td>
<td>3/13</td>
</tr>
<tr>
<td>Unchanged</td>
<td>0/1</td>
</tr>
</tbody>
</table>

Table 5.2 The ability of changes in the blood clearance of tin colloid (Ktc) and the liver uptake of tin colloid (Klu) to predict death between initial testing and repeat testing at 6 months. (The 4 deaths are expressed as a fraction of those patients in each group from the total of 21 patients) - By changes from the normal range between the 2 sets of tests.

<table>
<thead>
<tr>
<th>Changes between 2 sets of tests</th>
<th>Clearance Test of Tin Colloid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ktc</td>
</tr>
<tr>
<td>Normal to Abnormal</td>
<td>1/3</td>
</tr>
<tr>
<td>Abnormal to Normal</td>
<td>0/3</td>
</tr>
<tr>
<td>Remained Normal</td>
<td>0/7</td>
</tr>
<tr>
<td>Remained Abnormal</td>
<td>3/8</td>
</tr>
</tbody>
</table>
Tin Colloid - Liver to Spleen Activity Ratio

A scatter diagram of the liver to spleen ratio for those patients with liver disease with respect to survival or not for the first set of tests is shown in Figure 5.7. There were 5 patients with results of liver to spleen ratio greater than 3 with respective values of 4.9, 6.7, 8.6, 9.7, and 28.9. These are shown schematically at the top of the scatter diagram. There is no significant difference between the medians of survivors and those who died (NS, Mann Whitney).

Indocyanine Green

The clearance of indocyanine green is shown with respect to liver disease in figure 5.8 and there is a significant difference between the survivors and the deaths (p<0.01, Mann Whitney).

In Appendix 2 the change in value of Kicg between the two sets of tests is shown and tables 5.3 and 5.4 monitor the effect of these changes on the 4 deaths occurring after the second set of tests.
Figure 5.7 Scatter diagram of the liver to spleen activity ratio with respect to liver disease and within liver disease to death or survival. (First set of tests only)

Liver to Spleen Activity Ratio

Liver Disease

Total (n=25)

Liver Disease Alive (n=17)

Liver Disease Dead (n=8)

Median (IQR)

1.5 (1.5) 1.5 (1.2) 1.4* (4.2)

* no significant differences to alive group (Mann Whitney)
Figure 5.8 Scatter diagram of the clearance of indocyanine green (Kicg) with respect to liver disease and within liver disease by death or survival. (First set of tests only).

**ENCE OF IRINE GREEN**

<table>
<thead>
<tr>
<th>LIVER DISEASE</th>
<th>TOTAL</th>
<th>ALIVE</th>
<th>DEAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEDIAN (IQR)</td>
<td>0.09 (0.12)</td>
<td>0.13 (0.10)</td>
<td>0.03* (0.06)</td>
</tr>
</tbody>
</table>

*p<0.01 for difference to alive group (Mann Whitney)*
**Table 5.3** Ability of changes in the clearance of indocyanine green (Kicg) to predict death between initial and repeat testing 6 months later. The 4 deaths are expressed as a fraction of those patients in each group out of the total of 21 patients. (Changes between the 2 sets of tests).

<table>
<thead>
<tr>
<th>Changes between 2 sets of tests</th>
<th>Clearance of Indocyanine Green Kicg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased</td>
<td>2/7</td>
</tr>
<tr>
<td>Decreased</td>
<td>2/12</td>
</tr>
<tr>
<td>Unchanged</td>
<td>0/2</td>
</tr>
</tbody>
</table>

**Table 5.4** Ability of changes in the clearance of indocyanine green (Kicg) to predict death between initial and repeat testing 6 months later. The 4 deaths are expressed as a fraction of those patients in each group out of the total of 21 patients. (Changes from the normal range between the 2 sets of tests).

<table>
<thead>
<tr>
<th>Changes between 2 sets of tests</th>
<th>Clearance of Indocyanine Green Kicg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal to Abnormal</td>
<td>0/3</td>
</tr>
<tr>
<td>Abnormal to Normal</td>
<td>0/1</td>
</tr>
<tr>
<td>Remained Normal</td>
<td>0/3</td>
</tr>
<tr>
<td>Remained Abnormal</td>
<td>4/14</td>
</tr>
</tbody>
</table>
Intravenous Caffeine Clearances

The 3 parameters measured by intravenous caffeine clearance FSC, K1c and K2c are shown in figures 5.9, 5.10 and 5.11 with respect to controls and total liver disease and for those patients with liver disease with respect to survival or not.

Fasting serum caffeine is significantly different between liver disease and controls (p<0.01, Mann Whitney) and between the dead and alive groups (p<0.05, Mann Whitney). The second metabolic component of caffeine clearance, K2c, is also significantly different between controls and all patients within the liver disease patients divided by death or survival (p<0.01 and p<0.05 respectively, Mann Whitney). K1c is only significantly different between liver disease and controls (p<0.01, Mann Whitney). There is no significant difference between liver disease patients when divided by death or survival for K1c (NS, Mann Whitney).

In 5 patients because of their inability to refrain from an intake of caffeine containing foodstuffs for the duration of the test the FSC and K1c were repeated between 2 and 10 days after the initial test. The average difference from the line of identity was 0.70 for FSC and 0.26 for K1c, and the results are shown graphically in Appendix 3.

All tests were repeated at 6 months and the changes in the 3 parameters of caffeine with time are shown in Appendix 2. The differences over the six month period are summarised in Tables 5.5 and 5.6 with respect to the 4 deaths which occurred after the second set of tests.
**Figure 5.9** Fasting serum caffeine levels (FSC) with respect to controls and liver disease and within liver disease by death or survival. (First test of tests only)

<table>
<thead>
<tr>
<th></th>
<th>CONTROLS</th>
<th>LIVER DISEASE</th>
<th>LIVER DISEASE</th>
<th>LIVER DISEASE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=10)</td>
<td>(n=25)</td>
<td>(n=17)</td>
<td>(n=8)</td>
</tr>
<tr>
<td><strong>MEDIAN (IQR)</strong></td>
<td>1.0 (4.0)</td>
<td>16* (39)</td>
<td>5.0 (22)</td>
<td>38** (68)</td>
</tr>
</tbody>
</table>

* p<0.01 for differences to controls (Mann Whitney)
** p<0.5 for differences to alive (Mann Whitney)
Figure 5.10 Scatter diagram of the first rate constant of caffeine (K1c) for both controls and patients with liver disease and within liver disease by death or survival. (First set of tests only)

<table>
<thead>
<tr>
<th>Group</th>
<th>Controls (n=10)</th>
<th>Liver Disease (n=25)</th>
<th>Liver Disease (n=17)</th>
<th>Liver Disease (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (IQR)</td>
<td>0.17 (0.05)</td>
<td>0.07* (0.13)</td>
<td>0.11 (0.13)</td>
<td>0.05 (0.07)</td>
</tr>
</tbody>
</table>

* p<0.01 for differences to controls (Mann Whitney)
Scatter diagram of the second clearance rate constant ofiffeine (K2c) for both controls and patients with liver disease and thin liver disease by death or survival. (First set of tests only)

<table>
<thead>
<tr>
<th>Controls</th>
<th>Liver Disease Total</th>
<th>Liver Disease Alive</th>
<th>Liver Disease Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=10)</td>
<td>(n=25)</td>
<td>(n=17)</td>
<td>(n=8)</td>
</tr>
<tr>
<td>Median</td>
<td>0.0040</td>
<td>0.0019*</td>
<td>0.0023</td>
</tr>
<tr>
<td>(IQR)</td>
<td>0.0020</td>
<td>0.0029</td>
<td>0.0026</td>
</tr>
</tbody>
</table>

* p<0.01 for differences to controls (Mann Whitney)
** p<0.5 for differences to alive (Mann Whitney)
Table 5.5. Ability of changes in each caffeine function test to predict death between initial testing and repeat testing at 6 months. Deaths expressed as a fraction of those patients in each group out of a total of 21 patients. Changes between the 2 sets of tests.

<table>
<thead>
<tr>
<th>Caffeine Function Test</th>
<th>FSC</th>
<th>K1c</th>
<th>K2c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Changes Between</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased</td>
<td>4/10</td>
<td>1/10</td>
<td>0/11</td>
</tr>
<tr>
<td>Decreased</td>
<td>0/11</td>
<td>3/11</td>
<td>4/10</td>
</tr>
<tr>
<td>Unchanged</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 5.6. Ability of changes in each caffeine function test to predict death between initial testing and repeat testing at 6 months. Deaths expressed as a fraction of those patients in each group out of a total of 21 patients. Changes from the normal range between 2 sets of tests.

<table>
<thead>
<tr>
<th>Caffeine Function Test</th>
<th>FSC</th>
<th>K1c</th>
<th>K2c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Changes Between</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal to Abnormal</td>
<td>1/2</td>
<td>1/3</td>
<td>1/4</td>
</tr>
<tr>
<td>Abnormal to Normal</td>
<td>0/3</td>
<td>0/4</td>
<td>0/3</td>
</tr>
<tr>
<td>2 Sets of Tests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remained Normal</td>
<td>2/10</td>
<td>0/4</td>
<td>0/7</td>
</tr>
<tr>
<td>Remained Normal</td>
<td>1/6</td>
<td>3/10</td>
<td>3/7</td>
</tr>
</tbody>
</table>
**Salivary Caffeine Clearance**

The relationship between fasting serum and caffeine levels and fasting salivary caffeine levels is shown in Figure 5.12. In agreement with previous reports, saliva concentration averages about 75% of plasma caffeine levels\(^{168,203,204}\).

At other times of measurement the relationship between serum and saliva levels is more variable and are shown graphically in Appendix 4 at 60 and 240 minutes.

In Table 5.7 the respective paired values of K2c are listed for plasma and salivary caffeine clearance. There is little correlation between the pairs of results suggesting that the salivary clearance is of limited value when the salivary samples are collected at such a short time interval after administration.

**Disida Kinetics**

The two parameters of Disida clearance, uptake (K1d) and elimination (K2d) are shown in figures 5.13 and 5.14 with respect to controls and liver disease and within the liver disease group for death and survival for each test.

There is a significant difference between the liver disease group and the controls for both parameters (p<0.005 and p<0.01, Mann Whitney) and the significant difference is maintained between the deaths and the survivors. All tests were repeated at 6 months and in Appendix 2 the changes in Disida parameters are shown with time.

In tables 5.8 and 5.9 the changes in the parameters of Disida kinetics are summarised with respect to the 4 deaths which occurred after the second set of tests.
Figure 5.12 Relationship between fasting serum and salivary caffeine levels. (9 patients and 5 controls)
Table 5.7. Paired values of the second rate constant of caffeine clearance (K2c) evaluated from serum and saliva respectively for each patient. (14 patients only).

<table>
<thead>
<tr>
<th></th>
<th>Plasma Clearance</th>
<th>Salivary Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>K2c</td>
<td>0.0057</td>
<td>0.0038</td>
</tr>
<tr>
<td></td>
<td>0.0057</td>
<td>0.0032</td>
</tr>
<tr>
<td></td>
<td>0.0043</td>
<td>0.0036</td>
</tr>
<tr>
<td></td>
<td>0.0041</td>
<td>0.0021</td>
</tr>
<tr>
<td></td>
<td>0.0040 ***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0039</td>
<td>0.0024</td>
</tr>
<tr>
<td></td>
<td>0.0029</td>
<td>0.0020</td>
</tr>
<tr>
<td></td>
<td>0.0027 ***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0024</td>
<td>0.0016</td>
</tr>
<tr>
<td></td>
<td>0.0023</td>
<td>0.0006</td>
</tr>
<tr>
<td></td>
<td>0.0021</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>0.0019 ***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0011 ***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0010 ***</td>
<td></td>
</tr>
</tbody>
</table>

*** - No fall or a rise in salivary caffeine levels between 1 and 6 hours
Figure 5.13 Scatter diagram of the liver uptake of Disida (Kid) for both controls and liver disease and within liver disease for death or survival. (First set of tests only)

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Liver Disease Total</th>
<th>Liver Disease Alive</th>
<th>Liver Disease Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Size</td>
<td>n=10</td>
<td>n=25</td>
<td>n=17</td>
<td>n=8</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>0.20 (0.07)</td>
<td>0.14* (0.14)</td>
<td>0.14 (0.05)</td>
<td>0.08** (0.07)</td>
</tr>
</tbody>
</table>

* p<0.005 for differences to controls (Mann Whitney)
** p<0.01 for differences to alive group (Mann Whitney)
Figure 5.14 Scatter diagram of the hepatic elimination of Disida (K2d) for both controls and liver disease and within liver disease for death or survival. (First set of tests only)

<table>
<thead>
<tr>
<th></th>
<th>CONTROLS</th>
<th>LIVER DISEASE</th>
<th>LIVER DISEASE</th>
<th>LIVER DISEASE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=10)</td>
<td>(n=25)</td>
<td>(n=17)</td>
<td>(n=8)</td>
</tr>
<tr>
<td><strong>MEDIAN</strong></td>
<td>0.0025</td>
<td>0.0016*</td>
<td>0.0021</td>
<td>0.0008**</td>
</tr>
<tr>
<td><strong>(IQR)</strong></td>
<td>(0.0006)</td>
<td>(0.0013)</td>
<td>(0.0015)</td>
<td>(0.0004)</td>
</tr>
</tbody>
</table>

* p<0.005 for differences to controls (Mann Whitney)
** p<0.01 for differences to alive group (Mann Whitney)
Table 5.8 Ability of changes in each Disida function test to predict death between initial testing and repeat testing at 6 months. Deaths expressed as a fraction of those patients in each group from a total of 21 patients. (Changes between each set of tests).

<table>
<thead>
<tr>
<th>Changes Between</th>
<th>K1d</th>
<th>K2d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased</td>
<td>1/5</td>
<td>1/10</td>
</tr>
<tr>
<td>Decreased</td>
<td>3/16</td>
<td>2/10</td>
</tr>
<tr>
<td>Unchanged</td>
<td>0</td>
<td>1/1</td>
</tr>
</tbody>
</table>

Table 5.9 Ability of changes in each Disida function test to predict death between initial testing and repeat testing at 6 months. Deaths expressed as a fraction of those patients in each group from a total of 21 patients. (Changes from the normal range between each set of tests).

<table>
<thead>
<tr>
<th>Changes Between</th>
<th>K1d</th>
<th>K2d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal to Abnormal</td>
<td>0/6</td>
<td>1/2</td>
</tr>
<tr>
<td>Abnormal to Normal</td>
<td>1/1</td>
<td>0/3</td>
</tr>
<tr>
<td>Remained Normal</td>
<td>1/8</td>
<td>1/12</td>
</tr>
<tr>
<td>Remained Abnormal</td>
<td>2/6</td>
<td>2/4</td>
</tr>
</tbody>
</table>
**Fibronectin**

In total only 40 assays of fibronectin levels were available as 6 were accidentally destroyed in the laboratory (5 after initial testing and 1 after repeat testing). The serum fibronectin levels are shown in Figure 5.15 for 20 patients with liver disease for the first set of results and by death and survival for the 8 deaths that occurred after initial testing. There is no significant difference between the two groups (NS, Mann Whitney). In figure 5.16 the fibronectin levels are plotted against Ktc with no relationship demonstrated.

**Complement**

Scatter diagram for the serum levels of C3 and C4 are in Appendix 5. All levels were greater than normal and there was no significant difference between deaths and survivors (NS, Mann Whitney).
Figure 5.15 Scatter diagram of the plasma concentration of fibronectin with respect to liver disease and within liver disease to death or survival (On 20 patients from the first set of tests)

No significant differences between groups (Mann Whitney)
Figure 5.16 Comparison of the serum fibronectin levels with the blood clearance of tin colloid (Ktc). (40 results from both sets of tests)
The use of all Tests of Liver Function to Predict Liver Disease

In table 5.10 for each of the tests performed the number that were outside the normal range is expressed as a percentage.

There was a wide variety in the ability of the tests to predict liver disease. Only Kicg and serum fibronectin levels correctly predicted more than 75% of the abnormal population. Serum bilirubin, AST and albumin along with the prothrombin time and the liver excretion of Disida all fared badly, being abnormal in less than 40% of cases. Apart from serum alkaline phosphatase levels the conventional liver function tests did less well individually than the clearance tests in predicting liver disease.

However, out of the 46 measurements from both sets of tests, only on 4 occasions were all conventional liver function tests normal confirming that the combination fares better than any individual clearance test as a predictor of hepatic dysfunction. The conventional liver function tests are tabulated for each set of tests in Appendix 6.

Comparing all Liver Function Tests with Child -Pugh’s Clinical Grading

Table 5.11 illustrates the relationship of the medians of the liver function tests with Child-Pugh clinical grading at the time of testing. For the first set of tests there were patients in Child-Pugh groups A, B and C whereas on the second set of tests there were only patients in Child-Pugh grades A and B. All tests showed a difference between Child-Pugh grades A and B combined compared with C on the first set of tests but were not able to differentiate A from B. This inability to differentiate group A from group B was maintained on the second set of tests (NS, Mann Whitney).
Table 5.10 Use of all liver function tests to predict liver disease from both sets of tests.
(46 results in total).

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal Range</th>
<th>Number of Patients with Abnormal Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>4 - 25 (mmols/L)</td>
<td>18 (39%)</td>
</tr>
<tr>
<td>AST</td>
<td>8 - 40 (iu/L)</td>
<td>21 (46%)</td>
</tr>
<tr>
<td>AP</td>
<td>70 - 250 (iu/L)</td>
<td>32 (70%)</td>
</tr>
<tr>
<td>Albumin</td>
<td>24 - 48 (gm/L)</td>
<td>12 (26%)</td>
</tr>
<tr>
<td>Prothrombin Time</td>
<td>Less than 18 secs</td>
<td>17 (37%)</td>
</tr>
<tr>
<td>Ktc</td>
<td>0.12 - 0.24 (min⁻¹)</td>
<td>29 (63%)</td>
</tr>
<tr>
<td>K1c</td>
<td>0.12 - 0.24 (min⁻¹)</td>
<td>31 (67%)</td>
</tr>
<tr>
<td>K2c</td>
<td>&gt; 0.0023 (min⁻¹)</td>
<td>25 (54%)</td>
</tr>
<tr>
<td>K1d</td>
<td>0.12 - 0.24 (min⁻¹)</td>
<td>21 (46%)</td>
</tr>
<tr>
<td>K2d</td>
<td>&gt;0.0015 (min⁻¹)</td>
<td>16 (35%)</td>
</tr>
<tr>
<td>L to S Ratio</td>
<td>&gt;2</td>
<td>11 (24%)</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>&gt; 250 (iu/L)</td>
<td>35 (87%)*</td>
</tr>
</tbody>
</table>

* Only 40 results
Table 5.11. Comparison of the clearance tests of liver function with Child-Pugh’s grading for both sets of tests.

<table>
<thead>
<tr>
<th>Child-Pugh Grading</th>
<th>Ktc</th>
<th>Kicg</th>
<th>K1c</th>
<th>K2c</th>
<th>K1d</th>
<th>K2d</th>
</tr>
</thead>
<tbody>
<tr>
<td>First set of tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (n=9)</td>
<td>0.12</td>
<td>0.09</td>
<td>0.10</td>
<td>0.002</td>
<td>0.17</td>
<td>0.0020</td>
</tr>
<tr>
<td>B (n=13)</td>
<td>0.13</td>
<td>0.11</td>
<td>0.09</td>
<td>0.002</td>
<td>0.15</td>
<td>0.0015</td>
</tr>
<tr>
<td>C (n=3)</td>
<td>0.07</td>
<td>0.04</td>
<td>0.05</td>
<td>0.000</td>
<td>0.06</td>
<td>0.0006</td>
</tr>
<tr>
<td>Second set of tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (n=9)</td>
<td>0.12</td>
<td>0.10</td>
<td>0.10</td>
<td>0.002</td>
<td>0.13</td>
<td>0.0020</td>
</tr>
<tr>
<td>B (n=12)</td>
<td>0.12</td>
<td>0.09</td>
<td>0.09</td>
<td>0.002</td>
<td>0.12</td>
<td>0.0016</td>
</tr>
</tbody>
</table>

No significant differences between Child-Pugh A and B for either the first or second set of tests (Mann Whitney).
The use of all individual liver function tests to predict outcome by death - First set of tests only

In table 5.12 the ability of the individual liver function tests to predict ultimate outcome is shown. Each liver function test is divided into whether the test was inside or outside the normal range and the deaths of patients within these groups expressed in numbers.

Only the clearance of indocyanine green had all deaths in the abnormal range and was the most specific test. For most sensitive tests were the serum values of AST and albumin.

The ability of individual liver function tests to predict outcome by death - Both Sets of tests

In table 5.13 the liver function tests are again divided into normal or abnormal and the deaths predicted by each test are expressed as a fraction and a percentage. In this table the deaths are expressed as the 4 occurring between first and second testing of the patients and the 4 which occurred after the second set of tests. Only serum AST, the clearances of K1c and Kicg and serum fibronectin levels had all deaths in patients with abnormal results.
Table 5.12 Ability of each liver function test to predict outcome (death). Using the first set of tests to predict the 8 deaths occurring over the year of the study with the results expressed as a fraction of those patients in each group from a total of 25 patients.

<table>
<thead>
<tr>
<th>Liver Function Test</th>
<th>In Normal Range</th>
<th>Outside Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>1/12</td>
<td>7/13</td>
</tr>
<tr>
<td>AST</td>
<td>1/12</td>
<td>7/13</td>
</tr>
<tr>
<td>AP</td>
<td>1/8</td>
<td>7/17</td>
</tr>
<tr>
<td>Albumin</td>
<td>2/17</td>
<td>6/8**</td>
</tr>
<tr>
<td>Prothrombin Time</td>
<td>5/17</td>
<td>3/8</td>
</tr>
<tr>
<td>Ktc</td>
<td>1/10</td>
<td>7/15</td>
</tr>
<tr>
<td>Kicg</td>
<td>0/6</td>
<td>8/19</td>
</tr>
<tr>
<td>FSC</td>
<td>2/10</td>
<td>6/15</td>
</tr>
<tr>
<td>K1c</td>
<td>1/10</td>
<td>7/15</td>
</tr>
<tr>
<td>K2c</td>
<td>2/14</td>
<td>6/11</td>
</tr>
<tr>
<td>K1d</td>
<td>1/14</td>
<td>7/11</td>
</tr>
<tr>
<td>K2d</td>
<td>1/13</td>
<td>7/12</td>
</tr>
<tr>
<td>Fibronectin *</td>
<td>1/3</td>
<td>7/17</td>
</tr>
<tr>
<td>L to S Ratio</td>
<td>2/8</td>
<td>6/17</td>
</tr>
</tbody>
</table>

* only 20 results

** p=0.05 for differences to normal group (Fisher’s exact)
**Figure 5.13** Ability of individual liver function tests to predict death. Using the first set of rules to predict the 4 deaths in the first 6 months and the repeat test results (second set) to predict the subsequent 4 deaths. Deaths expressed as a fraction of the patients in each group with a total of 25 patients initially tested and 21 patients repeat tested.

<table>
<thead>
<tr>
<th>Liver Function Test</th>
<th>First Set of Tests</th>
<th>Second Set of Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0/12</td>
<td>4/13</td>
</tr>
<tr>
<td>AST</td>
<td>0/12</td>
<td>4/13</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>1/8</td>
<td>3/17</td>
</tr>
<tr>
<td>Albumin</td>
<td>0/11</td>
<td>4/14</td>
</tr>
<tr>
<td>Prothrombin Time</td>
<td>2/14</td>
<td>2/11</td>
</tr>
<tr>
<td>Ktc</td>
<td>0/10</td>
<td>4/15</td>
</tr>
<tr>
<td>Kicg</td>
<td>0/6</td>
<td>4/19</td>
</tr>
<tr>
<td>FSC</td>
<td>0/10</td>
<td>4/15</td>
</tr>
<tr>
<td>K1c</td>
<td>0/8</td>
<td>4/17</td>
</tr>
<tr>
<td>K2c</td>
<td>1/14</td>
<td>3/11</td>
</tr>
<tr>
<td>K1d</td>
<td>0/15</td>
<td>4/10</td>
</tr>
<tr>
<td>K2d</td>
<td>0/13</td>
<td>4/12</td>
</tr>
<tr>
<td>Fibronectin*</td>
<td>0/3</td>
<td>4/17</td>
</tr>
<tr>
<td>L to S Ratio</td>
<td>1/8</td>
<td>3/17</td>
</tr>
</tbody>
</table>

* only 40 results
The Ability of Changes Between the Two Sets of Tests to Predict Outcome

In table 5.14 the influence of changes between the two sets of tests is assessed in predicting the 4 deaths, which occurred after the second set of tests, are summarised.

The Ability of Combinations of Liver Function Tests to Predict Outcome

From the conclusion of Chapter 4 there was a suggestion that the combination of Kicg with Ktc may give a good prediction of outcome in patients with intrinsic liver disease. In Table 5.15 the predicted ability of combinations of clearance tests of liver function with respect to outcome are summarised.

Whilst the combination of clearance tests such that both tests are abnormal improves the predictive ability of individual clearance tests. The combination of blood clearance of tin colloid and the liver elimination of Disida was the most selective of death in these patients (p<0.001, Fisher’s exact).
Table 5.14 Ability of changes between initial testing and repeat testing 6 months later to predict death. There were 4 deaths in the 21 patients who had both sets of tests and deaths are expressed as a fraction of the patients in each group.

<table>
<thead>
<tr>
<th>Liver Function Test</th>
<th>Increased</th>
<th>Decreased</th>
<th>Unchanged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>4/12</td>
<td>0/9</td>
<td>0</td>
</tr>
<tr>
<td>AST</td>
<td>3/11</td>
<td>1/10</td>
<td>0</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>3/10</td>
<td>1/11</td>
<td>0</td>
</tr>
<tr>
<td>Albumin</td>
<td>2/13</td>
<td>2/7</td>
<td>0/1</td>
</tr>
<tr>
<td>Prothrombin Time</td>
<td>2/11</td>
<td>1/6</td>
<td>1/4</td>
</tr>
<tr>
<td>Ktc</td>
<td>1/7</td>
<td>3/13</td>
<td>0/1</td>
</tr>
<tr>
<td>Kicg</td>
<td>2/7</td>
<td>2/12</td>
<td>0/2</td>
</tr>
<tr>
<td>FSC</td>
<td>4/10</td>
<td>0/11</td>
<td>0</td>
</tr>
<tr>
<td>K1c</td>
<td>1/10</td>
<td>3/11</td>
<td>0</td>
</tr>
<tr>
<td>K2c</td>
<td>0/11</td>
<td>4/10</td>
<td>0</td>
</tr>
<tr>
<td>K1d</td>
<td>1/5</td>
<td>3/16</td>
<td>0</td>
</tr>
<tr>
<td>K2d</td>
<td>1/10</td>
<td>2/10</td>
<td>1/1</td>
</tr>
<tr>
<td>Fibronectin*</td>
<td>0/6</td>
<td>4/14</td>
<td>0</td>
</tr>
<tr>
<td>L to S Ratio</td>
<td>2/10</td>
<td>1/10</td>
<td>1/1</td>
</tr>
</tbody>
</table>

* Only 20 results
Table 5.15 Ability of combinations of liver function tests to predict death in which both of the liver function tests have to be abnormal. Using the first set of tests to predict the 8 overall deaths. Deaths are expressed as a fraction of the patients in that group with both liver function tests abnormal.

<table>
<thead>
<tr>
<th>Liver Function Test</th>
<th>Kieg</th>
<th>K1c</th>
<th>K2c</th>
<th>K1d</th>
<th>K2d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ktc</td>
<td>7/13</td>
<td>7/12</td>
<td>7/11*</td>
<td>7/10**</td>
<td>7/8****</td>
</tr>
<tr>
<td>Kieg</td>
<td>--</td>
<td>7/14</td>
<td>7/13</td>
<td>7/10**</td>
<td>7/10**</td>
</tr>
<tr>
<td>K1c</td>
<td>--</td>
<td>--</td>
<td>7/12</td>
<td>7/11*</td>
<td>7/11*</td>
</tr>
<tr>
<td>K2c</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>7/11*</td>
<td>7/11*</td>
</tr>
<tr>
<td>K1d</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>7/9***</td>
</tr>
</tbody>
</table>

* p<0.05 (Fisher’s Exact)

** p<0.01 (Fisher’s Exact)

*** p<0.005 (Fisher’s Exact)

**** p<0.001 (Fisher’s Exact)
DISCUSSION

Alcoholics are a notoriously difficult group to study and in other studies follow-up is rarely complete\(^ {186}\). In this part of the study patients with severe alcoholic liver disease have been studied with complete follow-up which has allowed 'tight' interpretation of the results.

**Tin Colloid**

As in Chapters 3 and 4 the blood clearance of tin colloid (Ktc) was a good test of liver function and there was a significant difference between survivors and non survivors after the initial tests (Figure 5.5) confirming its usefulness in the prediction of outcome and as a test of liver function.

Clearly, the liver uptake and blood clearance measure different parameters of Kupffer cell function (Fig 5.4). To measure the liver uptake, both uptake from the blood and the activity in the blood circulating within the liver are measured and the final clearance value is a combination of these parameters. The liver uptake was less discriminatory in selecting patients who died than blood clearance indicating that the blood clearance may be the better measure to use clinically of Kupffer cell function.

McClaren\(^ {193}\) had shown that the liver to spleen ratio was significantly decreased in patients with cirrhosis and the findings in this study concur with that. However, there was little correlation between blood clearance of tin colloid and L:S ratio and the L:S ratio was not significantly different between survivors and non survivors (NS, Mann Whitney). As with scan score that was investigated in Chapter 4, the L:S ratio, although an attractive
theoretical quantification of liver function from the static scan, did not provide as accurate a discrimination of outcome as the clearance tests of liver function.

**Indocyanine Green**

The plasma disappearance rate constant of indocyanine green was, as in Chapters 3 and 4, a good test of liver dysfunction and provided good discrimination between survivors and non survivors. In Chapter 5 only patients with severe liver disease were tested as in Chapters 3 and 4 and the reservations expressed in the discussion to Chapter 4 about the use of ICG clearance at only one dose still exist.

Two newer tests of liver function, the biexponential clearance of caffeine and the two clearances constants of Disida have been described.

**Disida**

The kinetics of clearance of Disida was initially quantified in dogs and confirmed in humans. The normal ranges were defined from the results in this study. Further work on larger population samples may be necessary to confirm or modify this normal range. There was a significant difference of both the liver uptake and the liver elimination between the controls and patients with liver disease, and between survivors and non survivors when analysed by all deaths on the initial rate constants. These results make the two clearance constants evaluated from Disida good tests of liver function.

Love identified the existence of two species within the Disida radiopharmaceutical which are both removed by the liver but at different rates. Further work may be necessary to optimise these parameters.
Caffeine

In the caffeine clearance test, the measurement of the plasma disappearance has been refined by using an intravenous bolus and was found to be biexponential with an initial undescribed fast component. In addition the fasting levels and first component of clearance have been validated by repeat measurements.

Caffeine clearance measured from either blood or saliva samples has been well described as a liver function test\textsuperscript{167, 168} but only the second, metabolically dependent component, has been described. The first component has a half life in the same range as Kicg and Ktc. Both of these tests are principally blood flow related (high extraction efficiency) and presumably so is the first component of caffeine clearance. This first component of the intravenous clearance represents the taking up of the caffeine by the hepatocyte whereas the second component has a much longer half life of at least 2 hours and relates to the metabolism of caffeine by demethylation\textsuperscript{157}.

In this work blood samples were taken every 5 minutes for the first 20 minutes allowing the first component to be evaluated from 4 samples only. As the half life of this rate constant in controls is from 2 to 5 minutes then some error may be introduced. Samples every 3 minutes from 3 to 15 minutes would have ensured greater accuracy.

Five minute samples were chosen as the total number of samples had to be rationalised and the small rise in serum level (Figure 5.1) at about 30 minutes was observed. This small rise was seen in all patients and probably represents leakage of a metabolite which was picked up on the assay. In this study an enzyme immune assay was
used but this has now been superseded by a more specific assay using high performance liquid chromatography which may eliminate this problem.

Some concern must be raised at the work of Renner\textsuperscript{167} in 1984 when they calculated the second lower exponential constant assuming a steady state serum concentration at 15 to 45 minutes after an oral dose. After oral ingestion of caffeine the time to reach peak plasma concentrations exhibits wide variations from 15 to 120 minutes\textsuperscript{157, 158, 206} with the longer times being in those patients with severe liver disease. In this study the use of an intravenous bolus has allowed precise analysis of the kinetics without relying on the variability of absorption from the gastrointestinal tract.

Caffeine appears to have extremely useful properties when used to measure liver function in that it can assess 2 separate liver functions. The fast component may be used to measure liver blood flow in a similar fashion to ICG although this was not pursued in this work.

The particular group of patients in this study all had alcoholic liver disease and for them the avoidance of caffeine containing beverages presents particular problems. They often maintain a good fluid intake and usually do this by tea or coffee in the day time and coca-cola or pepsi-cola at night. As all of these contain large amounts of caffeine, the patients only need a momentary loss of concentration to skew all the results. We opted only to take samples for 6 hours during which the patients were carefully supervised. Despite this 5 patients out of 46 consumed caffeine (3 drank tea or coffee and 2 ate chocolate) before the end of the test which had to be repeated. Many other authors\textsuperscript{83, 167}. 
have advocated overnight caffeine clearance but the inability of patients to abstain from caffeine could make this approach open to serious error.

Similar worries arise from the use of fasting serum caffeine as a test of liver function but this measurement in both plasma and saliva was an excellent test and could become a simple and easy initial test of liver disease. In our results the fasting serum caffeine level gave significantly different results comparing controls with those patients with liver disease. Again the inability of patients to refrain from imbibing caffeine containing foodstuffs is a major cause for concern about the accuracy and reliability of such a test although work by Hasegawa testifies to its usefulness.

The second metabolic component of caffeine clearance in control patients had a half life of at least 2 hours and in patients with liver disease the half life was up to 20 hours. These calculations were made from measurements over 6 hours and in the longer half lives this short period of study may cause some error. Much longer half lives of up to 48 hours have been observed in other studies but were not seen in this work despite testing patients with significant liver disease. Perhaps in the other studies the patients may have ingested caffeine while unsupervised which prolonged the half life.

Attempts to measure the second component of caffeine clearance by salivary samples were totally unsuccessful when compared to the accuracy of plasma clearance. Fasting salivary caffeine levels were in agreement with previous reports and were about 75% of plasma caffeine levels. The subsequent paired levels of plasma and saliva lost this comparability at 6 hours (Appendix 4) suggesting that a longer time span is necessary for plasma and salivary equilibration.
However on further analysis those patients whose salivary plasma levels at 6 hours were more than 80% of the 60 minute value did have significant liver disease in that their blood clearance was in the abnormal range. Clearly a clearance test over only 5 hours from salivary samples is not accurate but as a marker of poor liver function in which no blood samples are required may have a place in assessing patients and further work is necessary possibly combining this with fasting salivary levels. This has a particular attraction in children as it may avoid the use of needles.

Consideration was made of collecting a further caffeine sample at 24 hours but in the study design I felt that imposing a total of 36 hours of abstaining from caffeine containing products was too great an imposition on the volunteer patients.

**Complement and Fibronection**

The serum levels of both C3 and C4 were 100% accurate in diagnosing liver disease. The immunoelectrophoretic technique used measures both active and inactive fragments. Raised serum levels generally reflect an acute phase reaction but the serum level reflects the net utilisation, synthesis and removal. The raised serum levels are usually a direct result of increased synthesis of complement by the liver. Charlesworth\textsuperscript{207} showed in a study of 12 alcoholic cirrhotics that the serum levels of C3 and C4 were normal unless the patient was hypoalbuminaemic. The patients in his study had severe disease and the accumulation in the serum of complement fragments was due to low rates of removal by the Kupffer cells.

The low rate of removal of C3 and C4 would fit with the fibronectin levels which were low in the majority of patients. Many cell types have the capacity to synthesise and
secrete fibronectin but most, if not all, circulating fibronectin is produced by hepatocytes\textsuperscript{208, 209}. The depletion of plasma fibronectin is probably related to hepatocyte failure with decreased synthesis and secretion.

The low serum values of fibronectin and the high levels of C3 and C4 cannot both be compatible with hepatocyte failure unless the complement measured is mostly inactive and is present in the bloodstream because of the inability of the Kupffer cells of the liver to remove it. In this study the differences between active and inactive complement fragments was not investigated.

Naveau\textsuperscript{210} studied 102 alcoholic cirrhotics who were followed up for 1 year and showed that a decreased plasma fibronectin concentration is a powerful predictor of poor survival. Our results agree with this in that all patients who died had a low plasma fibronectin. However, nearly all patients had a low fibronectin level and so the specificity of this test is low.

It is disappointing that there was not a good correlation between the serum fibronectin level and the clearance of tin colloid. As opsonisation of colloidal particles by fibronectin plays an integral role in their removal from the blood stream the generally low levels of fibronectin are associated with a low clearance of tin colloid, particularly the blood clearance. Presumably the low levels of fibronectin were adequate to opsonise the tin colloid and the tin colloid clearance more accurately reflects the Kupffer cell dysfunction. As most fibronectin is synthesised by the hepatocytes this result compares favorably with the other synthetic liver function tests such as serum albumin and prothrombin time both of which predicted a much lower percentage of patients with liver
Individual and Combination of Clearance Tests

It is most noticeable from Table 5.10 that the clearance tests in general rank higher in their ability to predict liver disease than any of the conventional liver function tests used alone. At the time of testing only 4 out of 46 tests had completely normal biochemical liver function tests which confirms that collectively they are better than on an individual basis and confirm their present use as a battery of tests which are most valuable in the diagnosis of liver disease.

Most of the tables in this chapter have assumed the existence of a normal range for each of the tests. For conventional liver function tests and the clearance of indocyanine green the normal range is well defined. For the newer liver function tests, whilst attempts have been made to define normality these values may not be absolute and after further studies may need to be redefined. This need not detract from the identification of a threshold level for each test in which a value either above or below has clinical significance.

Child's classification and its modified scoring system introduced by Pugh called the Child-Pugh gradings are widely accepted as the mainstay of assessment of patients with portal hypertension having been used to select patients for injection sclerotherapy and portocaval shunting.
In general terms all of the liver function tests were reasonably good at predicting the first 4 deaths as these occurred in the patients with severe liver disease and 3 of these were in Child-Pugh grade C and 1 in Child-Pugh grade B (Table 5.12). However, the second 4 deaths occurred in patients with less severe liver disease as assessed by conventional liver function tests and Child-Pugh grading.

All of the clearance tests which are blood flow related (Ktc, Kicg and Klc) show a significant difference in the mean of their values between Child-Pugh’s grade C and the other groups but fail to show any significant differences between Child-Pugh’s A and B (Table 5.11). Of interest is that these tests (from table 5.12) had similar success in predicting outcome with at most 1 false positive and the other clearance tests with similar predictive ability are the two clearance constants of Disida, K1d and K2d.

There were only 4 deaths in the second 6 months but these were of particular interest in that they were in Child-Pugh grades A and B which carries a more favourable prognosis than Grade C and an ability to identify these patients would have been of value. From Table 5.14, a deterioration in the serum values of bilirubin and fasting caffeine included all 4 deaths as did a worsening of K2c, the second metabolic component of caffeine clearance. Whilst understanding that only small numbers are involved, these results indicate that parameters of liver function evaluated from caffeine appear to hold great promise. In defence of the other liver function tests, all of these patients were being treated by injection sclerotherapy and had abstained from alcohol except for 1 patient and perhaps deterioration may not have been detectable over the time interval of 6 months.
It may have been important to make prognostic factor evaluations of each liver function test using logistic regression analysis. However, the small numbers of patients in this study makes such analysis impossible. For binary analysis at least 10 patients (preferrably 15) are required in each group to enable reliable conclusions to be made from the results\textsuperscript{215}.

In Chapter 4 the combination of clearance of tin colloid and indocyanine green did not improve overall accuracy in predicting outcome but by selecting out the patients with intrinsic liver disease this combination could improve the predictive ability of individual results. In this chapter multiple liver function tests have been assessed. In the first instance overall differences could have been demonstrated using a test such as the Kruskal-Wallis test but this type of analysis was not pursued as I was interested in any pairwise differences irrespective of any overall difference. The best selection of patients for ultimate outcome (Table 5.15) was by the combination of the clearances from blood of tin colloid (Ktc) and elimination across the liver by Disida (K2d). With reservations because of the small numbers involved, these results suggest that in severe liver disease the combination of a measure of Kupffer cell function with a test of hepatocyte function may select out a cohort of patients with a poor prognosis.
CHAPTER 6

CLINICAL STUDIES 3(b)

In this chapter the effects of isosorbide dinitrate on liver function within a randomised trial of treatment of oesophageal varices are discussed. In particular the effect on blood flow dependent parameters as compared to metabolic parameters of liver function are evaluated. Note is also made of the clinical results of the trial.

CONTENTS

<table>
<thead>
<tr>
<th></th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1</td>
<td>Introduction</td>
</tr>
<tr>
<td>6.2</td>
<td>Patients and Methods</td>
</tr>
<tr>
<td>6.3</td>
<td>Results</td>
</tr>
<tr>
<td>6.4</td>
<td>Discussion</td>
</tr>
</tbody>
</table>
6.1 INTRODUCTION

Bleeding from oesophageal varices is the most life threatening complication of patients with liver disease causing portal hypertension. Prevention of the first variceal bleed is most likely to affect overall survival as short term mortality from a variceal bleed is approximately 60%\textsuperscript{216}.

Once a significant bleed has occurred acute variceal haemorrhage is mainly managed by endoscopic sclerotherapy\textsuperscript{211, 217, 218} although alternatives include endoscopic band ligation\textsuperscript{219}, operative shunting procedures\textsuperscript{214}, transjugular intrahepatic portosystemic shunts (TIPS)\textsuperscript{213} or pharmacological measures\textsuperscript{220}.

The options of shunting procedures are mostly confined to those patients who continue to bleed despite endoscopic and/or pharmacological intervention\textsuperscript{213, 214}. Portosystemic shunting by elective surgery or TIPS has fallen into disrepute as an elective procedure because shunted patients can trade death from bleeding for unpredictable encephalopathy and death from liver failure\textsuperscript{221}. There is an associated high morbidity and mortality from open portosystemic shunts and a high stenosis rate in TIPS\textsuperscript{213}. Other surgical procedures include oesophageal transection with injection sclerotherapy which reduces early rebleeding significantly but does not produce a survival advantage\textsuperscript{222}.

It is disappointing that in controlled trials there has been no reduction in mortality from injection sclerotherapy and common to all of these trials has been a high frequency of rebleeding episodes of up to 50%\textsuperscript{211, 223, 224}. These bleeding episodes may also be controlled by drugs which act by reducing portal pressure. Acute agents include vasopressin, glypressin, somatostatin, propranolol, glycercyl trinitrate, isosorbide dinitrate
and isosorbide mononitrate. Of these somatostatin and octreotide have received most evaluation in acute or primary variceal bleeding and are the drugs of choice\(^{225,226}\).

After the initial control of a haemorrhage from oesophageal varices, patients are at high risk for further bleeding and death. Therapy to prevent rebleeding is essential and the management of choice is injection sclerotherapy and/or drug therapy\(^{220,225}\). Sclerotherapy is still associated with a rebleeding rate as high as 50% and complications such as fever and oesophageal ulceration or stricture occur in up to 40% of patients with treatment related deaths in 1% to 2%.

Following control of the acute variceal bleed propanolol is at present the therapeutic treatment of choice for secondary prevention to reduce the bleeding episodes, numbers of deaths from rebleeding and the total number of deaths\(^{227}\). Propanolol has been shown to have a poor compliance amongst patients, suppresses the natural response of a patient to an acute bleed and may precipitate renal failure\(^{228}\). Direct trials comparing propanolol and injection sclerotherapy have shown little difference between the two treatments individually on rebleeding and survival\(^{229}\) but injection sclerotherapy plus propranolol may reduce rebleeding episodes. As stated previously there are major concerns about compliance with drug therapy in these types of patients. For those patients who attend there is no worry about compliance with injection sclerotherapy.

Propanolol which is the most commonly used drug has been shown to have no significant affect on liver function tests after 2 months of therapy\(^{230,231,232}\), and does reduce the rate of re-bleeding\(^{228,232}\).
The treatment of choice which is injection sclerotherapy is effective only if promptly applied by experienced personnel. It would be desirable if drug therapy was easy to use and efficacious in controlling bleeding especially in non-specialist centres where injection sclerotherapy may not be available.

Since Freeman\textsuperscript{233} showed that 1 month of treatment with isosorbide dinitrate produced a sustained fall in portal pressure the therapeutic options of this drug have needed to be explored. Isosorbide dinitrate has the theoretical advantage over propranolol as it does not blunt the natural response by the patient to a bleed and renal function should be preserved.

In common with these drugs used both individually and in combination with injection sclerotherapy in the treatment of oesophageal varices, isosorbide dinitrate is supposed to work by reducing portal pressures and this is achieved by reducing liver blood flow. If liver blood flow is decreased then isosorbide dinitrate may adversely affect blood flow dependent hepatic clearance constants. The prerequisite of any drug used in this way should be that it does not cause any deterioration in already compromised hepatic function and it was important to test the effects of isosorbide dinitrate.

The other unanswered questions are (i) does reducing portal pressure reduce the incidence of bleeding episodes from the oesophageal varices and (ii) does this ultimately improve the overall morbidity of the condition? In this report the numbers are too small and length of follow up too short to allow these important questions to be answered but results of a larger trial could provide this information.
Isosorbide dinitrate was given in addition to injection sclerotherapy in a randomised trial to patients with alcoholic liver disease complicated by oesophageal varices. Within the framework of this trial, the effect of isosorbide dinitrate on liver function has been assessed by a battery of both conventional and clearance tests of liver function.

In a similar trial to this study, Polston added sucralfate to injection sclerotherapy in a randomised fashion and showed a reduction in frequency of rebleeding, although mortality was unaffected. Sucralfate was used for its possible beneficial effect in preventing rebleeding from oesophageal mucosal ulceration.

In this particular study a homogenous group of patients with alcoholic, cirrhotic liver disease has been studied to reduce the errors incurred by studying patients with diseases of different aetiology.

6.2. PATIENTS AND METHODS

a) Patients

All patients who had liver function tests performed in Chapter 5 were entered at the time of their first set of tests.

b) Method

Patients were randomised after initial testing by sealed envelope to either treatment by injection sclerotherapy alone or by injection sclerotherapy plus oral isosorbide dinitrate 20 mgs four times per day.
Injection sclerotherapy was carried out using fibreoptic endoscopy (Olympus K series) and 5% ethanolamine oleate as the sclerosant. A freehand technique of intravariceal injection was used and up to 5 mls of sclerosant injected per varix. During a single session not more than 15 mls of sclerosant was injected. All injections were performed by endoscopists (n=2) trained and experienced in the technique.

After their first endoscopy patients had sclerotherapy initially every 1 to 3 weeks until the varices were eradicated and then had further endoscopies at longer intervals of up to 6 months. Patients were reviewed at least every 4 weeks for the first 6 months and at least every 8 weeks during the second six months. Patients were entered into this study at various stages of their treatment when initial liver function testing was performed.

In the original randomisation there were 13 patients in the isosorbide group and 12 in the control group. Eight out of the 13 patients experienced headaches on the full dose of ISDN. In 3 patients this resolved without changing the dose over a period of 3 to 7 days. In the 5 other patients with headaches the dosage was halved. In 3 patients this relieved their symptoms and they were steadily increased over a period of 2 to 4 weeks back to full dose without adverse sequelae. Two patients were unable to tolerate even reduced doses of ISDN and were excluded from the trial. This resulted in 12 patients in the IS alone (control) group and 11 patients in the IS plus ISDN group.

Table 6.1. summarises the distribution of the patients in each limb of the trial by age, sex and Child-Pugh clinical grading. It is noted that there are more females in the IS alone group.
The other medication taken by the patients is summarised in Table 6.2.

6.2c **Statistics**

Comparison between groups was made using the paired Wilcoxon test and comparisons of outcome by Fisher's exact test.
**Table 6.1.** Patient details of the 19 patients who completed the trial

<table>
<thead>
<tr>
<th></th>
<th>Injection Sclerotherapy</th>
<th>Injection Sclerotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alone (n=10)</td>
<td>Plus Isosorbide Dinitrate (n=9)</td>
</tr>
<tr>
<td>Mean Age (years)</td>
<td>58.3</td>
<td>60.2</td>
</tr>
<tr>
<td>SEX</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Child-Pugh Clinical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 6.2. Current drug therapy of the 19 patients who completed the trial

<table>
<thead>
<tr>
<th></th>
<th>Isosorbide Sclerotherapy</th>
<th>Injection Sclerotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alone</td>
<td>Plus Isosorbide Dinitrate</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Frusemide</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Brufen</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Cyanocobalamin Inj</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

The following drugs were each taken by only 1 patient: Heminevrin, ferrous sulphate, tolbutamide, temazepam, coproxamol and spironolactone
6.3 **Results**

In table 6.3, there is a flow chart of the progress of the trial. The deaths within 12 months are shown for each treatment group and there was no statistical difference (Fisher's exact test). All 8 deaths occurred as a result of progressive hepatic failure but the terminal event was complicated in 2 patients by bleeding from their oesophageal varices.

If all 25 patients are considered then there are no significant differences (NS, paired T test) between each set of liver function tests (Table 6.4) which were performed 6 months apart.

For the 19 patients who had two sets of tests and completed treatment the mean level of their liver function tests both before entering the trial and 6 months later are recorded in table 6.4.

Table 6.5. records the mean time intervals between and the number of injections at each attendance for injection sclerotherapy. There is no statistical difference between either of the treatment groups.

Episodes of bleeding from oesophageal varices for the patients are shown in table 6.6. There were more bleeds in the IS plus ISDN group but this did not achieve statistical significance (NS, Fisher’s exact).
Table 6.3  Flow chart of progress and outcome of patients over 1 year of follow up

25 patients

Injection sclerotherapy
  Alone
  (n=12)

FIRST SET OF TESTS

2 deaths after first testing and before second tests
  (n=10)

SECOND SET OF TESTS

3 further deaths after second set of tests
  (n=7)

Injection Sclerotherapy plus isosorbide dinitrate
  (n=13)

FIRST SET OF TESTS

2 patients unable to tolerate ISDN
  (n=11)

SECOND SET OF TESTS

2 deaths after first testing and before second tests
  (n=9)

SECOND SET OF TESTS

1 further death after second set of tests
  (n=8)

A total of 17 patients were alive at the end of 1 year follow up

(Includes 2 patients withdrawn from the IS and ISDN group)
Table 6.4 The mean value of each of the liver function tests of the patients who completed the trial were assessed initially and 6 months later.

<table>
<thead>
<tr>
<th>Test</th>
<th>Injection Sclerotherapy Alone (n=10)</th>
<th>Injection Sclerotherapy Plus Isosorbide Dinitrate (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 6 Months Later</td>
<td>0 6 Months Later</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>61 38</td>
<td>29 18</td>
</tr>
<tr>
<td>AST</td>
<td>36 35</td>
<td>40 38</td>
</tr>
<tr>
<td>Alkaline Phos.</td>
<td>164 120</td>
<td>130 111</td>
</tr>
<tr>
<td>Albumin</td>
<td>34 39</td>
<td>34 37</td>
</tr>
<tr>
<td>Prothrombin Time</td>
<td>1.4 1.3</td>
<td>1.2 1.3</td>
</tr>
<tr>
<td>Kieg</td>
<td>0.08 0.09</td>
<td>0.10 0.10</td>
</tr>
<tr>
<td>Tin Ktc</td>
<td>0.12 0.11</td>
<td>0.11 0.12</td>
</tr>
<tr>
<td>Colloid K1u</td>
<td>0.17 0.19</td>
<td>0.17 0.17</td>
</tr>
<tr>
<td>Disida K1d</td>
<td>0.17 0.14</td>
<td>0.12 0.09</td>
</tr>
<tr>
<td>K2d</td>
<td>0.0018 0.0017</td>
<td>0.0014 0.0018</td>
</tr>
<tr>
<td>Caffeine FSC</td>
<td>28 21</td>
<td>24 17</td>
</tr>
<tr>
<td>K1c</td>
<td>0.09 0.08</td>
<td>0.07 0.09</td>
</tr>
<tr>
<td>K2c</td>
<td>0.0028 0.0021</td>
<td>0.0030 0.0029</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>245 223</td>
<td>169 186</td>
</tr>
<tr>
<td>Liver to Spleen Ratio</td>
<td>4.48 3.88</td>
<td>1.46 1.16</td>
</tr>
</tbody>
</table>

NS, for changes between the 2 groups (Paired Wilcoxon test)
Table 6.5 Details of the injection sclerotherapy treatment in both groups.

<table>
<thead>
<tr>
<th></th>
<th>Injection Sclerotherapy</th>
<th>Injection Sclerotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alone (n=10)</td>
<td>plus Isosorbide Dinitrate (n=9)</td>
</tr>
<tr>
<td>Mean Time After First Bleed Before Entering Study (weeks)</td>
<td>10.4</td>
<td>12.5</td>
</tr>
<tr>
<td>Mean Time Between Endoscopy (weeks)</td>
<td>8.2</td>
<td>7.8</td>
</tr>
<tr>
<td>Total Number Of Injections</td>
<td>12.1</td>
<td>13.4</td>
</tr>
<tr>
<td>Mean Number of Injections Per Visit</td>
<td>2.0</td>
<td>2.1</td>
</tr>
</tbody>
</table>
Table 6.6 Numbers of episodes of rebleeding in each group over the 1 year of follow up.

<table>
<thead>
<tr>
<th></th>
<th>Injection Sclerotherapy</th>
<th>Injection Sclerotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alone (n=10)</td>
<td>Plus Isosorbide Dinitrate (n=9)</td>
</tr>
<tr>
<td>Bleed</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>No Bleed</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>

NS, Fisher’s exact
DISCUSSION

In Chapter 5 the limitations of assessing the severity of liver disease by conventional liver function tests were discussed. In this study the serum albumin and the prothrombin time were similar in the two groups and the mean serum bilirubin was higher in the IS alone group possibly suggesting that this group had more severe liver disease.

Perhaps the two groups were not matched in terms of severity of liver disease. From Table 6.1 both groups had 6 patients in Child-Pugh's B and of the remainder there is a similar number (3 and 4 respectively) in Child-Pugh's A indicating a similar distribution of severity of liver disease by this classification between the two groups.

In patients with alcoholic liver disease the major cause of further problems is that they continue to imbibe alcohol. Serum alcohol levels were not routinely measured but if clinically indicated and in cases of doubt were performed. At each attendance the patients were specifically quizzed about their alcohol intake and only 1 patient in the IS plus ISDN admitted to any alcohol intake and this lady patient was continuing to imbibe >100 mgs of alcohol daily. She was the only patient with a positive blood alcohol level out of 7 patients tested in the IS plus ISNN group.

In the IS alone group 2 patients admitted to partaking of alcohol with 1 male imbibing approximately 50 mgs of alcohol per day and the other less than 10 mgs. These admissions were confirmed on blood alcohol testing as these two patients were the only positive blood alcohol levels out of 8 patients tested. Although alcoholic patients are notoriously unreliable I spent a great deal of time with these patients during their liver function testing and managed to establish a good working relationship which included
their trust. On this basis I feel that their response to the questions about their alcohol intake are reliable and that none of the other 22 patients were drinking significant quantities of alcohol. In addition with over 75% of the patients I had substantiating evidence from either close relatives or close friends who regularly attended.

As isosorbide dinitrate reduces portal pressure by reducing liver blood flow\textsuperscript{235,236} it might be expected that the blood flow dependent clearance constants would be reduced in the treatment group. The four such parameters, Ktc, Kicg, K1d and K1c showed no statistical difference between the 2 groups indicating that there was no difference between the two groups (NS, paired Wilcoxon test).

Other drugs such as Cimetidine have been shown to reduce liver blood flow\textsuperscript{140} but there were similar numbers of patients in each group on such medication (Table 6.2).

Using the clearance tests the results for both groups using tin colloid were similar indicating that Kupffer cell malfunction was equally impaired in each group. The serum fibronectin level was lower in both sets of tests on the IS plus ISDN group which shows that despite lower levels of fibronectin the clearance tests of tin colloid were not affected. Possibly for the serum fibronectin alone to reduce Kupffer cell function requires a much lower serum level.

Overall the majority of liver function tests are similar between the two groups. The mean serum bilirubin and clearance of ICG are worse in the IS alone group whereas the mean serum fibronectin and liver uptake of Disida are worse in the IS plus ISDN group.
The conclusion is that the two groups have equally severe liver disease and that the oral administration of ISDN does not affect liver function.

It is striking from this study that there have been more episodes of bleeding in the treatment group who received ISDN in addition to the IS (Table 6.6). The two treatment groups were not ideally matched by sex with a preponderance of females in the IS alone group. This makes the finding of more bleeds in the IS plus ISDN group more surprising as female patients tend to do less well than males.

Care is needed in the interpretation of rebleeding rates as the time of entry of the patient into studies after an acute bleeding episode needs evaluating. The nearer in terms of days to the initial bleeding episode the patient is entered then the worse is the survival and the more likelihood of rebleeding. In this study only stable patients were admitted such that none was entered within 2 weeks of an acute bleeding episode and from Table 6.5 the mean time of entry was about 3 months after an acute bleeding episode.

It is possible that the administration of ISDN in addition to IS has increased the risk of bleeding from the oesophageal varices and it is interesting to speculate that ISDN reduces portal pressure by relaxing smooth muscle. This induces vasodilation which lowers arterial pressure and stimulates baroreceptors to bring about splanchnic vasoconstriction. It is the combination of vasoconstriction and a venous dilatation which leads to a reduced portal flow and hence a fall in portal pressure. This fall in portal blood flow is achieved without altering overall liver perfusion. With long term ISDN there is probably not a constant level of dilatation of smooth muscle with resultant swings in portal pressure. A portal hypertensive "surge" may cause a breach in the variceal wall,
leading to bleeding. Possibly the flux of portal pressure brought about by these haemodynamic changes induces the bleeds that would not occur if the portal pressure was more constant whether at a higher or a lower level.

It has been observed that varices appear to have a diurnal pattern of bleeding being maximal at night. Although not looked at in this study, it indicates that the success of drug therapy may partly depend on the time of administration.\(^{218}\)

It is well documented that IS does not affect liver function\(^{225}\) but could a difference in the treatment of the varices by IS have contributed to the higher number of bleeding episodes in the IS plus ISDN group? The answer to this has to be ‘yes’ as other studies have shown that a bleeding rate after eradication of the varices may approach 0%\(^{225, 239}\), but overall there is a rebleeding rate of upto 50% before the varices are eradicated\(^{211}\). In the IS alone group the only bleed was in a patient who was in Child-Pugh grade B and there were no bleeds in any other patients.

All treatment by IS was performed by two endoscopists who were both very experienced in the technique and were unaware to which group the patients had been allocated. It is difficult to project that the patients in the IS plus ISDN group had any different or worse treatment than the IS alone group.

In conclusion isosorbide dinitrate was poorly tolerated with problems in 8 (62%) of patients and in 2 of these despite dose reductions they were unable to tolerate the drug. These problems arose in patients under close medical supervision and it is extremely unlikely that patients would take this drug on a regular basis in the community.
Compliance has also been shown to be a problem with propanolol when given to patients with alcoholic liver disease\textsuperscript{228}, but ISDN even if it was a clinically equal or superior agent does not improve this problem.

Despite testing with a battery of conventional and clearance tests of liver function, isosorbide dinitrate was not shown to have any effect on liver function which is confirmed by other studies\textsuperscript{240,241}.

Although the numbers are small, the patients on isosorbide dinitrate did have a higher number of bleeds from their oesophageal varices. The efficacy of the drug may be questioned although ultimately a larger study will be necessary.
CONCLUSIONS

In Chapter 3 the blood clearance of tin colloid was shown to be a reproducible measure of liver function and a straightforward test to perform. Even if sophisticated gamma cameras are not available then a simple bedside counter could be used. The recent development of the Mediscint arm cuff (John Gaunt Scientific Limited, Eynsham, Oxford) which has been specifically designed to measure peripheral blood clearance has made the measurement of the clearance of tin colloid an easier and more attractive proposition as it can be performed at the bedside of patients or in the outpatient clinic.

The usefulness of the clearance of tin colloid (Ktc) was confirmed in Chapters 4 as a diagnostic liver function test. In this study the blood clearance of tin colloid (Ktc) was measured whereas previous studies had used the liver uptake. Direct comparisons of these two measurements in Chapter 5 showed that the blood clearance allowed better discrimination of the severity of liver disease in alcoholic liver disease than liver uptake and should be used for this purpose.

It is realised that the applications of quantitative liver function tests are not enormous and that biochemical liver function tests are often adequate in clinical practice especially in the diagnosis of liver disease. However, the prediction of severity of liver disease and outcome is of particular importance to the surgeon. In liver transplant surgery it is essential to be able to select out patients with liver dysfunction who would benefit from surgery and then after operation to monitor their progress. Operations on jaundiced patients have a higher morbidity and mortality than surgery on normal patients. Although biochemical tests of liver function tests are of some value it would be extremely helpful to have a quantitative test of tests of liver function which would aid in both selection of patients and optimising the timing of surgery. These tests may
enable a more critical approach to the relative merits of ERCP (endoscopic retrograde cholangiopancreatography) and surgery in the management of obstructive jaundice by allowing direct comparison of the severity of liver disease/damage in both groups and subsequent clinical course.

It was speculated in Chapter 4 that tin colloid clearance (Ktc) combined with indocyanine green clearance (Kicg) may provide a good assessment of outcome (death) in patients with severe liver disease if the patients with metastatic liver disease are excluded. In Chapter 5 the blood clearance of tin colloid (Ktc) combined with the clearance of indocyanine green (Kicg) was a good predictor of death but was improved by combining the liver elimination of Disida (K2d) with tin colloid clearance (Ktc).

The combination of a test of Kupffer cell function and a test of hepatocyte function produced the best results and is not unexpected as there is an interdependence between the hepatocyte and Kupffer cell. These conclusions suggest that the significant loss of both of these cell function is a grave sign in chronic cirrhotic liver disease and Canalese\textsuperscript{16} had already shown similar results but in acute hepatic failure. This finding deserves further evaluation in clinical studies.

The use of the newer compounds, Disida and caffeine, as liver function test agents has been investigated in this work. They are both easy to quantify, inexpensive tests and well tolerated by the patients.

In common with other workers\textsuperscript{168, 169}, the fasting serum or salivary caffeine level was an excellent measure of liver dysfunction and shows great promise as a routine clinical test as it is
extremely easy to collect. The measurement of the second, metabolic component if caffeine clearance was attempted on further salivary samples at 1 and 6 hours. This work was routinely discontinued as the difference in the concentrations of caffeine between the two samples did not allow a calculation of clearance in about half of the patients. On re-analysis of this data, the absence of a fall in concentration between the 2 samples of more than 20% may also be a marker of liver dysfunction. This simple addition to a fasting level may provide confirmatory evidence of liver disease and deserves further evaluation. Using a technique with oral caffeine administration and collecting salivary samples has particular attraction in children where intravenous cannulation or needling is avoided.

The ability to formulate both a fast and slow rate constant was first described in this work using an intravenous bolus which has allowed precise kinetics to be evaluated. Using an intravenous technique a readily defined dose can be administered and the patients monitored while the test is in progress. A better definition of the normal range of the first component is required and blood samples taken every 3 minutes for the first 15 minutes would help instead of every 5 minutes as was used in this study. The controls in this study were mainly young, fit adults. It would be expected that the clearance of caffeine like other clearance tests would reduce in older subjects and/or be dose dependant. Therefore definition of the normal range in different age bands may be necessary.

Problems with accidental taking of caffeine containing foodstuffs are a real hazard which was seen and identified in this study. The inability of patients to take a caffeine free diet would make interpretation of a single value of either fasting caffeine or caffeine clearance potentially risky. This suggests that a combination of fasting and clearance parameters may improve accuracy.
It is possible that the fast component of caffeine clearance could be utilised to measure liver blood flow although using the EMIT assay a small rise in plasma values was observed in all patients at about 30 minutes after intravenous injection (Figure 5.2) which probably represents some cross reactivity with a caffeine metabolite. Further work using high performance liquid chromatography analysis of caffeine concentrations may allow only caffeine to be analysed and eliminate this problem.

Disida kinetics have been evaluated in alcoholic liver disease and as with caffeine clearance were a good test of liver function (Table 5.10). The unique feature of HIDA scintigraphy is that simultaneous quantitative information is provided as well as the static pictures and Disida deserves further evaluation in different liver diseases such as primary biliary cirrhosis and sclerosing cholangitis. It is disappointing that over the duration of this work the routine use of radioisotope scanning in the investigation of liver and biliary disease has declined to be replaced by other modalities such as ultrasound scanning and MRI scanning.

Biochemical liver function tests individually or in combination did not perform as well in predicting outcome as the clearance tests of liver function (Table 5.10) but collectively they were most sensitive in the diagnosis of liver disease. There seems little possibility of the necessity of replacing these tests to screen generally for liver disease especially as they are cheap to perform using modern autoanalysers.

The opsonin, fibronectin, is important in phagocytosis and in this study a low serum level was a good marker of liver disease (Table 5.10) and needs further study as a synthetic test of liver function.
From Chapter 6 the conclusion that after a comprehensive battery of liver function tests there is minimal effect on liver function by oral isosorbide dinitrate is an important pre-requisite for the use of this drug in the treatment of oesophageal varices. However the findings of a poorly tolerated drug renders further widespread usage most unlikely and hepatologists may have to look elsewhere for a drug modality.

Combination therapy such as with nitroglycerin and vasopressin$^{242}$ in acute bleeding and nadolol plus isosorbide mononitrate$^{243}$ in chronic bleeding holds promise that the portal pressure lowering effects of nitrates can be utilised without the side effects of the therapy.

Just as definitive tests have evolved to diagnose and monitor diseases such as hepatitis with specific antibodies, there appears to be a place for clearance tests in cirrhotic liver disease to assess quantitatively liver cell damage. A combination of a Kupffer cell test and a hepatocyte test has given good results in this work with a combination of tin colloid clearance and Disida liver elimination proving the best combination to predict outcome in alcoholic liver disease. There are many functions of the liver and no single test or tests could be expected to be useful in all diseases. Therefore other combinations may need to be evaluated in other liver diseases.
<table>
<thead>
<tr>
<th>Appendix</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendix 1</td>
<td>169</td>
</tr>
<tr>
<td>Appendix 2</td>
<td>175</td>
</tr>
<tr>
<td>Appendix 3</td>
<td>184</td>
</tr>
<tr>
<td>Appendix 4</td>
<td>185</td>
</tr>
<tr>
<td>Appendix 5</td>
<td>187</td>
</tr>
<tr>
<td>Appendix 6</td>
<td>190</td>
</tr>
</tbody>
</table>
APPENDIX 1 - From Chapter 4

The blood clearance of tin colloid (Ktc) is compared with the biochemical measures of liver function; bilirubin, AST and alkaline phosphatase, and albumin and prothrombin ratio.
Figure A1-1 The relationship of tin colloid clearance (Ktc) with bilirubin (from Chapter 4 in 21 patients with liver disease)
Figure A1-2 The relationship of tin colloid clearance (Ktc) with aspartate aminotransferase (AST). From Chapter 4 in 21 patients with liver disease.
Figure A1-3 The relationship of tin colloid clearance (Ktc) and alkaline phosphatase (from Chapter 4 in 21 patients with liver disease).
Figure A1-4 The relationship of tin colloid clearance (Ktc) with serum albumin (from Chapter 4 in 21 patients with liver disease).

![Graph showing the relationship between tin colloid clearance (Ktc) and serum albumin (gms/L).]
Figure A1-5 The relationship of tin colloid clearance (Ktc) with the prothrombin ratio (from Chapter 4 in 21 patients with liver disease).
APPENDIX 2 - From Chapter 5

Changes in the clearance tests of liver function between the two sets of tests 6 months apart.
Figure A2-1 The changes in the blood clearance of tin colloid \((K_{tc})\) between the two sets of tests taken 6 months apart (from Chapter 5 in the 21 patients who had repeat tests).

<table>
<thead>
<tr>
<th></th>
<th>Median (IQR)</th>
<th>0.10 - 6 months</th>
<th>0.12 (0.08)</th>
<th>0.11 (0.06)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NS, paired Wilcoxon test</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure A2-2 The changes in the liver uptake of tin colloid between the two sets of tests taken 6 months apart (from Chapter 5 on the 21 patients who had repeat tests).

Uptake of colloid (Klu)

<table>
<thead>
<tr>
<th></th>
<th>0 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (IQR)</td>
<td>0.17 (0.10)</td>
<td>0.18 (0.09)</td>
</tr>
</tbody>
</table>

NS, paired Wilcoxon test
Figure A2-3 The changes in the clearance of indocyanine green (Kicg) between the two sets of tests taken 6 months apart (from Chapter 5 on the 21 patients who had repeat tests).

**Figure**

0.20

0.10

0.10 (0.10) 0.09 (0.10)

NS, paired Wilcoxon test
Figure A2-4 The changes in the fasting serum caffeine level (FSC) between the two sets of tests taken 6 months apart (from Chapter 5 on the 21 patients who had repeat tests).

MEDIAN (IQR) 0.10 (0.18) 0.09 (0.29)

NS, paired Wilcoxon test
Figure A2-5 The changes in the first clearance constant of caffeine (Klc) between the two sets of tests taken 6 months apart (from Chapter 5 on the 21 patients who had repeat tests).

For clearance
stant of
eine (Klc)

0.20

0.10

0

0 months 6 months

MEDIAN (IQR) 0.09 (0.14) 0.07 (0.10)

NS, paired Wilcoxon test
Figure A2-6 The changes in the second clearance constant of caffeine (K2c) between the two sets of tests taken 6 months apart (from Chapter 5 on the 21 patients who had repeat tests).

<table>
<thead>
<tr>
<th>0.0060</th>
<th>0.0050</th>
<th>0.0040</th>
<th>0.0030</th>
<th>0.0020</th>
<th>0.0010</th>
<th>0.0000</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 months</td>
<td>6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MEDIAN (IQR) 0.0021 (0.0023) 0.0022 (0.0026)

NS, paired Wilcoxon test
Figure A2-7 The changes in the first clearance constant of Disida (Kld) between the two sets of tests taken 6 months apart (from Chapter 5 on the 21 patients who had repeat tests).

<table>
<thead>
<tr>
<th>MEDIAN (IQR)</th>
<th>0 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.14 (0.06)</td>
<td>0.12 (0.10)</td>
</tr>
</tbody>
</table>

NS, paired Wilcoxon test
Figure A2-8 The changes in the second clearance constant of Disida (K2d) between the two sets of tests taken 6 months apart (from Chapter 5 on the 21 patients who had repeat tests).

**Disida (K2d)**

<table>
<thead>
<tr>
<th></th>
<th>0 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEDIAN</td>
<td>0.0017</td>
<td>0.0017</td>
</tr>
<tr>
<td>(IQR)</td>
<td>(0.0017)</td>
<td>(0.0014)</td>
</tr>
</tbody>
</table>

NS, paired Wilcoxon test
Appendix 3. The repeated measures of fasting serum caffeine (FSC) and the first clearance constant of caffeine (Klc). From Chapter 5 on the 5 patients who had repeat measurements.

![Graph of fasting serum caffeine (μmol/L) vs. time](image1)

![Graph of first clearance constant of caffeine (min⁻¹) vs. time](image2)
Appendix 4 The relationship between serum and saliva caffeine levels. From Chapter 5 and measured on 14 patients at 60 minutes and 360 minutes respectively.
Salivary caffeine (µmols/L)
Appendix 5

Scatter diagrams of the initial serum levels of C3 and C4 for both deaths and survivors

(From Chapter 5 on the 25 patients who were studied)
Figure A5-1 Scatter diagram of the serum level of C3 divided between death and survival. Twenty five patients were tested but results are only available on 22 patients and there were 8 deaths over the 1 year of follow up.

Liver disease Liver disease Liver disease
Total Alive Dead
(n=22) (n=14) (n=8)

MEDIAN (IQR) 3,300 (1,300) 3,200 (1,300) 3,100 (1,300)

NS, Mann Whitney
Figure A5-2 Scatter diagram of the serum level of C4 for the 25 patients tested and divided by death or survival. Results are only available in 22 patients and there were 8 deaths after 1 year of follow up.

<table>
<thead>
<tr>
<th>Liver disease</th>
<th>Total (n=22)</th>
<th>Alive (n=14)</th>
<th>Dead (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEDIAN (IQR)</td>
<td>1,100 (700)</td>
<td>1,100 (1,000)</td>
<td>1,300 (500)</td>
</tr>
</tbody>
</table>

NS, Mann Whitney
APPENDIX 6

The initial and repeat biochemical liver function tests of the patients tested in Chapter 5.
Table A6-1 The initial biochemical liver function tests of the 25 patients from Chapter 5.

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Bilirubin (μmols/L)</th>
<th>AST (iu/L)</th>
<th>Alkaline Phosphatase (iu/L)</th>
<th>Prothrombin Time (Secs)</th>
<th>Albumin (gms/L)</th>
<th>Child-Pugh Grading</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>14</td>
<td>401</td>
<td>13/12</td>
<td>48</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>84</td>
<td>163</td>
<td>14/12</td>
<td>35</td>
<td>B</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>15</td>
<td>467</td>
<td>18/12</td>
<td>32</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>10</td>
<td>281</td>
<td>13/12</td>
<td>40</td>
<td>B</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>58</td>
<td>639</td>
<td>14/12</td>
<td>35</td>
<td>A</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>26</td>
<td>295</td>
<td>14/12</td>
<td>34</td>
<td>A</td>
</tr>
<tr>
<td>7</td>
<td>27</td>
<td>35</td>
<td>298</td>
<td>18/12</td>
<td>37</td>
<td>A</td>
</tr>
<tr>
<td>8</td>
<td>26</td>
<td>25</td>
<td>313</td>
<td>17/12</td>
<td>35</td>
<td>B</td>
</tr>
<tr>
<td>9</td>
<td>32</td>
<td>42</td>
<td>175</td>
<td>13/12</td>
<td>52</td>
<td>A</td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td>18</td>
<td>254</td>
<td>14/12</td>
<td>36</td>
<td>B</td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>29</td>
<td>230</td>
<td>19/12</td>
<td>37</td>
<td>B</td>
</tr>
<tr>
<td>12</td>
<td>22</td>
<td>26</td>
<td>194</td>
<td>16/12</td>
<td>36</td>
<td>B</td>
</tr>
<tr>
<td>13</td>
<td>13</td>
<td>31</td>
<td>333</td>
<td>19/12</td>
<td>37</td>
<td>B</td>
</tr>
<tr>
<td>14</td>
<td>27</td>
<td>33</td>
<td>189</td>
<td>12/12</td>
<td>48</td>
<td>B</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
<td>75</td>
<td>519</td>
<td>19/12</td>
<td>32</td>
<td>B</td>
</tr>
<tr>
<td>16</td>
<td>6</td>
<td>30</td>
<td>288</td>
<td>12/12</td>
<td>44</td>
<td>A</td>
</tr>
<tr>
<td>17</td>
<td>35</td>
<td>33</td>
<td>267</td>
<td>19/12</td>
<td>35</td>
<td>B</td>
</tr>
<tr>
<td>18</td>
<td>93</td>
<td>71</td>
<td>123</td>
<td>18/12</td>
<td>21</td>
<td>C</td>
</tr>
<tr>
<td>19</td>
<td>7</td>
<td>17</td>
<td>258</td>
<td>17/12</td>
<td>33</td>
<td>B</td>
</tr>
<tr>
<td>20</td>
<td>7</td>
<td>22</td>
<td>190</td>
<td>13/12</td>
<td>36</td>
<td>A</td>
</tr>
<tr>
<td>21</td>
<td>549</td>
<td>74</td>
<td>1134</td>
<td>16/12</td>
<td>20</td>
<td>C</td>
</tr>
<tr>
<td>22</td>
<td>12</td>
<td>18</td>
<td>281</td>
<td>14/12</td>
<td>23</td>
<td>B</td>
</tr>
<tr>
<td>23</td>
<td>61</td>
<td>34</td>
<td>395</td>
<td>16/12</td>
<td>24</td>
<td>C</td>
</tr>
<tr>
<td>24</td>
<td>64</td>
<td>83</td>
<td>284</td>
<td>17/12</td>
<td>18</td>
<td>C</td>
</tr>
<tr>
<td>25</td>
<td>17</td>
<td>21</td>
<td>125</td>
<td>18/12</td>
<td>31</td>
<td>B</td>
</tr>
</tbody>
</table>
Table A6-2  The repeat liver function tests at 6 month of the patients in Chapter 5. (21 patients as 4 died between initial and repeat testing).

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Bilirubin (µmols/L)</th>
<th>AST (iu/L)</th>
<th>Alkalline Phosphatase (iu/L)</th>
<th>Prothrombin Time (Secs)</th>
<th>Albumin (gms/L)</th>
<th>Child-Pugh Grading</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>9</td>
<td>373</td>
<td>14/12</td>
<td>49</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>147</td>
<td>187</td>
<td>19/12</td>
<td>37</td>
<td>B</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>19</td>
<td>572</td>
<td>20/12</td>
<td>33</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>23</td>
<td>259</td>
<td>13/12</td>
<td>36</td>
<td>B</td>
</tr>
<tr>
<td>5</td>
<td>37</td>
<td>25</td>
<td>253</td>
<td>13/12</td>
<td>43</td>
<td>A</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>29</td>
<td>300</td>
<td>17/12</td>
<td>34</td>
<td>B</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>39</td>
<td>290</td>
<td>19/12</td>
<td>39</td>
<td>A</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>35</td>
<td>307</td>
<td>16/12</td>
<td>40</td>
<td>A</td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td>51</td>
<td>195</td>
<td>14/12</td>
<td>47</td>
<td>A</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>17</td>
<td>311</td>
<td>15/12</td>
<td>41</td>
<td>B</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>19</td>
<td>252</td>
<td>15/12</td>
<td>40</td>
<td>B</td>
</tr>
<tr>
<td>12</td>
<td>21</td>
<td>32</td>
<td>166</td>
<td>18/12</td>
<td>40</td>
<td>B</td>
</tr>
<tr>
<td>13</td>
<td>14</td>
<td>26</td>
<td>288</td>
<td>16/12</td>
<td>35</td>
<td>B</td>
</tr>
<tr>
<td>14</td>
<td>23</td>
<td>31</td>
<td>165</td>
<td>13/12</td>
<td>47</td>
<td>A</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>33</td>
<td>255</td>
<td>15/12</td>
<td>41</td>
<td>B</td>
</tr>
<tr>
<td>16</td>
<td>7</td>
<td>29</td>
<td>220</td>
<td>16/12</td>
<td>41</td>
<td>A</td>
</tr>
<tr>
<td>17</td>
<td>204</td>
<td>101</td>
<td>385</td>
<td>19/12</td>
<td>31</td>
<td>B</td>
</tr>
<tr>
<td>19</td>
<td>10</td>
<td>34</td>
<td>312</td>
<td>13/12</td>
<td>35</td>
<td>B</td>
</tr>
<tr>
<td>20</td>
<td>15</td>
<td>26</td>
<td>170</td>
<td>13/12</td>
<td>40</td>
<td>A</td>
</tr>
<tr>
<td>22</td>
<td>16</td>
<td>101</td>
<td>413</td>
<td>17/12</td>
<td>17</td>
<td>B</td>
</tr>
<tr>
<td>25</td>
<td>77</td>
<td>41</td>
<td>209</td>
<td>18/12</td>
<td>39</td>
<td>B</td>
</tr>
</tbody>
</table>
REFERENCES
1  Aschoff L.
Reticulo-endothelial system.

2  Van den Bergh A A H, Muller P.
Uber eine direkte und eine indirekte Diazoreaktion auf Bilirubin.

3  Ribbert H.
Die abscheidung intravenas injizierten gelosten karmis in den geweben.
Z Allg Physiol, 1904, 4, 201-214.

4  Nagoa K.
The fate of killed non haemolytic streptococci injected into the blood and the resulting cellular change.
J Infect Dis, 1920, 27, 327-362.

5  Cole P G, Lathe G H, Billing P H.
Separation of the bile pigments of serum bile and urine.

6  Billing B H, Lathe G H.
The excretion of bilirubin as an ester glucuronide giving the direct van den Bergh reaction.

7  Roberts W M.
Blood phosphatase and the van den Bergh reaction in the differentiation of the several types of jaundice.

8  King E J, Armstrong G L.
A convenient method for determining serum and bile phosphatase activity.
Can Med Ass J, 1934, 31, 376-381.
9 De Ritis F, Coltorti M, Giush G.
Attivita transaminasiche del siero nell'epatite virale.

10 Wroblewski F, Ladue J S.
Serum glutamic oxalacetic transaminase activity as an index of liver cell injury. A preliminary report.

11 Rappaport A M, Hiraki G Y.
The anatomical patterns of lesions in the liver.
Acta Anat (Basel), 1958, 32, 126-140.

12 Wisse E, De Zanger R B, Charles K et al.
The liver sieve: Consideration concerning the structure and function of endothelial fenestræ, the sinusoidal wall and the space of Disse.
Hepatology, 1985, 5, 683-687.

13 Kaplowitz N.
Drug induced hepatotoxicity.

14 Buchanon B J, Filkins J P.
Hypoglycaemic depression of reticulo-endothelial function.

15 Drivas G, Wardle E N.
Reticulo-endothelial dysfunction in diabetes and hyperlipidaemia.
16 Wardle E.N.
Kupffer cells and their function.
Liver, 1987, 7, 63-75.

17 Kawahara T, Sakisaki S, Yamauilis K, et al.
Effect of alcohol on cultured Kupffer cells.
In: Cells of the hepatic sinusoid, No 1, Kupffer cell foundation, 1986, 323-328.

18 Schidlt B, Bouteng R, Sollenbeg M.
Plasma substitute induced impairment of RES function.

19 Saba T M, Jaffe E.
Fibronectin synthesis by vascular endothelial cells and role after trauma in reticuloendothelial function.

20 Saba T M, Scovill W A.
Effect of surgical trauma on host defence.

21 Cuddy B G, Loegering D J, Blumenstock F A.
Depression of an in vivo clearance of hepatic macrophage complement receptors following thermal injury.

22 Rubli E, Bussard S, Frei E, Lundgard-Hansen P.
Plasma fibronectin and associated variables in surgical intensive care patients.

23 Hoyoux C, Foidart J, Rigo P, Mahieu P.
Effects of methyl prednisolone on the Fc receptor function of the human reticuloendothelial system in vivo.
24 Editorial.

The effect of dietary restriction on macrophage function evaluated by 125-iodine polyvinyl-pyrrolidone clearance.
Nutr Rev, 1977, 35, 82-84.


Endotoxaemia as a cause of pyrexia in immunosuppressed patients.

26 Beeson P.

Effect of reticulo-endothelial blockade on immunities of the Schwartzman reaction.

27 Holper K, Olca J, Kitahama A et al.

Effect of ischaemia on hepatic parenchymal and reticuloendothelial function in the baboon.
Surgery, 1974, 76, 423-432.

28 Sanders K D, Fuller G M.

Kupffer cell regulation of fibrinogen synthesis.

29 Mayanski D N.

Kupffer cells in liver injury and regeneration.

30 Gregiordais G,

The cancer potential of liposomes in medicine and pathology.


Lack of in vivo activation of the interferon system in hepatitis B antigen positive chronic active hepatitis.
32 Ali M, Nolan J P.
   Alcohol induced depression of reticulo-endothelial function in the rat.
   J Lab Clin Med, 1967, 70, 295-301

33 Wardle E N, Anderson A, James O.
   Kupffer cell phagocytosis in relation to BSP clearances in liver and inflammatory bowel disease.

34 Neuberger J M.
   Transplantation for alcoholic liver disease.

35 Pequignot G.
   Les problemes nutritionnelles de la societe industrielle.

36 Ricketts W E, Kirsner J G.
   Latent portal cirrhosis.
   Gastroenterology, 1951, 17, 184-193.

37 Rankin J G D, Orrego H, Deschenes J, et al.
   Alcoholic liver disease: the problems of diagnosis.

38 Morgan M Y.
   Alcoholic liver disease: its clinical diagnosis, evaluation and treatment.

39 Orrego H, Blake J E, Blendis L M, Medline A.
   Prognosis of alcoholic cirrhosis in the presence and absence of alcoholic hepatitis.
40 Brunt P W, Kew M C, Scheuer P J, Sherlock S.
Studies in alcoholic liver disease in Britain.
Gut, 1974, 15, 52-58.

41 Pares A, Caballena J, Bruguera M, Torres M, Rodes J.
Histological course of alcoholic hepatitis.
J Hepatol, 1986, 2, 33-42.

The morphology of cirrhosis; definition, nomenclature and classification.

43 Conn H O, Atterbury C E.
Cirrhosis.
In: Disease of the liver, Schiff L and Schiff L R (eds), J B Lippincott, Philadelphia, 1987,
pages 725-864.

44 Willis R A.
Secondary tumours of the liver.
178-192.

45 Jalan R, Hayes P.C.
Review Article; quantitative tests of liver function.
Aliment Pharmacol Ther, 1995, 9, 263-270.

Criteria for assessment of functional impairment in patients with cirrhosis of the liver.

47 Sherlock S, Dick R, Van Leeuwan D J.
Liver biopsy today. The Royal Free Hospital experience.
J Hepatol, 1984, 1, 75-79.
48 Child C G, Turotte J G,
Surgery and portal hypertension.

49 Pugh R N H, Murray-Lyon I M, Dawson J L, Pietroni M C, Williams R.
Transection of the oesophagus for bleeding oesophageal varices.

Clinical and statistical validity of conventional prognostic factors in predicting short term survival among cirrhotics.

51 Conn H O.
A peek at the Child-Turcotte classification.
Hepatology, 1981, 6, 673-676.

52 D'Amico G, Morabito A, Pagliaro L, Marubini E.
Survival and prognostic indicators in compensated and decompensated cirrhosis.

53 Schlichting P, Christenson E, Anderson P et al.
Prognostic factors in cirrhosis identified by Cox's regression model.

54 Bircher J.
Quantitative assessment of deranged hepatic function: A missed opportunity?

55 McIntyre N.
The limitations of conventional liver function tests.
56 Moseley R.
Evaluation of abnormal liver function tests.

57 Price C P, Alberti K G M M.

58 Kaplan M M.
Understanding serum enzyme tests in clinical liver disease:
In Davidson C S (Ed); Problems in liver disease, New York, Stratton Intercontinental Medical Book Corp, 1979, 79-85.

59 Wroblewski F.
The clinical significance of transaminase activities of serum.

60 Zimmerman H J, West M.
Serum enzyme levels in the diagnosis of hepatic disease.

61 Van Weas L, Lieber S.
Glutamate dehydrogenase: A reliable marker of liver cell necrosis in the alcoholic.

62 Ellis G.
Serum enzyme tests in diseases of the liver and biliary tree.

63 Kaplan M M.
Biochemical basis for serum enzyme abnormalities in alcoholic liver disease.
64 Demers L M, Hepner G.
Radioimmunoassay of bile acids in serum.

65 Kaplan M M.
Laboratory tests.

66 Betro M G.
Gamma-glutamyl transpeptidase in disease of the liver and bone.

67 Rosalki S B.
Gamma-glutamyl transpeptidase.

68 Burrows S.
Serum gamma-glutamyl transpeptidase. Evaluation in screening of hospitalised patients.

69 Olson J P.
Synthesis of coagulation factors by the in vitro perfused liver.
Blood, 1963, 22, 82-834.

70 Friedman P A.
Vitamin K-dependant proteins.

71 Ratnoff O D,
Haemostatic mechanisms in liver disease.
72 Kelly D A, Summerfield J A.
Haemostasis in liver disease.

73 Kelly D A, Tuddenham D G A.
Haemostatic problems in liver disease.

74 Cooper A D.
Role of the liver in the degradation of lipoproteins.
Gastroenterology, 1985, 88, 192-205.

75 McIntyre N.
Plasma lipids and lipoproteins in liver disease.

76 Scheinberg I H, Sternieb I

77 Potter B J, Chapman R W, Nunes R M, Sorrentino D, Sherlock S.
Transferrin metabolism in alcoholic liver disease.
Hepatology, 1985, 5, 714-721.

78 Feizi T.
Serum, immuglobulins in liver disease.

79 Kindmark C O.
Plasma protein patterns in hepatitis A and B.
In: Peeters H (Ed), Protides of the biological fluids, 23rd Colloquium Pergamon Press,
Measurement of serum bilirubin and its mono and diconjugates: application to patients with hepatobiliary disease.

81 Berry W, Reichen J.
Bile acid metabolism: its relation to clinical disease.

82 Simmonds W J.
Radioimmunoassay of conjugated choleyl bile acids in serum.

83 Preisig R.
Clinical evaluation of liver function.
In: MacSween R (Ed), Recent advances in Hepatology, Churchill Livingstone, Edinburgh, 1986, 1-12.

84 Lautt W W.
Hepatic vasculature: A conceptual review.
Gastroenterology, 1977, 73, 1163-1169.

85 Lautt W W, Legare D J, D'Almeida M S.
Adenosine as putative regulator of hepatic arterial flow (the buffer response).

86 Wilkinson G R, Shand D G.
A physiological approach to hepatic drug clearance.
Clin Pharmacol Ther, 1975, 18, 377-381.

Intrinsic hepatic clearance in cirrhosis.
88 Villeneuve J P, Huet R, Marleau D, Huet P M.
Estimation of hepatic blood flow with indocyanine green: Comparison between continuous infusion and single injection methods.

89 Bradley S E, Ingelfinger F J, Bradley G P et al.
The estimation of hepatic blood flow in man.
J Clin Invest, 1945, 24, 890-897.

90 Sherlock S, Dooley J.

91 Hofmann A F
The aminopyrine demethylation breath test and the serum bile acid level: nominated but not yet elected to join the common liver tests.

Lidocaine metabolite formation as a measure of peri-operative liver function.

93 Branch R A.
Drugs as indicators of hepatic function.
Hepatology, 1982, 2, 97-105.

94 Bumstein A V, Galambos J J.
14C-aminopyrine breath test in chronic liver disease. Preliminary diagnostic implications.

95 Keiding S.
Galactose clearance measurements and liver blood flow.
Gastroenterology, 1988, 94, 477-481.
96 Rosalki S, Dooley J.
Liver function profiles and their interpretation.

Aminopyrine N-demethylation: a prognostic test of liver function in patients with
alcoholic liver disease.
Gastroenterology, 1980, 80, 1145-1150.

98 Saunders J B, Wright N, Lewis K O.
Predicting outcome of paracetamol poisoning by using the 14C-aminopyrine breath test.

99 Ramsoe K, Andreason P B, Ranket L.
Functioning liver mass in uncomplicated and fulminant acute hepatitis.

100 Potter J, Hickman P, Lynch S et al.
Use of monoethylglycinexylidide as a liver function test in the liver transplant recipient.
Transplantation 1993, 56, 1385-1388.

101 Jalan R, Plevris J, Jalan A et al.
A pilot study of indocyanine green clearance as an early predictor of graft function.
Transplantation, 1994, 58, 196-200.

102 Bircher J, Ratzer R, Gikalov I, Kupfer A, Preisig R.
Aminopyrine breath test for evaluation of liver function. How to analyse the 14 CO2 data.
In: Hofer R (Ed), Radioaktive isotope in Klinik und Forschung, Vol 12, Egermann,
Vienna, 1976, 347.

103 Carlisle R, Galambos J T, Warren W D.
The relationship between conventional liver tests, quantitative function tests and
histopathology in cirrhosis.
104 Irving C S, Schoeller D A, Nakamua K I, Baker A L, Klein P D.
The aminopyrine breath test as a measure of liver function.

105 Eriksson L S, Kagedale B, Wahren J.
Effects of captopril on hepatic venous pressure and blood flow in patients with liver cirrhosis.

106 Thomas H C.
The immune response in hepatic cirrhosis: animal and human studies.

107 Ellison R T, Horsburgh C R, Curd J.
Complement levels in patients with hepatic dysfunction.

108 Saba T M.
Physiology and pathophysiology of the reticuloendothelial system.

109 Dumitrascu D L, Cotul S, Tamas S.
Liver blood flow (LBF) estimated by Kupffer cells clearance in health and disease.

110 Torrance H B, Gowenlock A H.
Radioactive colloidal clearance techniques to measure liver blood flow in man.

111 Bradley E L.
Measurement of hepatic blood flow in man.
Surgery, 1974, 75, 783-789.
112 Chiandussi L, Greco F, Cesano L, Muraton F, Vaccarino A, Corradi C.
A study of the kinetics of the reticuloendothelial system in normal and cirrhotic subjects
with the use of colloidal denatured alumbin labelled with 1131.

113 Taplin G V.
Dynamic studies of liver function with radioisotopes,

114 De Nardo S J, Bell G B, De Nardo G I et al.
Diagnosis of cirrhosis and hepatitis by quantitative hepatic and other reticuloendothelial
clearance rates.

115 Horisawa M, Goldstein G, Waxman A, Reynolds T.
The abnormal hepatic scan of chronic liver disease: its relationship to hepatic
haemodynamics and colloid extraction.
Gastroenterology, 1976, 71, 210-213.

116 Miller J, Diffey B L, Fleming J S.
Measurement of colloidal clearance rate as an adjunct to static liver imaging.

117 Houston A S, MacLeod M A.
Processing of liver dynamic studies with technetium-labelled sulphur colloid.

Reticuloendothelial system and hepatocyte function in fulminant hepatic failure.
Relation of renal impairment and haemorrhagic diathesis to endotoxaemia in fulminant hepatic failure.
Lancet, 1974, 1, 521-524.

120 Cavanagh D, Rao P S, Sutton D M C, Blumgart B D, Buchanon F.
Pathophysiology of endotoxic shock in the primate.

121 Adams F G, Horton P W, Selim S.M.
Clinical comparison of three liver scanning agents

122 Zivanovic M A, Meller S T, Trott N G, McCready V R.
A clinical comparison of four Tc-labelled radiopharmaceuticals for liver and spleen imaging.

123 Wilkins D J.
Interactions of charged particles with the reticuloendothelial system.

124 Whateley T G, Steele G.
Particle size and surface charge studies of a tin colloid radiopharmaceutical for liver scintigraphy.

125 Frier M, Whalley D R, Dean S A.
Particle size changes in tin colloid. Their influence on dynamic liver blood flow studies.
126 Chadwick S J D, Biglin J E J, Dudley H A F.
Kupffer cell clearance of technetium tin colloid in reticuloendothelial blockade and severe sepsis.

127 Fox I J, Brooker L G S, Heseltine D W, Essex H E, Wood E H.
A tricarbocyanine dye for continuous recordings of dilution curves of the whole blood independant of variations in blood oxygen saturation.

128 Wheeler H O, Chanston W I, Meltzer J I.
Hepatic uptake and biliary excretion of indocyanine green in the dog.

129 Caesar J, Shaldon S, Chiandussi L, Guevara L, Sherlock S.
The use of indocyanine green in the measurement of hepatic blood flow and as a test of hepatic function.

130 Baker K J.
Binding of sulfobromophthalein sodium and indocyanine green by plasma α1-lipoproteins.

131 Cornelius C E, Ben-Ezzer J, Anas I M.
Binding of sulfobromophthalein and other organic anions by isolated hepatic cell plasma membranes in vitro.

132 Hunton D B, Bollman J L, Hoffman H N.
The plasma removal of indocyanine green and sulfobromophthalein - effect of dosage and blocking agents.
133 Reyes H, Levi A J, Gatmaitan Z, Anas I M.
   Studies of Y and Z, two hepatic cytoplasmic organic anion binding proteins: effect of
drugs, chemicals, hormones and cholestasis.

134 Klaessen C D, Plaa G L.
   Plasma disappearance and biliary excretion of indocyanine green in rats, rabbits and dogs.

135 Cherrick G R, Stein S W, Leevy C M, Davidson S C.
   Indocyanine green: observations on its physical properties, plasma decay and hepatic
   extraction.

136 Hunton D B, Bollman J L, Hoffman H N.
   Studies of hepatic function with indocyanine green.
   Gastroenterology, 1960, 39, 713-716.

137 Paumgartner G.
   The handling of indocyanine green by the liver.

138 Michaelis L, Menten M L.
   Die kinetik der invertinwirkung.

139 Kojima H.
   Relation of the size of functional hepatic cell mass to the clearance of indocyanine green.

140 Daneshmend T K, Ene M D, Parks G, Roberts C J C.
   Effects of oral cimetidine on apparent liver blood flow and hepatic microsomal enzyme
   activity in man.
   Gut, 1984, 25, 125-128.
141 Clements D, West R, Elias E.
Comparsion of bolus and infusion methods for estimating hepatic blood flow.

142 Cohn J N, Khatri I M.
Hepatic blood flow in alcholic liver disease.

143 Branch R.A., James J A, Read A E,
The clearance of antipyrine and indocyanine green in normal subjects and patients with chronic liver disease.
Clin Pharmc Ther, 1976, 20, 81-89

144 Gottlieb M E.
Indocyanine green: Its use as an early indicator.

145 Matsumata T, Kanematsu T, Yoshida Y, Furuta T, Yanaga K, Sugimachi K.
The indocyanine green test enables prediction of postoperative complications after hepatic resection.

146 Krishnamurthy S, Krishnamurthy G T.
Nuclear hepatology: Where is it heading now?

147 Stadelnik R C, Matulo N M.
Clinical experience with 99m-Tc Disofenin.
Radiology, 1981, 140, 797-800.

148 Hernandez M, Rosenthall L.
A crossover study comparing the kinetics of Tc 99m labelled Diisopropyl and P_Butyl IDA analogs in patients.
149 Weissmann H S, Badia J, Sugarman L A et al.
   Spectrum of 99 Tc-m-IDA cholescintigraphic patterns in acute cholecystitis.

150 Wistow B W, Subramanian G, Gagna G M et al.
   Experimental and clinical trials of new 99m-Tc labelled heptobiliary agents.

151 Wistow B W, Subramanian G, Van Heertum R L et al.
   An evaluation of 99m-Tc-labelled hepatobiliary agents.

152 Fueger G F.
   The biokinetics of Tc-99m labelled hepatobiliary agents in humans.

153 Tarolo G L, Picozzi R, Palagi B, Cammelli F.
   Comparative quantitative evaluation of hepatic clearance of diethyl-IDA and para butyl-IDA in jaundiced and non jaundiced patients.

   Hepatocyte vs biliary disease: A distinction by deconventional analysis of Tc-99m-IDA time activity curves.

155 Love J E, Shaffer P, Fraser I A et al.
   Pharmacokinetic studies of Disida disposition. II. clinical studies.

156 Blanchard J, Sawers S J A.
   The absolute bioavailability of caffeine in man.
157 Arnaud M J, Welsch C.
Theophylline and caffeine metabolism in man.

Caffeine disposition after oral doses.

159 Parsons W D, Neims A H.
Effect of smoking on caffeine clearance.

Effects of cimetidine on caffeine disposition in smokers and nonsmokers.

161 Broughton L J, Rogers H J
Decreased systemic clearance of caffeine due to cimetidine.

162 Beach C A, Mayes D C, Guiler R C et al.
Inhibition of elimination of caffeine by disulfiram in normal subjects and recovering alcoholics.

163 Pathwarden R V, Desmond P V, Johnston R F et al.
Impaired elimination of caffeine by oral contraceptive steroids.

164 Arnaud M J.
The pharmacology of caffeine.
165 Statland B E, Demas T, Danis M.
Caffeine accumulation associated with liver disease.

166 Desmond P V, Patwardhan R V, Johnson R F, Schenker S.
Impaired elimination of caffeine in cirrhosis.

167 Renner E, Wietholtz H, Huguenin P, Arnaud M J, Preisig R.
Caffeine: A model compound for measuring liver function.

Overnight salivary caffeine clearance: A liver function test suitable for routine use.

169 Hasegawa M, Yamada S, Hirayama C.
Fasting plasma caffeine level in cirrhotic patients: Relation to plasma levels of catecholamines and renin activity.
Hepatolgy, 1989, 10, 973-977.

170 Vetter H, Falkner R, Neumayr A.
The disappearance rate of colloidal radiogold from the circulation and its application to the estimation of liver blood flow in normal and cirrhotic subjects.

171 Castell D O, Johnson R B
An index of portal-systemic collateral circulation in chronic liver disease.

172 Eddleston A L W F, Blendis L M, Osburn S B, Williams R.
Significance of increased ‘splenic uptake’ on liver scintiscanning.
Gut, 1969, 10, 711-716.
173 Waxman A D.
Scintigraphic evaluation of diffuse liver disease.

174 Popper H, Elias H, Petty D E.
Vascular pattern of the cirrhotic liver.

175 Huet P M, Chartrand R, Marleau D.
Extrahepatic uptake of Tc 99m-phytate: its mechanism and significance in chronic liver
disease.
Gastroenterology, 1980, 78, 76-80

176 Millett B, Chartrand R, Lavoie P et al.
The extrahepatic uptake of radioactive colloidal gold in cirrhotic patients as an index of
liver function and portal hypertension.

177 Triger D R, Boyer T D, Redekar A G, Reynolds T B, Waxman A D.
Differences in intrahepatic portal-systemic shunting in alcoholic and non alcoholic liver
disease as assessed by liver scan, portal pressure and E Coli antibodies.

178 Rogers A I, Feiss J S.
Application of the liver scan to diagnosis of oesophageal varices.

179 Shaldon S, Chiandussi L, Guevara L, Caesar J, Sherlock S.
The estimation of hepatic blood flow and intrahepatic shunted blood flow by colloidal
heat denatured human albumin labelled with 131I.
180 Xu Z L, Bucana C D, Fidler I J.
In vitro activation of murine Kupffer cells by lymphokines or endotoxins to lyse syngenic tumour cells.

181 Decker T, Kiderlen A F, Lohman-Matthes M L.
Liver macrophages as cytotoxic effector cells in extracellular cytotoxicity.

182 Manfoled I H, Triger D R, Underwood C J E.
Kupffer cell depletion in chronic liver disease.

183 Cooke A D, Parkin A, Wiggins P, Robinson P J, Giles G R.
Hepatic perfusion index and the evolution of liver metastases.

184 Robinson P J, Cooke D, Parkin A et al.
Hepatic perfusion index and the detection of hepatic metastases.

185 Tavill A S.
The synthesis and degradation of liver produced proteins.

186 Orrego H, Israel Y, Blake J D, Medline A.
Assessment of prognostic factors in alcoholic liver disease: Towards a global quantitative expression of severity.

187 Leevy C M, Smith F, Longueville J, Paumgartner G, Howard M M.
Indocyanine green clearance as a test for hepatic function. Evaluation by dichromatic ear densitometry.
188 Hofer R, Neumayr A, Parzer O, Vetter H.  
Bromsulphalein clearance und durchblutung derzirrhotischen leber während ein totallebevextraktbehandlung.  

189 Taplin G V, Hayashi J, Johnson D E, Dore E.  
Liver blood flow and cellular function in hepatobiliary disease: tracer studies with Radiogold and Rose Bengal.  

190 Kwon A H, Inada Y, Vetsuji S et al.  
Response of fibronectin to liver regeneration after hepatectomy.  
Hepatology, 1990, 11, 593-595.

191 Coleman D L.  
Regulation of macrophage phagocytosis.  

192 Wasnich R, Glober G, Hayashi T, Vicher T, Yeh F.  
Simple computer quantification of spleen to liver ratios in the diagnosis of hepatocellular disease.  

Dynamic liver scanning in cirrhosis.  

194 Almasio P L, Hughes R D, Williams R.  
Characterisation of the molecular forms of fibronectin in fulminant hepatic failure.  
Hepatology, 1986, 6, 1340-1345.

195 Gonzalez-Calvin J, Scully M F, Sanger Y et al.  
Fibronectin in fulminant hepatic failure.  
196 Thomas H C, De Villiers D, Potter B J et al.
Immune complexes in acute and chronic liver disease.

197 Potter B J.
Profiles of serum complement in patients with hepatobiliary diseases,
Digestion, 1978, 18, 371-374.

198 Kourilsky O, Leroy C, Peltier A P,
Complement and liver cell function in 53 patients with liver disease.

199 Corrao G, Ferran P, Zanlon A et al.

200 Borowsky S A, Strome S, Lott E.
Continued heavy drinking and survival in alcoholic cirrhotics,
Gastroenterology, 1981, 80, 1405 - 1409.

201 Zysset T, Wahllander A, Preisig R.
Evaluation of caffeine plasma levels by an automated enzyme immunoassay (EMIT) in comparison with a high performance liquid chromatographic method.
Ther Drug Monitoring, 1984, 6, 348-354.

202 Lachman P J, Hobart M J, Aston W P.
Complement technology.

Plasma and salivary pharmacokinetics of caffeine in man.
204 Zylber-Katz E, Granit L, Levy M.
   Relationship between caffeine concentrations in plasma and saliva.

205 Fraser I A, Love J, Staubus A E, et al.
   Pharmacokinetics of Disida disposition; Animal studies.

206 Cheng W S C, Murphy T, Smith M et al.
   Dose dependant pharmacokinetics of caffeine in humans; relevance as a test of
   quantitative liver function.

207 Charlesworth J A, Lawrence S.
   Acute hepatitis: significance of changes.
   Clin Exp Immunol, 1977, 28, 495-501

208 Mosher D F.
   Fibronectin and liver disease.
   Hepatology, 1986, 6, 1419-1421.

   Sinusoidal endothelial cells from guinea pig liver synthesise and secrete cellular
   fibronectin in vitro.
   Hepatology, 1987, 7, 856-858.

210 Naveau S, Poynard T, Abella A et al.
   Prognostic value of serum fibronectin concentration.
   Hepatology, 1985, 5, 819-823.

211 Westaby D, MacDougall B R D, Williams R.
   Improved survival following injection sclerotherapy for oesophageal varices: final
   analysis of a controlled trial.
   Hepatology, 1985, 5, 827-831.
212 Garden O J, Motyl H, Gilmour W H, Utley R J, Carter D C.
Prediction of outcome following acute variceal haemorrhage.
Br J Surg, 1985, 72, 91-95.

Long term follow up of transjugular intrahepatic portosystemic stent shunt (TIPSS) for
the treatment of portal hypertension: results in 130 patients.

214 Lacy A M, Nevasa M, Gilabert R et al.
Long term effects of distal splenorenal shunt on hepatic haemodynamics and liver
function in patients with cirrhosis: importance of reversal of portal blood flow.
Hepatology, 1992, 15, 616-622.

215 Simon R, Altman D G.
Statistical aspects of prognostic factor studies in oncology,
Br J Cancer, 1994, 69, 979-985.

216 Burroughs A K, D'Heygere F, McIntyre N.
Pitfalls in studies of prophylactic therapy for variceal bleeding in cirrhotics.
Hepatology, 1986, 6, 1407-1413.

217 Stanley A J, Bouchier I A D.
Portal hypertension: Pathophysiology and Management.

218 D'Amico G, Paglian L, Bosch J.
The treatment of portal hypertension - A meta-analytic review.

219 Gimson A E S, Ramage J K, Panoz M Z.
Randomised trial of variceal bleeding band ligation versus injection sclerotherapy for
bleeding oesophageal varices.
220 Williams S, Westaby D.
Management of Variceal Haemorrhage.

221 Reynolds T B, Ito S, Iwatsuki S.
Measurement of portal pressure and its clinical application.

222 Johnston G W.
Treatment of bleeding varices by oesophageal transection with the SPTU gun.

223 Korula J, Balart L A, Radvan G et al.
A prospective randomised controlled trial of chronic oesophageal variceal sclerotherapy.
Hepatology, 1984, 5, 584-589.

224 Soderlund C, Ihre T.
Endoscopic sclerotherapy versus conservative management of bleeding oesophageal varices; A 5 year prospective controlled trial of emergency and long term treatment.

225 Jenkins S A, Shields R, Davies M et al.
A multicentre randomised trial comparing octreotide and injection sclerotherapy in the management and outcome of acute variceal haemorrhage.

A prospective randomised controlled trial comparing the efficacy of somatostatin with injection sclerotherapy in the control of bleeding oesophageal varices.
J Hepatol, 1992, 16, 128-137.

Propranolol in the prevention of recurrent variceal haemorrhage in cirrhotic patients.
Gastroenterology, 1990, 98, 185-190.
228 Hayes P C, Davis J M, Lewis J A.
Meta analysis of propranolol in prevention of variceal haemorrhage.

229 Elsayed S S, Shiha G, Hamid M et al.
Sclerotherapy versus sclerotherapy and propranolol in the prevention of rebleeding from
oesophageal varices: a randomised study.
GUT, 1996, 38, 770-774.

230 Burroughs A K, Jenkins W J, Sherlock S et al.
Controlled trial of propranolol for the prevention of recurrent variceal haemorrhage in
patients with cirrhosis.

Comparison of three adrenoreceptor blocking agents in patients with cirrhosis and portal
hypertension.
Gut, 1984, 25, 73-78.

232 Lebrec D, Poynard T, Bernvau J et al.
A randomised controlled study of propranolol for prevention of recurrent gastrointestinal
bleeding in patients with cirrhosis. A final report.

233 Freeman J G, Barton J R, Record C O.
Effect of isosorbide dinitrate, verapamil and labetolol on portal pressure in cirrhotics.

234 Polston R J, Westaby D, Gimson A E S et al.
Sucralfate for the prevention of early rebleeding following injection sclerotherapy for
oesophageal varices.
235 Dawson J, Gertsch P, Mosmann F.
Endoscopic variceal pressure measurements: response to isosorbide dinitrate.
Gut, 1985, 26, 843-847.

236 Jones A L, Hayes P C.
Organic nitrates in portal hypertension.
Am J Gastroenterol, 1994, 89, 7-14.

Cirrhotics with variceal haemorrhage: The importance of the time interval between admission and the start of analysis for survival and rebleeding rates.
Hepatology, 1989, 9, 801-807.

238 Hallemans R, Naeije M.
Treatment of portal hypertension with isosorbide dinitrate alone and in combination with vasopressin.

239 MacDougall B R D, Westaby D, Theodossi A, Dawson J L, Williams R.
Increased long term survival in variceal haemorrhage using injection sclerotherapy.

240 Merkel C, Bolognesi M, Angeli P et al.
Lack of effect of verapamil and isosorbide dinitrate on the hepatic clearance of indocyanine green in cirrhosis.

241 Ikegami M, Toyonaga A, Tanikawa K.
Reduction of portal pressure by chronic administration in patients with cirrhosis: Effects on systemic and splanchnic haemodynamics and liver function.

242 Groszmann R J, Kravetz D, Bosch J et al.
Nitroglycerin improves the haemodynamic response to vasopressin in portal hypertension.
Hepatology, 1982, 2, 757-762.
243 Villaneuva C, Balanzo J, Novella M et al.

Nadolol plus isosorbide mononitrate compared with sclerotherapy for the prevention of variceal bleeding.