The use of computer-assisted experiments in the canine to support the development of a mathematical model for the hepatic removal of indocyanine green dye under normal and reduced hepatic blood flow conditions

Paul Joseph Antol
Iowa State University

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THE USE OF COMPUTER-ASSISTED EXPERIMENTS IN THE CANINE TO SUPPORT THE DEVELOPMENT OF A MATHEMATICAL MODEL FOR THE HEPATIC REMOVAL OF INDOCYANINE GREEN DYE UNDER NORMAL AND REduced HEPATIC BLOOD FLOW CONDITIONS

Iowa State University

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The use of computer-assisted experiments in the canine to support the development of a mathematical model for the hepatic removal of indocyanine green dye under normal and reduced hepatic blood flow conditions

by

Paul Joseph Antol

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major: Biomedical Engineering

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Professor-in-charge
Program in Biomedical Engineering

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For the Graduate College

Iowa State University
Ames, Iowa

1984
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FOREWORD

The most profound statement I could find with which to introduce this dissertation came from the book I probably consulted as much as any other during my graduate studies: Experimental Surgery by Markowitz, Archibald, and Downie (1964). The paragraph below was found on page 7 at the end of their introductory remarks:

Meliora sequi: This book aspires: we believe that the jack-of-all trades in our profession, the general surgeon, human or veterinary, will find it useful; so will that drawer of water, the urologist, and that hewer of wood, the orthopedist; so indeed will all workers in experimental biology—all, that is, except those incredible soothsayers of today, the Freudian psychoanalysts. These gentry may not read the stars, but they interpret dreams. True, they do not concentrate upon the entrails of chickens, but they do have more than a passing interest in the last inch of the human digestive tube. If you wish to know what to do until the psychiatrist arrives, you won't find it in this book.

You won't find it in this dissertation, either!

Paul J. Antol
March, 1984
INTRODUCTION

The rate of removal of an indicator substance has been clinically established as an indication of normal or abnormal hepatic function (Bradley et al., 1945). Many liver-specific dye substances such as bromosulfophthalein (BSP) or indocyanine green (ICG) have been developed with which to test the ability of the liver to remove an exogenous dye from the plasma. ICG dye has been shown to be an ideal test substance since its discovery in 1957 (Fox et al., 1957). ICG has two major advantages over other available dye compounds: 1) it undergoes no extrahepatic removal, and 2) it is completely recoverable in the bile. Because of these advantages, ICG is ideally suited as an indicator in the determination of hepatic blood flow.

In most methods available today, the determination of hepatic blood flow employs the indirect Fick method and involves catheterization of a hepatic vein (Leevy, 1965). Aside from the considerable patient risk involved in any catheterization procedure, the area sampled at a given time in the measurement of blood flow in the liver is small. Therefore, the important question becomes whether the mixing of the blood from the various lobes of the liver is uniform enough to ensure a sufficient distribution of indicator in the determination of hepatic blood flow in the sampled area. As will be seen later, this is not as important in humans as it is in canines due to the complexity of the canine hepatic venous system.

Since the total liver mass produces bile and ICG is completely recoverable in the bile, then the concentration of dye in the bile is
representative of the functional activity of the total liver mass, leaving no doubt it is indeed well-mixed.

The purpose of the present study is to develop a system by which the hepatic transit of ICG from blood to bile can be measured accurately. In order to ensure precision in the measurements, a data handling system will be developed that will center on a computer-controlled network of devices to aid in the collection of data.

This will result in curves of concentration of ICG vs. time for both blood and bile from an in vivo canine system. These curves will be compared to similar computer-generated curves from a mathematical model developed to simulate hepatic clearance of ICG. The model is based on a five-compartment system consisting of blood pool, splanchnic bed, hepatic sinusoids, hepatic cells, and bile pool. When inserting estimated literature values for hepatic blood flow, bile flow, tissue dye transfer rate constants, and respective tissue volumes, the model will predict dye concentration vs. time for each compartment. The data from both the in vivo studies and the mathematical model will be collected from two experimental states: 1) a normal state, and 2) a shunted state where portal venous blood is completely diverted into the vena cava through an Eck fistula or portal-caval shunt. The shunted state should result in a reduction in total hepatic blood flow of 40 to 50 percent and thus decrease the dye removal rate. From the analysis of the blood and bile curves generated by the in vivo data with support from the mathematical model, the dynamics of hepatic dye clearance may become better understood.
REVIEW OF LITERATURE

Physiology of Indocyanine Green

General characteristics

Indocyanine green (ICG) is a water-soluble, tricarbocyanine dye which was developed by Fox and associates in 1957. They demonstrated ICG to have an absorption maximum of 800 nm, which is also the wavelength where oxyhemoglobin and deoxyhemoglobin have equal absorbance characteristics. This allows the dye to be detected in the blood independent of oxygen saturation (Fox and Wood, 1957; Fox et al., 1957). ICG is unstable in aqueous solution, but is stable in plasma up to seven hours. Barbier and DeWeerdt (1964) postulated that the presence of plasma proteins prevented a cyclizing reaction from occurring, which greatly reduced the absorbance characteristics of the molecule.

When injected into the blood, ICG primarily binds to the alpha-1-lipoproteins (Baker, 1966) and the albumins (Cherrick et al., 1960) in the plasma, and to the Y and Z proteins of the hepatic cytoplasm (Levi et al., 1969). Paumgartner et al. (1970) demonstrated the uptake of ICG by the rat liver obeyed Michaelis-Menten kinetics, and doses of ICG less than 10 mg/kg would fall within the linear region of the Michaelis-Menten curve. It was concluded that dye was not removed from the plasma by simple diffusion, but rather by binding to a receptor or carrier substance similar to an enzyme-substrate mechanism. This supports the findings of Levi and coworkers (1969).
Studies which attempted to detect amounts of ICG in various other tissues such as kidney, muscle, and brain showed there is no uptake of dye by any tissue other than the liver (Rapaport et al., 1959; Cherrick et al., 1960; Leevy et al., 1963).

**Blood removal characteristics**

The clearance of ICG from the blood has been shown to be a single exponential decay in the rat, rabbit, dog, and man, and is dependent on dosage in all test animals (Wheeler et al., 1958; Hunton et al., 1960b, 1961; Klaassen and Plaa, 1969). In addition, Klaassen and Plaa have suggested the single exponential decay is due to the one-way uptake of dye; i.e., there is no backflow of dye from liver to blood. Table 1 is a summary of dye removal rates.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Dose (mg/kg)</th>
<th>PDR (range)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheeler et al. (1958)</td>
<td>1.05</td>
<td>6.6</td>
</tr>
<tr>
<td>Hunton et al. (1960b)</td>
<td>1.00</td>
<td>7.6 (5.5–9.8)</td>
</tr>
<tr>
<td>Hunton et al. (1961)</td>
<td>1.00</td>
<td>5.8 (4.5–7.4)</td>
</tr>
<tr>
<td>Klaassen and Plaa (1969)</td>
<td>1.00</td>
<td>10.0</td>
</tr>
<tr>
<td>Vogin et al. (1960)</td>
<td>0.50</td>
<td>9.7 (4.9–16.7)</td>
</tr>
</tbody>
</table>

$^a$Percentage Disappearance Rate (PDR) = \( \frac{\ln(C_1/C_2) \times 100}{t_2 - t_1} \) min$^{-1}$ where 

\( C_1 \) = ICG concentration at time 1 and 

\( C_2 \) = ICG concentration at time 2.

In addition to calculation of PDR, the percent retention at 20 minutes post-injection is another means of measuring ICG uptake. Percent retention is calculated as a percentage of the zero-time concentration found by extrapolation. A value of retention greater than 3% is considered abnormal (Cobb and Saunders, 1970).
Hunton et al. (1961) and Klaassen and Plaa (1969) demonstrated the relationship between dosage of ICG and PDR to be a nonlinear, inverse relationship. A later study by Iga and Klaassen (1979) showed a linear relationship between the dosage of ICG and the area under the concentration vs. time curve.

Hunton et al. (1961) also discussed the apparent competition for hepatic uptake of ICG by other substances such as BSP, bilirubin, and rose bengal. It would appear a similar transport mechanism exists which is shared by all of the various indicators and there is no preference of one substance over another for removal from the blood.

The rate of ICG uptake can also be affected by the presence of cardiovascular pathology. Hood et al. (1968) reported a 41% decrease from normal in the plasma clearance of ICG in dogs three to four days following surgical ligation of the left coronary artery. It was hypothesized that the more severe cardiac impairment immediately following myocardial infarction may be the cause of sustaining injury to the liver.

Cobb and Saunders (1970) reported percent retention values in humans ranging from 3.46 to 4.15% one month following mild to severe myocardial infarction, respectively. It was stated that the impairment appeared to continue for some time when, presumably, the circulation was normal, as indicated by presence of normal blood pressures and the lack of evidence of cardiac failure.

Edwards et al. (1972) conducted a study of human patients presenting varying degrees of reduced ventilatory capacity associated with chronic bronchitis. Twenty-one of 27 patients (78%) who showed a normal liver
function by a more conventional galactose tolerance test also showed an abnormal mean percent ICG retention of 4.7% (normal <= 3%), indicative of liver dysfunction. It was proposed that the common factor between these two tests was either a reduced hepatic blood flow, or a production of hepatic congestion preceding clinical heart failure. In support of comments made by Leevy et al. (1962), it was concluded that hypoxia per se should not affect ICG clearance. They did not rule out, however, that nutritional changes in these patients could play a role in the altered liver function.

Mechanism of Bile Formation

General characteristics

The bile canaliculi are the primary site of bile formation. The lumen of the canaliculi is separated from the adjacent cytoplasm by the unit membrane of the hepatocytes with the available area for diffusion augmented by the presence of microvilli. Therefore, the canicular network may be regarded as a single chamber separated from the hepatocytes by a thin membrane of large surface area (Wheeler, 1965). The movement of substances into the bile is difficult to ascertain due to the difficulty in measurement of the electrochemical conditions on both sides of the hepatocyte membrane. The supposition that bile is formed by an active transport or secretion process is based on one or more of three indirect types of evidence:

1) The concentration of a substance in the bile, e.g., bilirubin, is significantly higher than that of the plasma such that it
could not have entered the bile by passive diffusion alone.

2) A saturation maximum, or transport maximum, can be demonstrated which is consistent with the "carrier" hypothesis for active transport.

3) Bile transport can be inhibited by substances known to be either rapidly excreted in the bile or disruptive to transport processes in other tissues.

The major determinant of bile flow is the hepatic secretion of osmotically active organic anions into the bile canaliculi with the formation of bile itself contingent on perfusion of the hepatocytes by the blood. Appropriate concentrations of cations are simultaneously delivered into the bile to maintain electrochemical neutrality. The non-diffusible, osmotically active anions then initiate passive diffusion of water into the bile in order to maintain isotonicity (Roullier, 1964).

**Bile ICG excretion characteristics**

After ICG is removed from the blood, it is excreted into the bile. Cherrick et al. (1960) and Caesar et al. (1961) reported ICG in plasma and bile to have uniform chromatographic migration. Barbier and De Weerdt (1964) confirmed by infra-red spectrography there is no molecular change in the ICG that appears in the bile from the ICG injected in the plasma. Therefore, ICG does not undergo any conjugation as it transits the liver cell, unlike bilirubin and BSP. Hargreaves (1966) suggested ICG is excreted by a slightly different mechanism than bilirubin or BSP and may not require the UDP-trans-glucuronylase system for excretion.
Wheeler et al. (1958) showed ICG to be 97.3% recoverable in bile four to five hours after a single injection. Hunton et al. (1960a) reported 81.4% recovery after four hours and 95.3% recovery after eight hours with peak concentrations occurring at 1 to 1.5 hours following a single injection. Ketterer et al. (1960) reported an average recovery of 91% with several 100% recoveries after six to seven hours with peak concentrations occurring two to three hours after injection.

Klaassen and Plaa (1969) indicated bile flow in dogs to remain unaltered for up to two hours after injections of 1 or 4 mg/kg of ICG. However, Wheeler and Ramos (1960) emphasized there would be a progressive and often profound depression in the flow of bile unless steps were taken to replace the lost bile salts during any study where bile was removed and not replaced.

Hunton et al. (1960a,b) found when ICG was constantly infused, negligible amounts of dye could be measured in the hepatic lymph. However, immediately following biliary obstruction, the dye concentration in the hepatic lymph elevated directly concomitant to the level of dye in the plasma and never exceeded the plasma dye content. They suggested lymphatic ICG is dependent on plasma concentration, not on bile concentration, and furthermore, that ICG does not diffuse across the biliary mucosa during bile obstruction and therefore, the membrane remains relatively intact.

Wheeler et al. (1958) also reported when a dose of ICG is introduced directly into the small intestine, only 1.9% of the initial dose is measurable in the bile after four hours. They concluded intestinal reabsorp-
tion and recirculation of ICG would be insignificant compared with the dye decay in the plasma.

Determination of Hepatic Blood Flow

Most methods available today for measurement of hepatic blood flow are based on the indirect Fick principle and are complicated by the need for catheterization of a hepatic vein, as first described by Warren and Brannon (1944). In humans, a catheter is introduced into the median basilic vein of the forearm and, with the assistance of a fluoroscope, passed through the superior vena cava and right atrium into the inferior vena cava. Final placement of the catheter is usually into the right hepatic vein, which is less difficult than placement in the left hepatic vein. In the canine, the catheter passes directly into the inferior vena cava from the superior vena cava, bypassing the right atrium altogether (Miller et al., 1968) with final placement usually in the left hepatic vein.

Other more invasive methods exist such as that described by Hand et al. (1981) which employs the surgical application of continuous wave Doppler flow cuffs to both the hepatic artery and the portal vein. However, the discussion that follows will be directed toward methods which are relatively noninvasive. In a discussion by Leevy and Gliedman (1958), contraindications to hepatic vein catheterization in humans were discussed. These included severe debility, lack of cooperation, septicemia, and history of pulmonary embolization from a fibrillating right atrium. Furthermore, since the catheter may enter the right ventricle, the pro-
procedure was not recommended if arrhythmia, recent myocardial infarction, or active rheumatic myocarditis is presented. Finally, they stated complications normally seen in cardiac catheterization such as arrhythmia, conduction defects, syncope, air emboli, catheter knotting, and bacterial endocarditis have not occurred with hepatic vein catheterization in more than 1000 cases reported in the literature.

Indicator substances

Many substances have been developed with which to measure hepatic blood flow (HBF). Regardless of whether an infusion or injection method is employed, they allow HBF measurement by indicator-dilution and the application of the indirect Fick principle. These substances include Chromium-51 (Shoemaker et al., 1961; Thorne et al., 1979), Xenon-133 (Strandell, 1978), Iodine-131-Albumin (Shoemaker et al., 1961; Cohn et al., 1972), and para-aminohippuric acid (Katz and Bergman, 1969). The most popular substances with which to measure HBF have been BSP and ICG, which will be discussed below.

Infusion methods

The method of infusion of an indicator substance into the liver is perhaps the most popular method for determining hepatic blood flow (HBF) since it was first described by Bradley et al. (1945) for BSP. When the indicator substance is infused, the estimated hepatic blood flow (EHBF) is calculated from the following formula:

\[
EHBF = \frac{R}{P - H} \times \frac{1}{1 - \text{hematocrit}}
\]

(1)

where \( R \) = removal rate in mg/min and \( P - H \) = peripheral - hepatic vein concentration difference in mg/ml. Since the indicator infusion rate (I)
will not always achieve equilibrium with the hepatic system, a correction of the infusion rate must be performed to estimate $R$ accurately. If $I$ is greater than $R$, then the excess must be extracted; i.e., $R = I - \Delta P \times V$

where $\Delta P$ is the peripheral increment and $V$ is the plasma volume. If $I$ is less than $R$, then $R = I + \Delta P \times V$ (Bradley et al., 1945; Waldstein and Arcilla, 1958). Substituting into (1), the working formula becomes

$$EHBF = \frac{I + \Delta P \times V}{P - H} \times \frac{1}{1 - \text{hematocrit}}$$  \hspace{1cm} (2)

Because indicator substances such as ICG and BSP do not enter the red blood cells, the extra term involving the hematocrit is necessary to account for the cell to plasma ratio to give blood flow. Otherwise, the calculation would result in the hepatic plasma flow.

Numerous sites for infusion of dye substances have been used by various authors. ICG has been infused at a constant rate into the pulmonary artery (Ketterer et al., 1960), antecubital vein (Reemtsma et al., 1960), femoral artery (Caesar et al., 1961), or axillary vein (Stein et al., 1963). Samples of dye-containing blood may be arterial or venous, or the use of dichromatic ear densitometry (Leevy et al., 1967) may be employed. Table 2 contains a listing of normal hepatic blood flows as determined by ICG infusion.

Winkler et al. (1965) performed a study in humans to determine HBF by placing two catheters primarily in the right lobe of the liver, one cranially and the other caudally. Five test substances including ICG were infused simultaneously with samples being taken from both hepatic venous catheters. This method was employed in an attempt to set duplicate determinations of hepatic flow from the same liver. They stated the funda-
Table 2. Normal hepatic blood flows

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Clearance$^a$</th>
<th>EHBF$^a$</th>
<th>Authors</th>
</tr>
</thead>
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<td></td>
<td></td>
<td></td>
<td>ICG infusion</td>
</tr>
<tr>
<td>Dogs</td>
<td>3.3</td>
<td>36.9</td>
<td>Ketterer et al. (1960)</td>
</tr>
<tr>
<td>Dogs</td>
<td>---</td>
<td>38.0</td>
<td>Banaszak et al. (1960)</td>
</tr>
<tr>
<td>Humans</td>
<td>---</td>
<td>25.7</td>
<td>Reemtsma et al. (1960)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ICG injection</td>
</tr>
<tr>
<td>Dogs</td>
<td>3.2</td>
<td>37.6</td>
<td>Ketterer et al. (1960)</td>
</tr>
<tr>
<td>Dogs</td>
<td>40.0</td>
<td>47.0</td>
<td>Banaszak et al. (1960)</td>
</tr>
<tr>
<td>Dogs</td>
<td>8.4</td>
<td>60.0</td>
<td>Vogin et al. (1960)</td>
</tr>
<tr>
<td>Humans</td>
<td>---</td>
<td>24.1</td>
<td>Reemtsma et al. (1960)</td>
</tr>
</tbody>
</table>

$^a_{ml/min*kg}$

mental problem is how local changes in liver perfusion are related to change in hepatic extraction and vice versa. If local differences in extraction do occur, it cannot be ascertained if the area is one of high perfusion or low extraction. Therefore, the confidence level of whether a hepatic venous sample is well mixed is not great.

Caesar et al. (1961) stated that because ICG is removed only by the liver, it allows for the specific measurement of hepatic, not splanchnic, blood flow. Random changes in plasma levels caused by dye absorption by the gut are eliminated due to the absence of enterohepatic circulation, which is important in patients with extrahepatic shunts of splanchnic blood.

Leevy et al. (1962) concluded ICG permits estimation of HBF in many patients with hepatic pathology in whom it is impossible to use BSP. They also stated hepatic hypoxia induced by either glucogen administration or
breathing ten percent oxygen does not influence hepatic extraction or hepatic blood flow when ICG is used. They found the best results for estimating HBF using the clearance method after a single injection of ICG, as compared to clearance of radioactive colloidal gold.

Injection methods

The method for calculating HBF using a single injection was first described by Dobson and Jones (1952) using radioactive colloidal chromic phosphate. It involves measuring plasma levels of an indicator substance serially for twenty minutes after injection while also measuring hepatic venous levels through a hepatic vein catheter. From the plasma concentrations, the clearance (Cl) rate may be calculated, and from the hepatic venous concentrations, the extraction ratio (ER) may also be calculated. The EHBF can then be computed from the following formulae:

\[
Cl = \frac{M}{C_0} \times \frac{0.693}{T_{1/2}}
\]  

(3)

\[
ER = \frac{a - HV}{a}
\]  

(4)

\[
EHBF = \frac{Cl}{ER}
\]  

(5)

where \( M \) = mass of dye injected, \( a \) = arterial concentration, \( C_0 \) = dye concentration at zero time, \( HV \) = hepatic venous concentration, and \( T_{1/2} \) = half-time where concentration is one-half of \( C_0 \). The term \( M/C_0 \) is the blood volume and 0.693 is equivalent to \( \log \left( C_0/C_{1/2} \right) \). Table 2 contains a listing of normal hepatic blood flows as determined by ICG injection.

If the extraction ratio is not known, then the value for EHBF can be no more than the value of clearance found in (3). However, EHBF will be less than Cl when ER is less than 1.0 (Waldstein and Arcilla, 1958). No
suitably ideal substance has been found to have an ER of 1.0 up to the present time. See Table 2 for normal hepatic flows determined by ICG injection.

Reemtsma et al. (1960) reported a close correlation between hepatic blood flows measured by both the infusion and single injection methods within human individuals from control and cirrhosis groups. They proposed the use of a mean extraction ratio of 0.88 in normal human subjects which obviates the need for hepatic vein catheterization.

Banaszak et al. (1960) used a slightly different method to calculate HBF in dogs by single injection of ICG. They defined hepatic dye clearance by means of the use of a geometric average for plasma dye content, i.e.,

\[ V_b \times \frac{C_1 - C_2}{\sqrt{C_1 C_2}} \times \frac{1}{(t_2 - t_1)} \]  
(6)

rather than the logarithmic average as proposed by Dobson and Jones (1952). The results obtained were not significantly different from Ketterer et al. (1960), who used a logarithmic average.

Noncatheterization methods

Waldstein and Arcilla (1958) proposed a noncatheterization method employing galactose as a test substance. Following an intravenous dose of 200 mg%, it was observed the characteristic plasma decay curve would produce a rectilinear phase followed by a curvilinear phase. Although galactose is primarily removed from the blood by the liver, the kidney also excretes enough galactose that the concentration in the urine must be taken into account to avoid errors. They suggested that at the point of transition between phases, the hepatic vein concentration would become
zero and the extraction ratio would then become 1.0. Thus, hepatic blood flow would be calculated as the ratio of the plasma removal rate during the rectilinear phase to the concentration at the phase transition point. It was concluded in this case there would be no need for hepatic vein catheterization since the extraction ratio could be determined from the peripheral decay curve alone.

McLean et al. (1979) investigated a method by which hepatic blood flow could be determined by measuring two substances with very different decay characteristics in the same individual. Compounds with a high intrinsic clearance such as ICG have a high extraction ratio and clearance is dependent on hepatic blood flow, whereas compounds with a low intrinsic clearance such as antipyrine (AP) have a low extraction ratio and clearance is limited by the ability of the liver to clear the compound independent of blood flow. They proposed a noninvasive kinetic method combining average values for intrinsic clearance of ICG and AP in normal populations with values of clearance obtained in a test subject to estimate hepatic blood flow. The information obtained would also allow the prediction of the degree of shunting and the fraction of functional liver mass. This technique is limited, however, to what is termed the intact hepatocyte hypothesis in which chronic liver disease is associated with a reduction in cell mass with the individual cells experiencing normal function and perfusion. It was concluded any deviation from this hypothesis would introduce errors in the estimation of hepatic blood flow.
Portal–Caval Shunting and Hepatic Blood Flow

**Anatomical considerations**

In a review article by Greenway and Stark (1971), it was stated that normal HBF as measured by BSP clearance was 43 ml/min*kg in conscious dogs and 39 ml/min*kg in dogs anesthetized with sodium pentobarbital. However, when measured using electromagnetic flowmeters, normal HBF was 31 ml/min*kg in conscious dogs and 36 ml/min*kg in anesthetized dogs. The contribution of the hepatic artery to total HBF was measured at 28% in conscious dogs and 29% in anesthetized dogs. Total HBF was 25% of the cardiac output. This was followed by a discussion of the distribution of hepatic arterial blood and of whether this blood perfuses the same sinusoidal beds as the portal blood. It was concluded that there are both common and separate channels for arterial and portal blood with the majority of the sinusoids being perfused by both. However, a small part of the hepatic vascular bed is perfused only by portal blood at one instant while another small part, including the vessels around the biliary tracts and the connective tissue, are perfused only with arterial blood. Furthermore, it was stated that in the normal liver, channels other than the sinusoids do not exist between the hepatic artery and hepatic vein or between the portal vein and the hepatic vein.

These observations confirmed a more in-depth study by McCuskey (1967), who observed living liver tissue of frogs, rats, mice, and rabbits at various monochromatic light wavelengths through a special microscope/TV camera combination.
Effects of portal-caval shunts on HBF

The need for portal-caval shunting in a clinical situation has been outlined previously (Antol, 1980). The discussion in the literature on the effects of portal-caval shunting on HBF and hepatic function is mostly limited to the conditions where patients already have abnormal hepatic dysfunction previous to shunt surgery. As a result, it is a rarity to find an instance in the literature of portal-caval shunting in normal subjects.

In a study by Reynolds (1970), it was stated that a substantial decrease in HBF of 40-50% will occur after shunt surgery, with a greater decrease following a side-to-side (SS) shunt as opposed to an end-to-side (ES) shunt. They also discussed the findings of previous authors on the response of the hepatic artery after removal of portal blood flow during portal occlusion, and indicated a substantial rise in hepatic arterial flow (HAF) as measured by electromagnetic flowmeters. Normal humans undergoing cholecystectomy were found to have a 62% increase in HAF when the portal vein was clamped, while humans undergoing portal-caval shunt demonstrate a 20% increase in HAF. Other authors cited in this paper found a mean increase in HAF of 48% in eleven patients after an ES anastomosis, compared to a mean increase of 100% in eight patients following an SS anastomosis.

Burchell et al. (1976) reported a significant increase of 60% in HAF measured in 47 patients who received either an ES anastomosis or an SS anastomosis. Within those patients, there was also a correlation between patients who had a smaller increment in HAF and the presence of encepha-
therapy. Likewise, there was a correlation between patients experiencing small increments in HAF and survival while hospitalized. It was suggested from these observations that the increase in HAF following portal-caval shunting was due to the lowering of the sinusoidal pressure, and that failure to do so would result in the further development of complications.

Johnson and Lambert (1967) studied changes in cardiac output in normal dogs following an ES anastomosis and observed a 35% increase over control dogs. It was stated that this effect was in accordance with Starling's law in that the shunted blood would increase the pre-loading of the right ventricle, thus increasing the force of contraction and the volume output from the left ventricle.

In a later article, Reynolds (1974) concluded that porto-systemic shunting would never become an ideal surgical procedure due to the bypassing of the detoxification action of the hepatic system and the removal of the nutritive portal inflow.

Mathematical Models of the Hepatic System

With the advance of larger, faster digital computers, an increasing number of studies are being made in which problems of hepatic function, blood flow and clearance capabilities are solved mathematically.

Richards et al. (1959) developed a mathematical model that predicted the content of BSP in the liver and bile given information obtained from a peripheral decay curve for BSP. The model was based on the solutions of a system of three first-order differential equations relating the change in mass of dye with respect to time for blood, liver, and bile. There were
two assumptions underlying this model: 1) all rates of transfer of BSP are proportional to the amounts of BSP from which the transfer occurs, and 2) the amount of BSP transferred to extrahepatic or extravascular tissue is negligible. After a standard intravenous dose of BSP at 5 mg/kg, the resultant decay curve was analyzed graphically to determine the four constants needed by the constraint equations. The solutions yielded values for the mass transfer coefficients between the three compartments, and a proportionality factor relating the concentration in the plasma to the total amount of dye in the circulation. It was the authors' contention that this model would present more information than the standard forty-five minute retention of BSP alone. However, whether the second assumption, i.e., the negligible transfer of BSP to extrahepatic or extravascular tissue, is completely valid is subject to dispute, as indicated by Leevy et al. (1962), and therefore, the quantitative accuracy of this method is questionable.

Luxon et al. (1982) noted the model suggested by Richards et al. (1959) failed because it ignored concentration profiles in the sinusoid and mixing artifacts in the peripheral circulation.

Giorgi and Segre (1973) conducted kinetic studies using BSP in normal rats and rats intoxicated with carbon tetrachloride (CCl₄). The results of plasma decay and bile accumulation were fitted to a model consisting of four compartments and five transfer constants. The assumptions governing this model were more precise than Richards et al. (1959) in that they accounted for BSP losses in the extrahepatic space. The liver was modeled as two compartments because 1) the compartment representing BSP in the
blood is third-order with respect to BSP in the bile, and 2) the bile kinetics of BSP require four exponential terms. The transfer constants were determined by simultaneously fitting observed BSP levels in the blood and bile to the model. It was concluded the changes in transfer constants agreed with known effects of CCl₄, namely the intrahepatic transfer and uptake of BSP were reduced.

Iga et al. (1980) conducted studies similar to Giorgi and Segre (1973), only with the use of ICG instead of BSP. In addition, a four-compartment open model was developed to derive the transfer constants. Two schemes were proposed in which to classify the relationship between dye kinetics and compartmental arrangement. Scheme 1 included the sinusoid in the plasma compartment where transfer between compartments represents the translocation of the sinusoidal plasma membrane of the hepatocyte and the rate of transfer is much less than the plasma flow. Scheme 2 placed the sinusoid in the liver compartment where transfer corresponds to the hepatic plasma flow and where there is a rapid equilibration between the sinusoid and hepatocytes, and the rate of transfer is approximately the hepatic plasma flow. Observations using ICG were made on normal rats and on CCl₄-intoxicated rats in which the sinusoidal membrane was known to be damaged. Since the product of the hepatic uptake constant and the blood volume is the hepatic blood flow, this product was compared to the measured hepatic blood flow and was found to be much smaller. It was concluded that Scheme 1 was the better representation, in which the permeability of the plasma sinusoidal membrane of the hepatocytes was the rate-determining step.
Numerous studies have been conducted in which the general pharmacokinetics of hepatic clearance have been examined (Rowland et al., 1973; Perrier and Gibaldi, 1974; Wilkinson and Shand, 1975; Bass et al., 1976, 1978; Bass and Bracken, 1977; Bracken and Bass, 1978; Bass and Robinson, 1979). Given the complexity of the mathematics employed by these authors, a detailed discussion of these papers is beyond the scope of this project. However, in general there is agreement that clearance of any drug is the result of an exponential decay process. Furthermore, the blood-liver-bile system can be represented with a minimum of three compartments up to a maximum of twenty-five million compartments, which is the projected number of individual sinusoids (Bass et al., 1978).

Recent work by Forker and Luxon (1978, 1982) has resulted in the development of a distributed model which takes into account intrahepatic concentration profiles, nonuniform blood flow distribution in the sinusoids, and delayed mixing in the peripheral circulation. The results indicated conventional "lumped" models underestimated the rate constants for hepatic uptake, but returned accurate estimates for steady-state plasma clearance and excretion rate constant (Luxon and Forker, 1982).

Summary

From the review of literature above, the characteristics of hepatic clearance of ICG from the blood and its hepatic excretion into the bile have been outlined. The available methods for the determination of hepatic blood flow using ICG have also been detailed. The effects of
portal-caval shunting on the hepatic system have been discussed. Finally, studies involving mathematical modeling of the hepatic system were presented.

The intention of this collection of literature is to give support to the experimental design of the project to be discussed in the remainder of this dissertation. The understanding of the mechanism of hepatic dye removal in the living system is crucial to the ability to design a mathematical model for hepatic dye removal, and further, to design an experimental system by which this model may be tested in the living system.
MATERIALS AND METHODS

Experimental Design

The design of this project has three objectives:

1. Development of a mathematical model for hepatic blood clearance and bile accumulation of ICG.

2. Development of a data-handling system with which to digitize and process data obtained from in vivo experiments.

3. The implementation of those in vivo experiments in the canine in which plasma dye clearance and bile accumulation curves are obtained under conditions of normal hepatic blood flow and reduced hepatic blood flow as a result of a portal-caval shunt.

Objective 1 involves production of data sets from the mathematical model representing dye concentration versus time for each of the five compartments modeled. Of particular interest will be the curves from the blood and bile compartments since these will be compared to those obtained from in vivo experiments.

Objective 2 involves the design of the data-handling system to 1) assist in the initial set-up of each experiment, 2) control the execution of each experiment, and 3) present the data from each experiment as results in a form such that further detailed processing is unnecessary.

Objective 3 involves performing the experimental studies in mongrel dogs. Following injection of ICG, the parameters to be measured from each animal in addition to blood and bile dye concentration versus time will be cardiac output, hematocrit, and average bile volume per five minute inter-
val, i.e., average bile flow. For each experiment, values for rate of dye disappearance, blood volume, and hepatic dye clearance will be obtained from the blood dye concentration data, while values of peak time, peak concentration, amount of dye excreted vs. time, and percent dye recovery versus time will be obtained from the bile dye concentration data.

Development of the Mathematical Model

The mathematical model for hepatic dye clearance and bile excretion prepared in this research has been summarized previously (Antol, 1980; Antol and Engen, 1981). It is an extension of a model for hepatic dye clearance defined previously (Antol, 1980, 1981) and is similar in design to the model proposed by Iga et al. (1980). It employs a five-compartment system consisting of blood pool, splanchnic bed (gut, spleen, and portal venous system), hepatic sinusoids, hepatocytes, and bile pool (Figure la). The model is governed by a system of five first-order differential equations (Figure lb) and was based on eight assumptions, as indicated by the literature.

1. No site of dye removal from the blood exists other than the liver.
2. The dye undergoes no generation or destruction.
3. The bulk of the dye is not removed from the blood in a single pass through the liver.
4. Portal blood flow is two-thirds of the total hepatic blood flow.
5. Dye concentration in the hepatic lymph is negligible.
Figure 1. Compartmental diagram of mathematical model (A) and the system of five governing first-order differential equations (B) for normal hepatic clearance of ICG.
6. Transport of dye from the sinusoids to the hepatocytes is proportional to the sinusoid dye concentration only and reverse transport is negligible.

7. Transport of dye from the hepatocytes to the bile pool is an active transport process and reverse transport is also negligible.

8. All blood and tissue compartments are well-mixed and, therefore, the dye content of each compartment is homogeneous at any instant in time.

In addition, the effects of reduced hepatic blood flow as a result of a portal-caval shunt have been modeled. This effect was programmed such that any amount of blood entering the liver from the splanchnic bed can be shunted back to systemic circulation. The eight assumptions listed above remain the same in the shunted case (Figure 2).

The equations governing each blood compartment of the mathematical model are based on the "Fick principle" and are therefore constructed such that the following premise is obeyed: The compartment volume times the change in concentration with respect to time is equal to the flow through that compartment times the difference between the concentrations entering and exiting the compartment. This premise is shown as the following:

$$ V \frac{dC}{dt} = Q(C_i - C_0) $$

In the equation for the hepatic sinusoids, an extra term appears which is the removal or "clearance" term of dye solute from the blood to the hepatocytes, and is controlled by the sinusoid-hepatocyte transfer rate constant, $K_H$. From the sixth assumption, dye transport into the hepato-
Figure 2. Compartmental diagram of mathematical model (A) and the system of five governing first-order differential equations (B) for shunted hepatic clearance of ICG.
cytes is dependent only on the sinusoid dye concentration \( (C_s) \) and is independent of the hepatocyte dye concentration \( (C_h) \). Therefore, the hepatocyte will theoretically hold any and all dye it receives without allowing any dye to be transported back into the sinusoid compartment.

The hepatocyte equation is based on the assumption that the hepatocytes are a homogeneous, well-stirred tissue bed. Therefore, the change in hepatocyte dye concentration with respect to time is determined by the difference between the rate of dye transported into the cells from the sinusoids and the rate of dye transported from the cells into the bile.

The bile pool equation is complex in that the change in bile concentration with respect to time is determined by both a transport process into the bile pool and a flow-related process out of the bile pool. It is assumed that the transport of dye from the hepatocytes into the bile is an active process which has been documented in the literature (Roullier, 1964). Therefore, the hepatocyte-bile transfer rate constant \( (K_{LB}) \) is indicative of an active transport phenomenon, and transport of dye into the bile is likewise independent of bile dye concentration \( (C_{LB}) \) and also irreversible.

Table 3 is a listing of parameters, literature values, and literature sources used for development of the initial conditions of the mathematical model. These values are averages as determined by the authors listed. Where possible, the given parameter has been determined in the same manner as that which will be used in this research; e.g., liver blood flow was determined by the single ICG injection method.
Table 3. Initial conditions for mathematical model of hepatic dye removal in dogs as reported in the literature

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model symbol</th>
<th>Literature value</th>
<th>Source</th>
<th>Calculated value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (BW)</td>
<td>---</td>
<td>18.8 kg (41.4 lbs)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>---</td>
<td>45</td>
<td>Schalm et al. (1975)</td>
<td>---</td>
</tr>
<tr>
<td>Dye dosage</td>
<td>---</td>
<td>1.0 mg/kg</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Liver blood flow</td>
<td>---</td>
<td>44.3 ml/min*kg</td>
<td>Banaszak et al. (1960)</td>
<td>0.833 L/min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Murray and Nebel (1959)</td>
<td></td>
</tr>
<tr>
<td>Liver plasma flow</td>
<td>$Q_L$</td>
<td>24.4 ml/min*kg</td>
<td>---</td>
<td>0.458 L/min</td>
</tr>
<tr>
<td>Bile flow</td>
<td>$Q_{LB}$</td>
<td>0.00735 ml/min*kg</td>
<td>Wheeler and Ramos (1960)</td>
<td>0.000138 L/min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Klaassen and Plaa (1969)</td>
<td></td>
</tr>
<tr>
<td>Blood volume</td>
<td>---</td>
<td>86.5 ml/kg</td>
<td>Stekiel et al. (1960)</td>
<td>1.626 L</td>
</tr>
<tr>
<td>Plasma volume</td>
<td>$V_B$</td>
<td>52.1 ml/kg</td>
<td>Stekiel et al. (1960)</td>
<td>0.979 L</td>
</tr>
<tr>
<td>Splanchnic plasma volume</td>
<td>$V_P$</td>
<td>Equal to $V_S$</td>
<td>Bradley (1963)</td>
<td>0.182 L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Murray and Nebel (1959)</td>
<td></td>
</tr>
<tr>
<td>Sinusoidal plasma volume</td>
<td>$V_S$</td>
<td>9.7 ml/kg</td>
<td>Murray and Nebel (1959)</td>
<td>0.182 L</td>
</tr>
<tr>
<td>Hepatocyte volume</td>
<td>$V_H$</td>
<td>4.2% BW-V_S-V_LB</td>
<td>Antol (1980)</td>
<td>0.602 L</td>
</tr>
<tr>
<td>Bile canalicular volume</td>
<td>$V_{LB}$</td>
<td>0.32 ml/kg</td>
<td>Wheeler and Ramos (1960)</td>
<td>0.006 L</td>
</tr>
<tr>
<td>Sinusoid-hepatocyte +</td>
<td>$K_H$</td>
<td>3.3 ml/min*kg or</td>
<td>Ketterer et al. (1960)</td>
<td>0.062 L/min</td>
</tr>
<tr>
<td>transfer rate constant</td>
<td></td>
<td>40.0 ml/min*kg</td>
<td>Banaszak et al. (1960)</td>
<td>0.752 L/min</td>
</tr>
<tr>
<td>Hepatocyte-bile transfer rate</td>
<td>---</td>
<td></td>
<td>empirically determined</td>
<td>---</td>
</tr>
<tr>
<td>constant + equivalent to &quot;clearance&quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The value of $K_H$ is equivalent to the clearance parameter as measured in the literature, although the range of reported clearances in Table 3 was broad enough that all three are given.

The value of $K_{LB}$ will be estimated empirically such that the model will respond in the same manner as indicated by the literature; i.e., blood is completely cleared of dye in 30–40 minutes and accumulations in the bile are complete in four or more hours.

The computer program of this mathematical model was written in FORTRAN and entered on a VAX/VMS 11/780 computer (Digital Equipment Corp., Maynard, MA). The solutions of the model system were obtained using a library subroutine which employs a rational extrapolation of Graggs' modified midpoint rule to solve systems of differential equations. As a result, the data sets returned by the program represent predicted dye concentration vs. time for all five modeled compartments. In Appendix A, a listing of the mathematical model program is given.

Development of the Data-Handling System

**Equipment requirements**

The data-handling system was constructed around a PDP-8e computer (Digital Equipment Corp., Maynard, MA) which serves as the experimental controller. In addition, a PDP-11/23 computer system was used for data storage at the end of each experiment through a direct communication link with the PDP-8e system. A block diagram of the basic experimental equipment arrangement is shown in Figure 3.
Figure 3. Block diagram of the basic experimental set-up for the data-handling system
Blood and bile dye concentrations were determined using a Gilford 103-IR densitometer (Gilford Instrument Laboratories, Oberlin, OH) which employed a continuous flow cell chamber. Output from the densitometer was fed into an A/D channel (±1.4 V maximum) of the PDP-8e.

Since the flow cell would be exposed to heparinized blood continuously for periods up to one hour, a procedure was derived to prevent formation of thromboses within the cell. A solution was prepared consisting of a one-inch bead of Silastic adhesive, Type A (Dow Corning, Inc., Midland, MI) dissolved in 30 ml hexane, as described by Miller (1982). With the cell dismantled, the Silastic solution was flushed through the cell and the connecting fittings and allowed to dry overnight. A thin film of Silastic, known to greatly reduce thrombosis, would be deposited on all surfaces in contact with the blood. To further decrease the possibility of thrombogenesis, the glass windows of the flow cell were replaced with Plexiglas windows which were dipped in the siliconizing agent Prosil-2B (PCR Research Chemicals, Inc., Gainesville, FL).

In parallel with the densitometer output was a Soltec 1241-3 strip chart recorder (Soltec Corp., Sun Valley, CA) which produced a back-up hard-copy of the dye dilution curve for determining cardiac output. In addition, the recorder contained a remote start feature which was linked to a relay in the computer to allow computer control of the chart.

Arterial blood was pumped from the left side of the heart, through the flow cell, and returned to the right side of the heart using a Sarns Model 3500 roller pump (Sarns, Inc., Ann Arbor, MI). The overall flow rate of blood through this system was set at 40 ml/min.
The bile collected from the animal was passed through a drop detector/cut-off valve assembly into a Fractomette 200 fraction collector (Buchler Instruments, Fort Lee, NJ). Upon command from the computer, a computer relay would cause fractions to change at five-minute intervals.

The drop detector circuit (Figure 4) consisted of a single packaged, matched IR-LED/photodetector pair (Vactec VTL-11, Vactec Optoelectronics, St. Louis, MO, or equivalent) mounted on a Plexiglas support which was secured on a buret clamp. The voltage on the collector of the photodetector is normally biased high due to the low light transmission of the LED. As a drop of liquid enters the detector gap, a "lens" effect occurs and focuses the LED emission on the photodetector, resulting in a marked drop in the collector voltage. This in turn would initiate the timing cycle of a 555 timer (Signetics Corp., Sunnyvale, CA) configured in its monostable mode to produce a 36 ms pulse. The output pulse of the drop detector circuit was connected to a 0-5V sense line on the PDP-8e computer resulting in a high-low logic status being returned to the controlling program.

The cut-off valve assembly was an accessory to the fraction collector that was modified to allow the computer to control its function; namely, to ensure a bile drop is not missed. The circuit designed for this device is shown in Figure 5.

The timing of the computer program events and the interactions with the peripheral devices were governed by a 30 Hz external clock circuit (Figure 6). An overall clock rate of 30 Hz was chosen to allow for accurate digitization of the cardiac output dye-dilution waveform. The
Figure 4. Schematic diagram of drop detector circuit; see text for description of detector.
Figure 5. Schematic diagram of controlling circuitry for cut-off valve
Figure 6. Schematic diagram of 30 Hz external clock circuit
clock circuit relies on the 60 Hz line frequency as its reference and employed an MFC 6050 dual toggle flip-flop (available as ECG 767, GTE-Sylvania, Williamsport, PA) as a divide-by-two frequency divider.

Computer program design

Six separate computer programs were written in BASIC to calibrate, control, record data, and compute results for each animal experiment. The six programs are shown in more detailed flow-charts in Appendix B and the actual program listings are given in Appendix C. A brief summary of the programs will be given in the following text.

The program DATCAL allows the calibration of the densitometer for dye concentration in blood and bile samples and stores the standard curve data on the floppy disk.

The program DATGET, the actual data-acquisition program in the series, provides prompts to configure the equipment in its starting modes, then gives a 60-second countdown to injection of the dye. When the dye has been injected, an internal program trigger waits until dye appears at the detector, then begins to sample the plasma dye-dilution curve at 30 Hz for 40 seconds. Subsequent plasma readings of the densitometer output are taken at one-minute intervals while bile drop totals are recorded at five-minute intervals.

Bile dye concentration for each fraction is determined by manually timing the passage of diluted samples through the pump and flow cell such that the sample is present in the flow cell when the computer records the densitometer output at the next one-minute mark. Those experiments in
which dye concentration in the hepatic venous blood was also measured were processed in this same manner.

At the conclusion of the experiment, the data is stored in two separate files with cardiac output data separate from blood and bile data.

The program CALOUT reads the calibration data files from the disk, performs a linear regression on the standard data for both blood and bile, and then computes and stores the slope and intercept information on the disk. The calibration data set is then transferred to the PDP-11 system.

The program DATOUT reads the standard slope-intercept files and the data file for both blood and bile from a given experiment. The experimental data are then converted to blood dye concentration, bile volume per five minute interval, and bile dye concentration. These results are then transferred to the PDP-11.

Finally, the program CRDOUT analyzes the dye-dilution curve in a manner similar to previously described methods (Lin, 1979; Cole and Crawford, 1980). After computing the estimated downslope to eliminate the effect of recirculation, the area under the curve and resultant cardiac output is computed. The complete data set is then transferred to the PDP-11 and the program sequence ends.

Experimental Procedure

Sixteen mongrel dogs of both sexes (16.3–23.6 kg) were tested in this research project. Each animal was anesthetized initially with 22 mg/kg Surital (Thiamylal, Parke-Davis, Morris Plains, NJ) and following insertion of an endotracheal tube, anesthesia was continued using Metophane
(Methoxyflurane, Pitman Moore, Inc., Washington Crossing, NJ)—Nitrous Oxide—Oxygen (0.1:2:1). Ventilation was assisted with a Bird respirator (Bird Corp., Palm Springs, CA) which was configured to assist rather than control ventilation. The use of a respirator was necessary to ensure proper anesthetic and metabolic gas exchange and to prevent any unnoticed apneic episodes due to sensitivity to Metophane, which may result in respiratory and/or cardiac failure in mid-experiment.

The surgical procedure is detailed in Appendix D. All animals were subjected to ligation of the cystic duct and to cannulations of the common bile duct, femoral artery and femoral vein, and were evaluated for blood dye removal and bile excretion. Nine animals were normals, two were given portal-caval shunts, three had hepatic vein cannulations, and two had both hepatic vein cannulations and portal-caval shunts.

Approximately 30–60 minutes after closure of the surgical sites, thus allowing sufficient time for coagulation of peripheral vessels in the surgical area, each animal was injected with 100 units/kg heparin solution (United States Biochemical Corp., Cleveland, OH). Additional heparin was injected over the duration of the experiment to maintain as close a non-thrombogenic state as possible.

In each experiment, a 25-mg vial of Cardio-Green (ICG, Hynson, Wescott, and Dunning, Baltimore, MD) was diluted with 1.0 ml distilled water to give a stock concentration of 25 mg/ml. From this dye stock, 20 μl was diluted to 200 μl (1:10) for blood standards and 50 μl was diluted to 200 μl (1:4) for bile standards. Three 10 ml blood standards were prepared in duplicate by adding aliquots of 1:10 diluted dye to give
concentrations of 2.5, 5, and 10 mg/L. Two 25 ml bile standards were prepared in duplicate in 25 ml volumetric flasks containing 2.0 ml canine blood plasma. Aliquots of 1:4 diluted dye were added to each flask followed by a sufficient quantity of saline to give concentrations of 5 and 10 mg/L.

When calibration was complete, the bile catheter was connected to the drop detector/cut-off valve assembly and the output from the detector was verified with both a storage oscilloscope and the data acquisition program to ensure the signal was being correctly propagated to the computer.

The stock dye solution was prepared at 25 mg/ml in order to keep the injection volume small (mean = 0.78 ml). This results in a very sharp dye pulse upon rapid injection which closely approximates the Dirac delta function. The dose of injected dye was 1 mg/kg.

The first 60 minutes of a given experiment was noneventful with the computer in complete control. After that time, the pump was stopped and the densitometer and cannulas were flushed with heparinized saline. Both cannulas were then connected to Statham P23Dc (arterial) and PR23ID (venous) pressure transducers (Statham Laboratories, Inc., Hato Rey, Puerto Rico) to allow monitoring of blood pressures in the latter stages of the experiment when the potential for acidotic shock becomes more critical. In the animals that did show signs of shock, especially those with hepatic vein cannulations, infusions of 8.4% sodium bicarbonate solution (Med-Tech, Elwood, KS) were usually sufficient to restore pressure. In some animals, however, the bicarbonate infusion was not completely effective and, therefore, it was also necessary to administer
calcium gluconate solution (Abbott Laboratories, North Chicago, IL) to increase cardiac contractility.

In those animals in which portal-caval shunts and hepatic vein cannulations were performed, a 2.5 ml/min intravenous drip of lactated ringers solution was infused to further stabilize the animal and to counter hypovolemia due to hemorrhage in the shunt and hepatic vein area. The amount of fluid infused never exceeded the amount of fluid collected from the abdominal cavity at the end of an experiment, which ranged from 500-750 ml.

After 110 minutes elapsed time, preparation was begun for bile sample analysis. In a similar manner to the preparation of the bile standards, 2.0 ml canine blood plasma was added to 25-ml volumetric flasks. Following addition of 200 µl from each bile fraction, saline was added to the mark, giving a 1:125 dilution. Samples were measured until the dye content had fallen to 10-15% of the peak concentration, at which time the experiment was declared completed.
RESULTS AND DISCUSSION

Animal Experiments

The sixteen animals were divided into two groups for statistical analysis. Group I consisted of nine normal and two portal-caval shunted animals. Group II consisted of the animals with an hepatic vein cannulation, and contained three normal and two shunted animals. The procedure for hepatic vein cannulation was never performed without difficulty. Unfortunately, both the large variability of the data and the small sample size in Group II have contributed to inability to resolve any true significance from this group. The manipulation of the liver lobes may have introduced enough variability into the data to cause the inaccuracies in the results of Group II. Therefore, the validity of the data in Group II is in question. This is certainly evident in the values for EHBF and EHBF/body weight, which are far below accepted normal levels of EHBF as determined by a single injection of ICG. As a result, the discussion of the animal results to follow will be centered on the Group I results.

The individual results from each animal experiment for blood ICG removal and bile ICG accumulation in both groups are listed in Appendix E.

Two computer programs were written in BASIC for the analysis of the experimental data. The listings of the programs KTEST and BILTES are given in Appendix F. The program KTEST was designed to read in the blood concentration files, perform a linear regression of the data within a designated range, and calculate the slope, y- and x-intercepts, and the hepatic clearance of ICG in addition to the other measured blood param-
ters. The program BILTES was designed to read the bile concentration and bile volume files and calculate peak time, peak concentration, and mean bile flow in addition to other measured bile parameters. Table 4 is a listing of all measured and calculated parameters from the experimental data. The means and standard errors from the experimental data are given in Table 5. All indications of significance in the following text were obtained using the Student's t-test with $p \leq 0.05$ as the minimum level of significance (Steel and Torrie, 1980). The average values given in the following text will be shown as mean ± standard error of the mean.

The measured values for body weight were $18.6 \pm 0.7$ kg ($n = 9$) for the normal animals and $19.5 \pm 0.9$ kg ($n = 2$) for the shunted animals, with an average group weight of $18.8$ kg ($n = 11$). The hematocrit, which was measured during the calibration procedure, averaged $41 \pm 2\%$ in the normals and $49 \pm 1\%$ in the shunted animals. Overall, there was little variation in the size of the animals between normal and shunted dogs.

The measured cardiac output in the normal animals was $0.156 \pm 0.014$ L min$^{-1}$ kg$^{-1}$, which is in the normal range for dogs (Schalm et al., 1975; Swenson, 1977). However, the cardiac output dropped significantly ($p < 0.01$) by a factor of two in the shunted animals to $0.073 \pm 0.008$ L min$^{-1}$ kg$^{-1}$. This finding is contradictory to the findings of Johnson and Lambert (1967), who reported an increase of 35% in cardiac output in normal dogs given portal-caval shunts. This difference may be explained by the fact that the dogs studied by Johnson and Lambert had chronic shunts and cardiac output measurements were not performed until four weeks after the surgery. In the case presented here, the animals were tested for
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Method of calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output</td>
<td>CO</td>
<td>directly from CRDOUT program</td>
</tr>
<tr>
<td>Blood concentration</td>
<td>CB</td>
<td>directly from DATOUT program</td>
</tr>
<tr>
<td>Blood y-intercept</td>
<td>IB</td>
<td>intercept from ln (CB) vs. time</td>
</tr>
<tr>
<td>Initial blood concentration</td>
<td>CB0</td>
<td>antilog of IB</td>
</tr>
<tr>
<td>Dye dosage</td>
<td>D</td>
<td>total dye injected determined by 1 mg/kg x body weight (kg)</td>
</tr>
<tr>
<td>Blood volume</td>
<td>VB^a</td>
<td>D divided by CB0</td>
</tr>
<tr>
<td>Plasma decay rate</td>
<td>KB</td>
<td>slope of ln (CB) vs. time</td>
</tr>
<tr>
<td>Clearance</td>
<td>Cl^a</td>
<td>KB x VB</td>
</tr>
<tr>
<td>Area under blood concentration curve</td>
<td>AUCB</td>
<td>( \sum_{t=0}^{T_{BCO}} CB_t )</td>
</tr>
<tr>
<td>Time to zero blood concentration</td>
<td>T_{BCO}</td>
<td>IB divided by KB</td>
</tr>
<tr>
<td>Bile volume/5 min interval</td>
<td>VBL5</td>
<td>directly from DATOUT program</td>
</tr>
<tr>
<td>Mean bile flow</td>
<td>Q_{LB}^a</td>
<td>VBL5 divided by 5</td>
</tr>
<tr>
<td>Bile concentration</td>
<td>CLB</td>
<td>directly from DATOUT program</td>
</tr>
<tr>
<td>Peak time</td>
<td>PT</td>
<td>directly from CLB vs. time</td>
</tr>
<tr>
<td>Peak concentration</td>
<td>PCLB</td>
<td>directly from CLB vs. time</td>
</tr>
<tr>
<td>Area under bile curve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total concentration</td>
<td>AUCLB</td>
<td>( \sum_{t=0}^{T_{LBO}} CLB_t )</td>
</tr>
<tr>
<td>Total dye mass</td>
<td>AUCLB0</td>
<td>( \sum_{t=0}^{T_{LBO}} CLB_{BL} )</td>
</tr>
<tr>
<td>Ascending conc.</td>
<td>AUCLBU</td>
<td>( \sum_{t=0}^{T_{LBO}} CLB_{LU} )</td>
</tr>
<tr>
<td>Ascending dye mass</td>
<td>AUCLBMU</td>
<td>( \sum_{t=0}^{T_{LBO}} CLB_{MU} )</td>
</tr>
<tr>
<td>Ascending slope</td>
<td>KLB</td>
<td>slope of ln (CLB) vs. time from t = 0 to t = T_{LBO}</td>
</tr>
<tr>
<td>Descending slope</td>
<td>KLD</td>
<td>slope of ln (CLB) vs. time from t = 100 to t = T_{LBO}</td>
</tr>
<tr>
<td>Bile y-intercept</td>
<td>ILB</td>
<td>intercept from ln (CLB) vs. time from t = 100 to t = T_{LBC0}</td>
</tr>
</tbody>
</table>

^aMathematical model parameters.
Table 4. (Continued)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Method of calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial bile conc.</td>
<td>(C_{\text{LBO}})</td>
<td>\text{antilog of } I_{\text{LB}}</td>
</tr>
<tr>
<td>Time to zero bile concentration</td>
<td>(T_{\text{LBCO}})</td>
<td>(I_{\text{LB}} \text{ divided by } K_{\text{LBD}})</td>
</tr>
<tr>
<td>Dye recovery</td>
<td>(%R)</td>
<td>((\Sigma C_{\text{LB}} V_{\text{BL5}/D}) \times 100 \text{ from } t = 0 \text{ to } t = T_{\text{F}})</td>
</tr>
<tr>
<td>Final measured time</td>
<td>(T_{\text{F}})</td>
<td>\text{time of last measured bile sample when experiment ended}</td>
</tr>
<tr>
<td>Mean blood concentration</td>
<td>(\overline{C}_{\text{B}})</td>
<td>(\text{AUC}<em>{\text{B}} \text{ divided by } T</em>{\text{BO}})</td>
</tr>
<tr>
<td>Mean bile concentration</td>
<td>(\overline{C}_{\text{LB}})</td>
<td>(\text{AUC}<em>{\text{LB}} \text{ divided by } T</em>{\text{LBCO}})</td>
</tr>
<tr>
<td>Volume of bile canaliculi</td>
<td>(V_{\text{LB}}^a)</td>
<td>(D \text{ divided by } C_{\text{LBO}})</td>
</tr>
<tr>
<td>Mean dye residence time</td>
<td>(\overline{T}_{\text{DR}})</td>
<td>(V_{\text{LB}}/Q_{\text{LB}})</td>
</tr>
</tbody>
</table>

Cardiac output within three hours after the creation of the shunt. It is possible that the change in normal hepatic circulation might result in an onrush on the cardiopulmonary vasculature of a vasodilatory substance contained in portal blood and may be acutely responsible for the decrease observed in cardiac output. When the blood pressure transducers were connected to the cannulas at 60 minutes post dye injection, both arterial and venous blood pressures were within the normal region of pressure. Heart rate was monitored throughout the experiment and usually remained between 110-130 bpm. It is also possible that the heparinization of the animal 1.5-2 hours prior to beginning the experiment caused enough fluid loss that the animal was in hypovolemic shock. This point is doubtful since if hypovolemia was indeed producing a state of shock in the animal, it is
Table 5. Averages from animal studies and mathematical model for ICG removal in normal and shunted cases

<table>
<thead>
<tr>
<th></th>
<th>C_{BO} (mg L^{-1})</th>
<th>BV (L)</th>
<th>BV/BW (L kg^{-1})</th>
<th>K_{B} (min^{-1})</th>
<th>Cl (L min^{-1})</th>
<th>AUC_{B} (mg min L^{-1})</th>
<th>T_{BCO} (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>14.2 ± 0.127</td>
<td>2.36</td>
<td>0.127</td>
<td>-0.0889</td>
<td>-0.206</td>
<td>1347</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>(1.1)</td>
<td>(0.20)</td>
<td>(0.010)</td>
<td>(0.0068)</td>
<td>(0.018)</td>
<td>(209)</td>
<td>(2.0)</td>
</tr>
<tr>
<td>Model</td>
<td>11.9 ± 0.130</td>
<td>2.77</td>
<td>0.147</td>
<td>-0.0925</td>
<td>-0.256</td>
<td>897</td>
<td>25</td>
</tr>
<tr>
<td>Shunt</td>
<td>15.7 ± 0.130</td>
<td>2.51</td>
<td>0.130</td>
<td>-0.0690*</td>
<td>-0.171</td>
<td>2421**</td>
<td>40**</td>
</tr>
<tr>
<td></td>
<td>(2.8)</td>
<td>(0.38)</td>
<td>(0.026)</td>
<td>(0.0055)</td>
<td>(0.013)</td>
<td>(180)</td>
<td>(0)</td>
</tr>
<tr>
<td>Model</td>
<td>11.9 ± 0.147</td>
<td>2.77</td>
<td>0.147</td>
<td>-0.0544</td>
<td>-0.151</td>
<td>2759</td>
<td>45</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Q_{LB} (ml min^{-1})</th>
<th>P_{T} (min)</th>
<th>P_{C} (mg L^{-1})</th>
<th>AUC_{LB} (mg min L^{-1} x 10^{6})</th>
<th>AUC_{LBM} (mg min L^{-1})</th>
<th>AUC_{LBU} (mg min L^{-1})</th>
<th>K_{LBU} (min^{-1})</th>
<th>K_{LBD} (min^{-1})</th>
<th>C_{LBO} (mg L^{-1})</th>
<th>T_{LBO} (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.162 **</td>
<td>42</td>
<td>1203</td>
<td>1.53</td>
<td>1052</td>
<td>61189</td>
<td>52.6</td>
<td>0.1491</td>
<td>-0.0201</td>
<td>4610</td>
</tr>
<tr>
<td></td>
<td>(0.018)</td>
<td>(4)</td>
<td>(147)</td>
<td>(0.32)</td>
<td>(92)</td>
<td>(13615)</td>
<td>(12.0)</td>
<td>(0.0163)</td>
<td>(0.0029)</td>
<td>(1371)</td>
</tr>
<tr>
<td>Model</td>
<td>0.160</td>
<td>45</td>
<td>1226</td>
<td>1.74</td>
<td>1412</td>
<td>50951</td>
<td>41.3</td>
<td>0.1144</td>
<td>-0.0229</td>
<td>6602</td>
</tr>
<tr>
<td>Shunt</td>
<td>0.082 *</td>
<td>39</td>
<td>1882</td>
<td>2.22</td>
<td>843</td>
<td>275573*</td>
<td>111.7*</td>
<td>0.1144</td>
<td>-0.0150</td>
<td>3127</td>
</tr>
<tr>
<td></td>
<td>(0.031)</td>
<td>(6)</td>
<td>(392)</td>
<td>(0.59)</td>
<td>(80)</td>
<td>(75101)</td>
<td>(14.5)</td>
<td>(0.0704)</td>
<td>(0.0023)</td>
<td>(77)</td>
</tr>
<tr>
<td>Model</td>
<td>0.080</td>
<td>45</td>
<td>1678</td>
<td>4.94</td>
<td>2000</td>
<td>322090</td>
<td>130.4</td>
<td>0.0470</td>
<td>-0.0115</td>
<td>3597</td>
</tr>
</tbody>
</table>

^a Mean ± SEM.

^b n = 8.

* p < 0.05.

** p < 0.01.
highly unlikely that the animal would have survived an experiment that could last another four hours. Mild neurogenic shock or shock induced by the handling of the gut during shunt surgery cannot be ruled out as possibilities as well. Therefore, since blood pressures were not measured at the time of dye injection, the reason for the decreased cardiac output becomes difficult to ascertain.

**Blood results**

The plasma decay rate \( (K_B) \) in the normal animals is within accepted ranges reported by Hunton et al. (1960b, 1961) and Wheeler et al. (1958) for the rate of decay of ICG from the blood. The measured dye clearance in the normal dogs is between the values reported by Ketterer et al. (1960) and Banaszak et al. (1960). The value for \( K \) in the shunted animals is significantly lower \( (p < 0.05) \) than the normals, which also accounts for the significant increase in area under the curve \( (p < 0.01) \) and time to zero blood concentration \( (p < 0.01) \). Dye clearance in the shunted animals demonstrates a decreasing trend \( (p < 0.16) \). These changes are concomitant with the decrease in cardiac output discussed earlier.

The blood volume to body weight ratio was higher at 13% than that which is normally accepted in the literature, namely 8-10%. Even though the animals had unknown clinical histories, they were not anemic since the mean hematocrits were normal. Stekiel et al. (1960) demonstrated that, within experimental error, blood volume determined by ICG was equal to blood volume determined by Evans' blue dye. Therefore, the blood volume to body weight ratio of 13% was most likely a normal condition in these animals.
Bile results

The measured value for mean bile flow was 0.162±0.018 ml min\(^{-1}\) or
0.0086 ml min\(^{-1}\) kg\(^{-1}\). This value is in accordance with that reported by
Klaassen and Plaa (1969) of 0.008 ml min\(^{-1}\) kg\(^{-1}\) for normal dogs. Klaassen
and Plaa also reported a peak concentration time (P\(_T\)) of 60 minutes,
whereas Wheeler et al. (1958) reported a P\(_T\) of 102 minutes. The litera­
ture values for peak concentration time are contrasted in this study by a
P\(_T\) of 42 minutes in the normal animals, which is as much as 50% faster
than the literature values. Bile flow is significantly reduced (p < 0.05)
to 0.082±0.031 ml min\(^{-1}\), a factor of two in animals with portal-caval
shunts. Peak concentration and area under the total dye mass curve in the
shunted animals show trends toward significance (p < 0.15 and p < 0.13,
respectively). This is most certainly due to the small number of animals
given shunt surgery.

The area under the up-slope concentration curve and the area under
the up-slope mass curve are also significantly larger (p < 0.05). All
other measured values with respect to bile excretion of ICG are not sig­
nificantly different.

In measuring ICG recovery, it was observed that 81.1% of the dye is
removed in 162 minutes on average in the normal animals while 65.2% of the
dye is recovered in a similar amount of time in the shunted animals.
Hunton et al. (1960a) indicated an average 81% recovery of ICG from the
bile after 240 minutes, while Wheeler et al. (1958) obtained an average
80% recovery in approximately 210 minutes. It can be shown here that not
only is the ICG being accumulated in the bile space at a faster rate in
the normal animals, but also the presence of the portal-caval shunt causes this rate of accumulation to decrease. However, it is difficult to say if this effect is due strictly to the shunt, or again to the fact that the cardiac output was reduced and, therefore, perfusion of the liver was reduced proportionally. In any case, the hepatic blood flow (HBF) has been reduced. It is impossible to know quantitatively how much the HBF has been reduced without a second, independent method to measure blood flow (e.g., electromagnetic flowmeters).

The more rapid recovery of dye in the present study could also be related to the type of anesthetic used. In most ICG clearance studies reviewed, the principle anesthetic was pentobarbital. Goodman and Gilman (1975) state that although barbiturates do not affect hepatic function, they do affect the rate of synthesis of the Y and Z proteins responsible for uptake of hepatic anions such as ICG and the rate of biliary excretion of ICG. Additionally, cardiac output is reduced while total peripheral resistance increases.

Following premedication with thiamylal, the principal anesthetic used in this study was methoxyflurane-nitrous oxide-oxygen (0.1:2.0:1.0). With regard to methoxyflurane, both Goodman and Gilman, and Soma (1971) state that hepatic impairment is slight and transitory, and there is tendency to reduce cardiac output as well. The effect of nitrous oxide on the liver is regarded as negligible, but data are lacking with respect to the combination of nitrous oxide and methoxyflurane. It should be noted that the discussions in the literature of methoxyflurane and its effects are respective to the use of methoxyflurane alone, which requires much higher concentrations than when used concomitant with nitrous oxide.
Mathematical Model

It was observed that the initial conditions for the mathematical model calculated from the literature were decidedly different from the equivalent parameters measured in the animal experiments. Therefore, in order to test the model properly such that it might more closely agree with the animal results, the initial conditions of the model were assigned the values from the animal experiments performed herein. Table 6 is a listing of the differences between the calculated literature values and the actual values programmed into the model. The combined output of the five compartments of the mathematical model are shown in Figure 7 for the normal case and Figure 8 for the shunted case.

The value for hepatocyte volume (\(V_H\)) was reduced by approximately two from 0.602 L to 0.304 L because the mathematical model would not respond similarly to the animal results with the larger value of \(V_H\). The literature value for hepatocyte volume was determined at necropsy strictly on a wet weight per body weight basis and may not be an accurate measurement of hepatocyte cell volume. It is difficult to find an accurate prediction of hepatic cell volume, since most reported weights are also most likely wet weights determined at necropsy. Bloom and Fawcett (1975) state the average liver weight in the human to be 1.5 kg. If the average human body weight is assumed to be 70 kg, then the liver would be 2.1% of the body weight. Miller et al. (1968) state the mass of the liver to be 3.38% of the body weight in the dog. Therefore, it is certain that the estimate of 4.2% of the body weight as an estimate of hepatic cell volume was overestimated. The volume of intercellular connective tissue in the liver
Table 6. Comparison of calculated literature parameters and actual parameters from animal studies used in initial conditions of mathematical model for ICG removal

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Literature value</th>
<th>Calculated literature value</th>
<th>Actual value used in model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (BW)</td>
<td>---</td>
<td>45</td>
<td>18.8 kg</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>45</td>
<td>45</td>
<td>43</td>
</tr>
<tr>
<td>Dye dosage</td>
<td>---</td>
<td>0.833 L min⁻¹</td>
<td>0.475 L min⁻¹</td>
</tr>
<tr>
<td>Hepatic blood flow</td>
<td>44.3 ml min⁻¹kg⁻¹</td>
<td>0.458 L min⁻¹</td>
<td></td>
</tr>
<tr>
<td>Hepatic plasma flow (Q_L)</td>
<td>---</td>
<td>0.00138 L min⁻¹</td>
<td>0.000162 L min⁻¹</td>
</tr>
<tr>
<td>Bile flow (Q_LB)</td>
<td>0.00735 ml min⁻¹kg⁻¹</td>
<td>0.000138 L min⁻¹</td>
<td>[0.000081 L min⁻¹]</td>
</tr>
<tr>
<td>Blood volume</td>
<td>9.2% BW</td>
<td>1.73 L</td>
<td>2.44 L</td>
</tr>
<tr>
<td>Plasma volume (V_P)</td>
<td>---</td>
<td>0.985 L</td>
<td>1.39 L</td>
</tr>
<tr>
<td>Splanchnic plasma volume (V_P)</td>
<td>equal to V_S</td>
<td>0.182 L</td>
<td>0.182 L</td>
</tr>
<tr>
<td>Sinusoidal plasma volume (V_S)</td>
<td>9.7 ml kg⁻¹</td>
<td>0.182 L</td>
<td>0.182 L</td>
</tr>
<tr>
<td>Hepatocyte volume (V_H)</td>
<td>4.2% BW-V_S-V_LB</td>
<td>0.602 L</td>
<td>0.304 L</td>
</tr>
<tr>
<td>Bile canaliclar volume (V_LB)</td>
<td>0.32 ml kg⁻¹</td>
<td>0.00602 L</td>
<td>0.007 L</td>
</tr>
<tr>
<td>Sinusoid-hepatocyte transfer rate constant (K_H)</td>
<td>3.3 ml min⁻¹kg⁻¹ or 40.0 ml min⁻¹kg⁻¹</td>
<td>0.062 L min⁻¹ [0.171 L min⁻¹]</td>
<td></td>
</tr>
<tr>
<td>Hepatocyte-bile transfer rate constant (K_LB)</td>
<td>---</td>
<td>empirically determined [0.0520 L min⁻¹]</td>
<td></td>
</tr>
</tbody>
</table>

[ ] = values used in shunted case
Figure 7. Resultant output of mathematical model for hepatic removal of ICG for the normal case: A, predicted dye concentration vs. time from blood pool (B), splanchnic bed (P), hepatic sinusoids (S), and hepatocytes (H); B, predicted dye concentration vs. time from bile pool compartment (LB)
Figure 8. Resultant output of mathematical model for hepatic removal of ICG for the shunted case: A, predicted dye concentration vs. time from blood pool (B), splanchnic bed (P), hepatic sinusoids (S), and hepatocytes (H); B, predicted dye concentration vs. time from bile pool compartment (LB)
would account for the excess volume in the value of $V$ after the volumes of the sinusoid and bile canaliculi have been deleted. In using the lower number for hepatocyte volume, the model behaved in a manner comparable to the animal data in terms of $P_T$, $P_{CLB}$, and $T_{LBCO}$.

The hepatocyte-bile canalicular transfer rate constant ($K_{LB}$) was empirically determined in order to cause the model to respond as closely as possible to the animal data. In the normal case, animal data and model data were consistent when the value for $K_{LB}$ was established to be 6.9% of the sinusoid-hepatocyte transfer rate constant ($K_H$).

The shunted case, however, exhibited a twofold decrease in bile flow, and showed no significant difference in peak concentration time. Therefore, the hepatocyte-bile transfer rate constant ($K_{LB}$) would have to increase four times in order to maintain a similar pattern of bile ICG excretion.

Bile is formed by the active secretion of organic anions such as ICG and the rate of bile flow is directly proportional to the rate of anion secretion (Roullier, 1964). In the present study, it was found that in order for the model data for bile excretion to fit the animal data for bile excretion in the shunted case, there would have to be an inverse relationship between bile flow and the rate of anion excretion. Since this is contradictory to the accepted mechanism for bile formation, it is necessary to conclude that the data from the shunted animals may be unreliable. The animals in this case may have been physiologically unstable as reflected by the 50% decrease in cardiac output.
In addition to the mean and standard error values from the animal studies, Table 5 also contains the equivalent results from the mathematical model as compared to the animal studies. It can be observed by inspection that even though the model results are not identical to the animal results, it does react similarly with respect to the inflections in the blood and bile response of the normal animal studies. Figures 9-12 demonstrate graphically the relationships between the results of the animal studies and mathematical model.

Table 7 is a comparison of specific blood and bile parameters. These parameters were computed in an attempt to observe differences between the normal and shunted animals in the analysis of the mathematical model results. Since it is known that a portal-caval shunt does alter the blood flow through the hepatic system, these ratios were chosen as a possible indication of how much change has occurred. Only the difference in mean blood concentration of ICG ($C_B$) between normal and shunted animals was determined to be significant. The ratio of mean bile concentration ($C_{LB}$) to mean blood concentration ($C_B$) is an index of the average concentrating capability of the liver and measured 83.9 in normal animals which decreased insignificantly to 64.5 in the shunted animals. The ratio of $C_{LBO}$ to $C_{BO}$ is an indication of the maximum concentrating capability of the liver as ICG passes from the blood to the bile, which was measured at 359 in the normals and decreased to 207 in the shunted animals.

The ratio of the blood decay slope ($K_B$) to the bile ascending slope ($K_{LBU}$) is less than 1.0 in the normal animals and slightly over 1.0 in the shunted animals, which is also concurred by the model. A possible expla-
Figure 9. Comparison of animal and model blood decay curves for both normal (A) and shunted (B) cases.
Figure 10. Comparison of animal and model bile dye accumulation slopes for both normal (A) and shunted (B) cases
Figure 11. Comparison of animal and model peak times and peak concentrations for both normal (A) and shunted (B) cases
Figure 12. Comparison of animal and model bile dye decay slopes for both normal (A) and shunted (B) cases.
Table 7. Comparison of special parameters between blood and bile from animal studies and mathematical model in normal and shunted cases

<table>
<thead>
<tr>
<th></th>
<th>( \overline{C}_B ) (mg L(^{-1}))</th>
<th>( \overline{C}_{LB} ) (mg L(^{-1}))</th>
<th>( \overline{C}_{LB}/\overline{C}_B )</th>
<th>( K_{B}/KLBU )</th>
<th>( V_{LB} ) (L)</th>
<th>( T_{DR} ) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>43.3(^a) (4.2)</td>
<td>3338 (531)</td>
<td>83.9 (21.3)</td>
<td>0.6282 (0.0459)</td>
<td>359(^b) (126)</td>
<td>0.0078(^b) (0.0024)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>36</td>
<td>4531</td>
<td>125.9</td>
<td>0.7904</td>
<td>570</td>
<td>0.0028</td>
</tr>
<tr>
<td>Shunt</td>
<td>60.5(^*) (4.5)</td>
<td>3907 (331)</td>
<td>64.5 (0.7)</td>
<td>1.0203 (0.6767)</td>
<td>207</td>
<td>0.0060</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>63.2</td>
<td>6968</td>
<td>110.3</td>
<td>1.1145</td>
<td>310</td>
<td>0.0052</td>
</tr>
</tbody>
</table>

\( ^a \text{Mean \pm (SEM).} \)

\( ^b \text{n = 8.} \)

\( ^* \text{p < 0.02.} \)
nation for this is that in the normal case, as the concentration of dye in the blood decreases, the concentration of dye in the bile accumulates at a rate faster than the blood. However, in the shunted case, due to the decreased perfusion of the liver, the rate of decrease of dye in the blood is roughly the same as the rate of increase in the bile.

If it is assumed that $C_{LB0}$ is the concentration that would result if the calculated dose of dye could be injected directly into the bile canicular space, then dividing $C_{LB0}$ into the dose of given dye will yield the volume of the bile canicular space, $V_{LB}$. Furthermore, dividing $V_{LB}$ by the mean bile flow, $Q_{LB}$, will yield the mean dye residence time; i.e., the average time the dye resides within the bile space, assuming the dye is homogeneously marking the bile with respect to time.

In the present study, it becomes possible to directly estimate the bile canicular volume by a specific experimental method. The implications of this with regard to the presence or absence of hepatic pathology would need to be researched in much greater detail.

In the study by Wheeler and Ramos (1960), it was stated that in animals previously given cholecystectomy, anywhere from 6-10 ml of bile could be withdrawn from the common bile duct by aspiration at the time of catheterization. Although a specific value for the bile canicular volume was not given, the value of 0.32 ml/kg was estimated by dividing the median of 8 ml of common duct bile by the mean body weight of the animals used in that study. This estimate is certainly inaccurate since it is improbable that the bile in the bile canaliculi could also be evacuated.
SUMMARY AND CONCLUSIONS

Sixteen mongrel dogs were tested for hepatic removal of indocyanine green dye in normal animals and animals with portal-caval shunts. Characteristics of blood dye clearance and bile dye accumulation were studied. Parameters measured in the blood included cardiac output (CO), dye decay rate ($K_B$), blood volume ($V_B$), area under the curve (AUC$_B$), and time to zero concentration (T$_{BO}$). Parameters measured in the bile included mean bile flow ($Q_{LB}$), peak time ($P_T$), peak concentration ($P_{CLB}$), area under the curve (AUC$_{LB}$), and bile concentration at time zero ($C_{LBCO}$).

The major findings of this study are as follows:

1) Response of the mathematical model was similar to the response from the studies of normal animals when programmed with initial parameters taken from the animal studies. Individual parameters in the mathematical model deviated less than 20% from equivalent parameters in the animal studies.

2) The number of animals given portal-caval shunts was too small to give indications of significance in parameters that, in theory, should have been different, such as peak bile concentration or blood clearance.

3) The data-handling system worked well in the acquisition of the experimental data, but problems did exist, such as clotting in the densitometer flow cell and inaccuracies in bile sample dilution.

4) The variance between animals was too large in certain parameters. It is possible that part of this variance may be due to procedure errors cited above. The ability to get greater significance and allow an analy-
sis of variance to eliminate interactions between animals requires not only more animals, but repeated measurements within animals.

5) Aside from the procedural difficulties, there were interesting findings in the animal results. Cardiac output decreased two-fold (p < 0.01) following a portal-caval shunt. It is entirely possible that the acute portal-caval shunt results in the release of vasoactive substances due to manipulation of the gut during surgery. A chronic study may be indicated in this case.

6) Dye decay rate in the plasma decreased (p < 0.05) which results in an increase in area under the blood concentration curve and time to zero concentration in the shunted animals. This implies that decreased liver perfusion will cause an increase in the time necessary to clear the dye from the blood.

7) In the shunted case, the mean bile flow was reduced two-fold (p < 0.05) while the area under the ascending bile curve increased (p < 0.05). Since bile flow is directly dependent on the blood perfusion, it stands to reason that bile flow would decrease as well. It is difficult to state, however, if this decrease is a direct result of the shunt, or a direct result of decreased cardiac output and is unaffected by the shunt.

8) The method with which to measure the volume of the bile canalicular space was realized. If no other information is obtained, knowing that the bile dye content is decreasing and then measuring the log rate of change in dye concentration vs. time will allow computation of the bile space.
9) The effects of methoxyflurane-nitrous oxide as an anesthetic on the hepatic system appear to be minimal with respect to dye clearance as seen by the relatively small differences between the animal data and the mathematical model.
RECOMMENDATIONS FOR FUTURE WORK

The present study presents some interesting possibilities for future research regarding the effects of portal-caval shunting on the hepatic system in general and on the characteristics of ICG removal specifically:

1) Repeat studies with more balanced numbers of animals under both normal and shunted conditions need to be performed. The results found in this study should be investigated more in depth, since several parameters were found to be insignificant due to the low number of animals used, particularly in the shunt studies.

2) Studies should be undertaken to ascertain if the dynamics of the cardiovascular system as measured by determination of the cardiac output are altered acutely during portal-caval shunt surgery. Rapid, multiple measurements of cardiac output which are correlated with blood pressures before, during, and after surgery would give enough information to discern the extent of alteration in cardiovascular performance.

3) Refinement of the data-handling system employing more accurate devices with which to gather experimental data. This includes a fiber-optic dye measurement system employing a solid-state infrared laser diode with which to detect ICG concentration in both blood and bile. A system such as this has several advantages in that it would not require a lengthy calibration procedure with every experiment and, therefore, would reduce the total experimental procedural time by at least two hours. Furthermore, determination of dye concentrations in the blood and the bile could be taken at a greater frequency which would allow the analysis of the data to be correlated more accurately.
4) Experimentation with the mathematical model should be performed to investigate the response of increasing the amount of dye injected and the effects on the analysis of the blood decay and bile accumulation curves. Further studies might simulate the presence of hepatic pathology by affecting the nature of the transfer rate constants. Particularly, the effects of hepatic cirrhosis might be studied by decreasing the sinusoid-hepatocyte transfer rate constant. These investigations could be substantiated by concurrent animal studies as well.

5) Chronic studies in animals on the effects of the shunt with respect to ICG removal need to be performed as well to determine the response of the dye to abnormal hepatic tissue as opposed to decreased blood flow.

6) Both acute and chronic studies in ICG removal characteristics should be performed involving experimentally induced hepatic pathology, such as administration of CCl₄ to induce cirrhosis of the liver. This effect may or may not be different from the abnormalities caused by the portal-caval shunt.

7) Finally, it may be interesting to study the specific effects of methoxyflurane-nitrous oxide on the cardiac output and hepatic function as measured by ICG removal during pre- and post-shunt conditions.
What are some of the qualifications of a good teacher? In the first place, he sticks to principles and keeps his mind on the goal . . . he does not qualify every generalization and insert periphrases in every other statement. Nor does he pulverize the noble structures of science to a dust, until they are no longer beautiful and serve but to repel the imaginative spirits that they should attract . . . A good teacher, again, is an artist in the use of words; is an advocate in pleading a cause; is a judge in weighing evidence; and above all things is an actor holding an audience. . . .

Knowledge of the technique of surgery is born only of prolonged practice. It seems obvious that it is much wiser and kinder to cultivate this art upon the lower animals than upon human beings. To achieve the requisite dexterity and facility in handling tissues and instruments demands patience and practice. Although some few appear to find these qualifications inherent, most of us can attain them only after persistent repetition, concentration, and devotion to the art we desire to possess. Fortunately, no study could prove more enthralling and stratifying, and simultaneously profitable. As in all surgery, there is sufficient of all the elements of satisfaction and pleasure in the performance of these procedures, to make the hardest efforts seem easy (Markowitz, Archibald, and Downie, 1964, pp. 644, 645).
Vivat acadamia, vivant professores;
Vivat acadamia, vivant professores;
Vivant membrum quodlibet, vivant membræ quaelibet,
Semper sint in flore! Semper sint in flore!

Gaudeamus igitur, iuvenes dum sumus;
Gaudeamus igitur, iuvenes dum sumus;
Post iucundam iuventutem, post molestam senectutem
nos habebit humus, nos habebit humus!

(Christian Wilhelm Kindleben, 1781)
BIBLIOGRAPHY


ACKNOWLEDGMENTS

It is difficult to look back and realize that my career as a student is finally over with this writing. I rejoice! During my graduate studies, many people have touched my life that are too numerous to mention by name other than to say I will retain many fond memories from the past six years.

To my major professor, Dr. Richard L. Engen, who has had to put up with me almost from the beginning, who had to listen to me moan about how rough it was, I give my deepest thanks for not allowing me to give up and encouraging me to continue and keep going.

To my committee members, Dr. Richard C. Seagrave, Dr. Bernard J. White, Dr. Donald C. Beitz, Dr. Malcolm H. Crump, and Dr. Thomas R. Rogge, who have also had to put up with me and listen to my jokes, I give my warmest regards, thanks, and appreciation for allowing me to learn from you.

To my chief confidant, Dr. Pamela K. McAllister, without whom I could not have performed this research project, and who had to stand across the operating table during all my experiments and listen to me swear when the bleeding started, I extend my most heartfelt appreciation and thanks.

To the faculty and graduate students of the Biomedical Engineering Program, who gave me mostly moral support, and the rest immoral support, I give my best regards and my old joke collection, which I'm having made into wallpaper for the coffee room so you'll never forget them ... ever!

To my typist, Charmian G. Price, whose masterful skill at the keyboard helped me sleep at night (after the effects of the caffeine were
gone, of course) and not worry about getting this manuscript in its final form, I give my sincerest gratitude.

To my wife, Sue, whose editorial skills were most helpful and most welcomed, who probably didn't realize in what she was getting herself involved with regards to being a "dissertation widow," I give all my love and promise you'll never have to bring me dinner in the middle of a surgical experiment again (I hope!).

To my family and friends who supported me throughout my career as a "professional student," and who also thought I would never finish "school," as it was often called, I give my love, thanks, and promise to get a job (someday!).
APPENDIX A: FORTRAN SOURCE LISTING OF MATHEMATICAL MODEL

(the subroutine ODES is the library subroutine that produces solutions to systems of differential equations)
LIVER MODEL TEST PROGRAM-----WRITTEN 12-13-82

******************************************************************************
*                      FLUID CONCENTRATIONS                         *
*   *            X(1) = CONC. IN BLOOD POOL   *                      *
*   *            X(2) = CONC. IN PORTAL VEIN  *                      *
*   *            X(3) = CONC. IN LIVER SINUSOIDS  *                 *
*   *            X(4) = CONC. IN LIVER HEPATOCYTES  *               *
*   *            X(5) = CONC. IN LIVER BILE    *                      *
******************************************************************************

PROGRAM LIVMOD

COMMON /EQNS/ NEQN
COMMON /PART/ RHO,SIG,DOSE
COMMON /RATE/ QL,QHA,QP,QLB,KH,KLB
COMMON /VOLU/ V(5)
COMMON /TIME/ FT,DELTA,DT
COMMON /SUMS/ SUM(5)

REAL X(10),T,DT,ERRLM(2),KH,KLB

EXTERNAL CLIV,ODES,ODESH

OPEN(UNIT=5,FILE='LIVIN.NUM',STATUS='OLD')
OPEN(UNIT=6,FILE='LIVOUT.DAT',STATUS='NEW')

CALL ENTDAT

T=0.0
N=5

DO 5 I=1,5
    SUM(I)=0.0
    CONTINUE

ERRLM(1)=1.E-5
ERRLM(2)=1.E-6

CALL INIVAL(X)

CALL OUTPUT(X,T)

DO 10 J=1,FT,DELTA
    CALL ODES(CLIV,X,N,T,T+DELTA,DT,ERRLM,ODESH)
    T=T+DELTA
    CALL OUTPUT(X,T)
10    CONTINUE
WRITE(*,12)(SUM(I),I=1,5)
12 FORMAT(4(2X,F9.3),2X,F12.3)
CLOSE(UNIT=5)
CLOSE(UNIT=6)
STOP
END

SUBROUTINE OUTPUT(X,T)
COMMON /EQNS/ NEQN
COMMON /SUMS/ SUM(5)
REAL X(5),T,KH,KLB
DO 15 I=1,5
   IF (X(I).LT.0.0) X(I)=0.0
15 CONTINUE
DO 17 I=1,5
   SUM(I)=SUM(I)+X(I)*T
17 CONTINUE
WRITE(6,20) T,(X(I), I=1,NEQN)
20 FORMAT(2X,F7.2,5(2X,F9.3))
RETURN
END

SUBROUTINE CLIV(T,X,N,RHS)
COMMON /RATE/ QL,QHA,QP,QLB,KH,KLB
COMMON /PART/ RHO,SIG
COMMON /VOLU/ V(5)
REAL X(5),RHS(5),T,RHO,KH,KLB,SIG
RHS(1)=((QL*X(3))+(SIG*QP*X(2))-(QHA*X(1))-(QP*X(1)))/V(1)
RHS(2)=((QP*X(1))-(RHO*QP*X(2))-(SIG*QP*X(2)))/V(2)
RHS(3)=((RHO*QP*X(2))+(QHA*X(1))-(QL*X(3))-(KH*X(3)))/V(3)
RHS(4)=((KH*X(3))-(KLB*X(4)))/V(4)
RHS(5)=((KLB*X(4))-(QLB*X(5)))/V(5)
RETURN
END
SUBROUTINE ENTDAT

COMMON /EQNS/ NEQN
COMMON /PART/ RHO,SIG,DOSE
COMMON /RATE/ QL,QHA,QP,QLB,KH,KLB
COMMON /TIME/ FT,DELTA,DT
COMMON /VOLU/ V(5)

REAL KH,KLB,RHO,SIG,DOSE
INTEGER NEQN

READ(5,*) NEQN
READ(5,*) RHO,DOSE
READ(5,*) QLP,QLB,KH,KLB
READ(5,*) FT,DELTA,DT
READ(5,*) (V(I), I=1,5)

SIG=1.0-RHO
QP=0.6666666667*QLP
QHA=0.3333333333*QLP
QL=QHA+RHO*QP
RETURN
END

SUBROUTINE INIVAL(X)

COMMON /PART/ RHO,SIG,DOSE
COMMON /VOLU/ V(5)
REAL X(5),DOSE

X(1)=DOSE/V(1)
X(2)=0.0
X(3)=0.0
X(4)=0.0
X(5)=0.0

RETURN
END
APPENDIX B: FLOW CHARTS OF DATA ACQUISITION PROGRAMS

DATCAL - dye calibration program
DATGET - main data acquisition program
CALOUT - standard curve determination program
DATOUT - raw data conversion program
CRDOUT - cardiac output determination program
CALFACTORS OUT

ENTER EXPT DATE

READ DATA FILE FOR X, Y

COMPUTE $e_x$, $e_x^2$, $e_{xy}$, $e_{y^2}$, $e_y$

COMPUTE - SLOPE - INTERCEPT - CORRELATION

SHOW - SLOPE - INTERCEPT - CORRELATION

STORE SLOPE AND INTERCEPT ON DISK

TRANSFER DATA TO PDP-11

BIE STDs?

N

CHAIN TO DATA OUT

A
DATA OUT

ENTER EXPT DATE

READ BLOOD SLOPE/INTERCEPT

READ BILE SLOPE/INTERCEPT

READ DATA FILE

SHOW WEIGHT - HEMATOCRIT - FINAL VALUE

CONVERT BLOOD DATA TO CONC

A

INPUT SPACING FACTORS FOR KEY PLOT

PLOT BLOOD DATA

PLOT OK?

TRANSFER TO PDP-11

CONVERT 'DRP TOTALS TO VOLUMES

INPUT SPACING FACTOR

B

C
C

COMPUTE
- SLOPE
- INTERCEPT
- CORRELATION

SHOW
- SLOPE
- INTERCEPT
- CORRELATION

O.K.,?

ENTER NEW LIMITS

REPLT
CURVE

COMPUTE
\[ y = e^{nt} x e^{s(t-t)} \]
FROM UPLMT TO FINAL VAL

PLOT
CALCULATED CURVE

E

COMPUTE AREA UNDER CURVE \( \varepsilon x y \)

READ ANIMAL WEIGHT

COMPUTE DOSAGE OF DYE

COMPUTE CARDIAC OUTPUT MEAN TRANSIT TIME

COMPUTE CENTRAL BLOOD VOLUME

SHOW CARDIAC OUTPUT MTT CBV

F
## APPENDIX C: BASIC SOURCE LISTINGS OF DATA ACQUISITION PROGRAMS WRITTEN FOR PDP-8e/OS8 COMPUTER

<table>
<thead>
<tr>
<th>User defined functions</th>
<th>Peripheral device</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIS(X,Y,M$)</td>
<td>X-Y plotting oscilloscope</td>
</tr>
<tr>
<td>TIM(W)</td>
<td>Clock/timing function</td>
</tr>
<tr>
<td>ADC(C,0)</td>
<td>A/D channel function</td>
</tr>
<tr>
<td>SSW(C)</td>
<td>Sense switch function</td>
</tr>
<tr>
<td>RLQ(C)</td>
<td>Relay control function</td>
</tr>
<tr>
<td>SLN(C)</td>
<td>Sense line function</td>
</tr>
</tbody>
</table>
10 DIM Y(11),X(11),Z(11),A$(10)
20 UDEFDIS(X,Y,M$),TIM(W),ADC(C,0),SSW(C)
30 PRINT TAB(20);"#### DATA CALIBRATION PROGRAM ####" \ PRINT
40 E$=DAT$(X) \ PRINT TAB(34);SEG$(E$,1,5)&"/83"
50 IF K>0 THEN 70
60 D$="BD"
70 C$=SEG$(E$,2,2)&SEG$(E$,4,5) \ A$="C"&D$&C$&".DA"
80 PRINT \ PRINT "ENTER NUMBER OF STANDARDS ( N<=10 )"; 
90 PRINT \ PRINT TAB(21);"**'-* SET CLOCK TO 30-HZ *****" 
100 PRINT TAB(15);"*** DENSITOMETER FILTER #";F+1","SCALE FACTOR";F+2; "***"
110 FOR J=2 TO N+1
120 PRINT \ PRINT "ENTER CONCENTRATION FOR THIS STANDARD (PRESS RETURN 
130 INPUT X(J) \ IF X(J)<=0 THEN 140 \ GO TO 480
140 PRINT \ PRINT "PRESS RETURN TO BEGIN SAMPLE RECORD"; \ INPUT B$
150 W=TIM(-30) \ REM 30-HZ CLOCK RETURNS TIME IN SECONDS
160 PRINT \ PRINT "WAITING FOR SAMPLE TO ARRIVE" 170 IF K=1 THEN 240
180 I=ADC(8,0)
190 IF I<1 THEN 210
200 GO TO 180
210 I=ADC(8,0)
220 IF I>0 THEN 240
230 GO TO 210
240 FOR I=1 TO 10
250 W=TIM(0) \ Z(I)=ADC(8,0)
260 IF Z(I)>=0 THEN 280
270 Z(I)=0
280 PRINT I,Z(I)
290 W=TIM(30)
300 NEXT I \ M=0
310 PRINT \ PRINT "DO YOU WISH TO OMIT DATA (Y/N)"; \ INPUT B$
320 IF B$="N" THEN 350
330 IF B$="Y" THEN 370
340 GO TO 310
350 IF M>0 THEN 400
360 A=1 \ B=10 \ GO TO 400
370 M=1 \ PRINT \ PRINT "ENTER RANGE OF NUMBERS TO BE SAVED"; \ INPUT A,B
380 R=0 \ FOR I=A TO B \ R=R+Z(I) \ PRINT I,Z(I),R \ NEXT I \ PRINT
390 PRINT J-1,X(J);"MG/L",R/(B-A+1),"AVERAGE OF N=";B-A+1;"POINTS" \ GO 
400 TO 310
410 R=0 \ FOR I=A TO B \ R=R+Z(I) \ NEXT I \ PRINT \ PRINT \ 
420 PRINT J-1,X(J);"MG/L",Y(J),"FINAL AVERAGE OF N=";B-A+1;"POINTS"
430 PRINT \ PRINT "PRESS RETURN TO CONTINUE CALIBRATION ROUTINE"; \ 
440 INPUT B$
450 NEXT J \ PRINT \ PRINT \ FILEVN#1:A$
460 PRINT TAB(10);"*** STORING DATA IN FILE ";A$;" ***"
470 PRINT \ PRINT "NUMBER OF STANDARDS STORED=";N+1 \ PRINT #1:N+1 \ 
480 FOR J=0 TO N+1 \ PRINT X(J);"MG/L",Y(J) \ PRINT #1:X(J),Y(J) \ NEXT J
490 CLOSE #1 \ GO TO 540
480 PRINT \ PRINT "ZERO ADJUST FOR DENSITOMETER"
490 PRINT \ PRINT "TOGGLE SENSE SWITCH 2 TO CONTINUE"
500 W=TIM(0) \ L=ADC(8,0)
510 IF SSW(2)<0 THEN 120
520 PRINT L,
530 W=TIM(15) \ GO TO 500
540 IF K>0 THEN 600
550 PRINT \ PRINT "DO YOU HAVE BILE STANDARDS ALSO"; \ INPUT B$
560 PRINT \ PRINT \ K=1 \ D$="BL" \ F=2
570 IF B$="Y" THEN 10
580 IF B$="N" THEN 600
590 GO TO 550
600 PRINT \ PRINT \ CHAIN "SYS:DATGET.SV" \ END
10 REM \ REM \ REM
20 DIM A$(10), C$(10), X(1200), Y(270), N(60), U(60)
30 UDEFDIS(X, Y, M$), TIM(W), ADC(C, 0), SSW(C), RLY(C), SLN(C)
40 PRINT TAB(20); "#### DATA ACQUISITION PROGRAM ####" \ PRINT
50 D$ = DAT$(X) \ PRINT TAB(34); SEG$(D$, 1, 5) & "/83" \ PRINT
60 F$ = SEG$(D$, 2, 2) & SEG$(D$, 4, 5) \ A$ = "BB" & F$ & ".DA" \ C$ = "HRT" & F$ & ".DA"
70 PRINT \ PRINT "ENTER WEIGHT (LBS)" ; \ INPUT HI
80 PRINT \ PRINT "ENTER HEMATOCRIT" ; \ INPUT H2
90 PRINT \ PRINT "ENTER SPACING FOR PLOT" ; \ INPUT M \ PRINT
100 PRINT \ PRINT "ENTER SAMPLE TO DENSITOMETER TIME DELAY" ; \ INPUT A
110 PRINT \ PRINT "ENTER DYE INJECTION TIME DELAY" \ INPUT B \ PRINT
120 PRINT TAB(15); "***** SET EXTERNAL CLOCK TO 30-HZ FREQUENCY ******" \ PRINT
130 PRINT TAB(18); "#### DENSITOMETER FILTER # 1, SCALE FACTOR 2 ####" \ PRINT
140 R = RLY(1) \ PRINT "RELAY(1) ENABLED...BILE FLOW ESTABLISHED" \ PRINT
150 R = RLY(-2) \ PRINT "RELAY(2) DISABLED...TUBE RESET READY" \ PRINT
160 PRINT "DROP COUNTER CHECK...TOGGLE SENSE SWITCH 2 TO CONTINUE" \ N = 0
170 PRINT "SENSE SWITCH 1 CONTROLS BILE RELAY FOR ADJUSTMENT" \ PRINT
180 IF SLN(1) < 0 THEN 220
190 IF SSW(2) < 0 THEN 270
200 R = RLY((SSW(1) + 0.5) * 2)
210 GO TO 180
220 N = N + 1 \ PRINT N,
230 IF N > 200 THEN 260
240 IF SLN(1) < 0 THEN 240
250 GO TO 180
260 R = RLY(2) \ W = TIM(20) \ R = RLY(-2) \ N = 0 \ GO TO 180
270 IF SSW(2) < 0 THEN 270
280 PRINT \ PRINT "ZERO LEVEL CHECK...TOGGLE SENSE SWITCH 2 TO CONTINUE" \ PRINT
290 GOSUB 1220
300 H3 = H1 * .453592 \ H4 = H3 / 25 \ PRINT \ PRINT \ PRINT
310 PRINT "ANIMAL WEIGHT = "; H1; " LBS OR "; H3; " KG " \ PRINT
320 PRINT "DYE DOSAGE FOR THIS ANIMAL IS"; H3 / 25; " ML ( @ 25 MG/ML)" \ PRINT
330 PRINT \ PRINT "PRESS RETURN TO BEGIN 60-SEC. COUNTDOWN" ; \ INPUT B$
340 IF SSW(1) < 0 THEN 370 \ IF SSW(2) < 0 THEN 370 \ IF SSW(3) < 0 THEN 370
350 IF SSW(4) < 0 THEN 370 \ IF SSW(5) < 0 THEN 370 \ IF SSW(6) < 0 THEN 370
360 GO TO 390
370 PRINT \ PRINT "$$$$$$ COUNTDOWN ABORTED! CHECK SENSE SWITCHES!!$$$$$$" \ GO TO 330
380 PRINT \ I = 60 \ D = DIS(0, -1, ") \ K = 0
400 W = TIM(0)
410 IF I = 30 THEN 470
420 IF I = 25 THEN 500
430 IF I = 10 THEN 550
440 PRINT "T-MINUS:"; I \ I = I - 1
450 W = TIM(30)
460 GO TO 400
470 PRINT \ PRINT "T-MINUS:"; I, "BILE FLOW CUTOFF NOW!" \ R = RLY(-1)
480 PRINT \ I = I - 1 \ W = TIM(30)
490 GO TO 400
500 FOR J = 30 TO 300 STEP 30 \ GOSUB 1300
GO TO 710
066 IF I=60 THEN 1000
068 R=RTX(20) \ R=RTX(2)
070 \ I=I+1 \ A=0
071 \ PRINT \ O=(I)A \ (1)A=(A)
086 PRINT \ N=(I) \ Z=(I) \ PRINT \ GO TO 910
090 \ IN[3] \ SS=6+I \ THEN 940
090 \ SS=6 \ THEN 840
090 \ SS=6 \ THEN 1020
090 \ SS=6 \ THEN 1020
090 \ SS=6 \ THEN 1020
090 \ SS=6 \ THEN 1020
090 \ SS=6 \ THEN 1020
100 IF SS=3 THEN 780
100 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
100 \ PRINT \ 
110 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
110 \ PRINT \ 
120 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
120 \ PRINT \ 
130 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
130 \ PRINT \ 
140 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
140 \ PRINT \ 
150 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
150 \ PRINT \ 
160 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
160 \ PRINT \ 
170 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
170 \ PRINT \ 
180 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
180 \ PRINT \ 
190 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
190 \ PRINT \ 
200 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
200 \ PRINT \ 
210 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
210 \ PRINT \ 
220 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
220 \ PRINT \ 
230 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
230 \ PRINT \ 
240 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
240 \ PRINT \ 
250 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
250 \ PRINT \ 
260 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
260 \ PRINT \ 
270 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
270 \ PRINT \ 
280 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
280 \ PRINT \ 
290 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
290 \ PRINT \ 
300 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
300 \ PRINT \ 
310 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
310 \ PRINT \ 
320 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
320 \ PRINT \ 
330 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
330 \ PRINT \ 
340 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
340 \ PRINT \ 
350 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
350 \ PRINT \ 
360 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
360 \ PRINT \ 
370 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
370 \ PRINT \ 
380 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
380 \ PRINT \ 
390 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
390 \ PRINT \ 
400 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
400 \ PRINT \ 
410 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
410 \ PRINT \ 
420 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
420 \ PRINT \ 
430 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
430 \ PRINT \ 
440 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
440 \ PRINT \ 
450 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
450 \ PRINT \ 
460 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
460 \ PRINT \ 
470 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
470 \ PRINT \ 
480 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
480 \ PRINT \ 
490 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
490 \ PRINT \ 
500 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
500 \ PRINT \ 
510 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
510 \ PRINT \ 
520 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
520 \ PRINT \ 
530 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
530 \ PRINT \ 
540 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
540 \ PRINT \ 
550 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
550 \ PRINT \ 
560 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
560 \ PRINT \ 
570 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
570 \ PRINT \ 
580 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
580 \ PRINT \ 
590 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
590 \ PRINT \ 
600 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
600 \ PRINT \ 
610 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
610 \ PRINT \ 
620 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
620 \ PRINT \ 
630 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
630 \ PRINT \ 
640 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
640 \ PRINT \ 
650 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
650 \ PRINT \ 
660 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
660 \ PRINT \ 
670 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
670 \ PRINT \ 
680 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
680 \ PRINT \ 
690 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
690 \ PRINT \ 
700 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
700 \ PRINT \ 
710 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
710 \ PRINT \ 
720 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
720 \ PRINT \ 
730 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
730 \ PRINT \ 
740 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
740 \ PRINT \ 
750 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
750 \ PRINT \ 
760 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
760 \ PRINT \ 
770 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
770 \ PRINT \ 
780 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
780 \ PRINT \ 
790 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
790 \ PRINT \ 
800 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
800 \ PRINT \ 
810 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
810 \ PRINT \ 
820 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
820 \ PRINT \ 
830 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
830 \ PRINT \ 
840 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
840 \ PRINT \ 
850 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
850 \ PRINT \ 
860 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
860 \ PRINT \ 
870 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
870 \ PRINT \ 
880 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
880 \ PRINT \ 
890 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
890 \ PRINT \ 
900 PRINT \ IN[3] \ +I+1 \ A=0 \ I=I+1
900 \ PRINT \
97

1000 PRINT "*** DENSITOMETER FILTER # 3, SCALE FACTOR 4 ***" \ PRINT
1010 GO TO 710
1020 REM FILE OUTPUTS AND PROGRAM END
1030 R=RLY(-1) \ R=RLY(-2)
1040 FILEVN#2:C$
1050 PRINT TAB(10);"*** STORING DATA IN FILE ";C$;" ***" \ PRINT
1060 PRINT G(0),A,S \ PRINT #2:G(0),A,S \ PRINT
1070 FOR J=1 TO S \ PRINT X(J), \ PRINT #2:X(J) \ NEXT J
1080 CLOSE #2 \ PRINT \ PRINT
1090 PRINT "PRESS RETURN FOR BLOOD & BILE DATA"; \ INPUT B$ \ PRINT
1100 FILEVN#1:A$
1110 PRINT TAB(10);"*** STORING DATA IN FILE ";A$;" ***" \ PRINT
1120 PRINT "FINAL TIME =";I;" MIN","# TUBES =";T-1,"# BILE SAMPLES =";V-1
1130 PRINT \ PRINT "ANIMAL WEIGHT =";H3;" KG"," HEMATOCRIT =";H2
1140 PRINT \ PRINT #1:H3,H2,I,T-1,V-1 \ PRINT \ PRINT
1150 PRINT "PRESS RETURN FOR BLOOD VALUES" \ INPUT B$ \ PRINT
1160 FOR J=1 TO I \ PRINT Y(J), \ PRINT #1:Y(J) \ NEXT J \ PRINT
1170 PRINT "PRESS RETURN FOR DROP TOTALS" \ INPUT B$ \ PRINT
1180 FOR J=1 TO T-1 \ PRINT N(J), \ PRINT #1:N(J) \ NEXT J \ PRINT
1190 PRINT "PRESS RETURN FOR BILE VALUES" \ INPUT B$ \ PRINT
1200 FOR J=1 TO V-1 \ PRINT U(J), \ PRINT #1:U(J) \ NEXT J \ CLOSE #1
1210 PRINT \ PRINT \ PRINT \ GO TO 1540
1220 Z=ADC(8,0) \ PRINT Z, \ FOR A7=1 TO 15
1230 IF J=0 THEN 1250 \ IF SLN(1)=0 THEN 1240 \ GOSUB 1270
1240 IF W-J>=55 THEN 1260
1250 W=TIM(0) \ W=TIM(1) \ NEXT A7 \ IF SSW(2)<0 THEN 1260 \ GO TO 1220
1260 PRINT \ PRINT \ A8=1 \ RETURN
1270 N=N+1
1280 IF SLN(1)<=0 THEN 1280
1290 RETURN
1300 Z=ADC(8,0)
1310 IF Z>=0 THEN 1330
1320 Z=0
1330 D=DIS(K,Z*.5,"") \ K=K+1 \ RETURN
1340 S=0 \ P=M \ PRINT \ PRINT "WAITING FOR APPEARANCE OF DYE" \ PRINT
1350 R=RLY(3)
1360 Z=ADC(8,0)
1370 W=TIM(1)
1380 IF Z<2 THEN 1400
1390 GO TO 1360
1400 Z=ADC(8,0)
1410 W=TIM(1)
1420 IF Z>5 THEN 1440
1430 GO TO 1400
1440 G(0)=TIM(0) \ PRINT "START DATA SAMPLING"
1450 FOR Q=1 TO 1200
1460 IF W=59 THEN 1530
1470 Z=ADC(8,0)
1480 IF Z>=0 THEN 1500
1490 Z=0
1500 IF Q<=P THEN 1520
1510 D=DIS(K,2*:.5,"") \ K=K+1 \ P=P+M
1520 S=S+1 \ X(S)=Z \ W=TIM(Q) \ NEXT Q
1530 R=RLY(-3) \ RETURN
1540 PRINT TAB(20);'##### MAIN PROGRAM HAS ENDED #####' \ PRINT \ PRINT
1550 PRINT \ PRINT TAB(14);'##### PRESS RETURN TO BEGIN DATA OUTPUT #####'
1560 INPUT B$ \ PRINT \ PRINT \ PRINT \ PRINT \ PRINT
1570 CHAIN "SYS:CALOUT.SV" \ END
10 DIM A$(10),X(11),Y(11)
20 PRINT TAB(20);"#### CALIBRATION FACTORS OUTPUT PROGRAM ####" 
   PRINT
30 PRINT \ PRINT "DO YOU WANT TODAY'S DATA"; \ INPUT B$ \ PRINT
40 IF B$="Y" THEN 60 \ IF B$="N" THEN 50 \ GO TO 30
50 PRINT "ENTER EXPERIMENT DATE"; \ INPUT F$ \ PRINT \ PRINT \ GO TO 70
60 F$=DAT$(X)
70 PRINT TAB(39);SEG$(F$,1,5)6c"/83" \ PRINT \ PRINT
80 IF T>0 THEN 100
90 D$="BD"
100 C$=SEG$(F$,2,2)&SEG$(F$,4,5) \ A$="C"&D$&C$&".DA" \ FILEN#1:A$
110 PRINT TAB(10);"*** OPENING FILE C"&D$&C$&".DA FOR DATA INPUT ***"
120 INPUT #1:N \ PRINT \ PRINT "THIS FILE HAD";N;"STANDARDS" \ PRINT
130 A=0 \ B=0 \ C=0 \ D=0 \ E=0
140 FOR J=0 TO N
150 INPUT #1:X(J),Y(J)
160 PRINT X(J);;"MG/L",Y(J)
170 A=A+X(J) \ B=B+X(J)2 \ C=C+X(J)*Y(J) \ D=D+Y(J)2 \ E=E+Y(J)
180 NEXT J \ CLOSE #1
190 PRINT \ PRINT "*** LINEAR REGRESSION ANALYSIS ***" \ PRINT
200 L=C-A2/E/N \ K=B-A2/N \ M=D-E2/N \ I=(E-S*A)/N \ R=L/SQR(K*M)
210 PRINT TAB(10);"SLOPE =";S \ PRINT \ PRINT TAB(10);"INTERCEPT =";I
220 PRINT \ PRINT TAB(10);"CORRELATION COEFFICIENT =";R \ PRINT
230 A$="L"&D$&C$&".DA" \ PRINT "STORING SLOPE/INTERCEPT VALUES IN FILE " 
   &A$
240 FILEN#2:A$ \ PRINT #2:S,I \ CLOSE #2 \ PRINT \ PRINT
250 PRINT "***** OPEN PDP-11 FILE "&A$"T" NOW *****" \ INPUT B$
260 PRINT \ PRINT "PRESS RETURN FOR DATA" \ INPUT B$ \ PRINT
270 PRINT S,I,R \ PRINT
280 FOR J=1 TO N \ PRINT X(J);;"MG/L",Y(J),(Y(J)-I)/S \ NEXT J
290 PRINT \ PRINT "***** CLOSE PDP-11 FILE *****" \ INPUT B$
300 PRINT \ PRINT \ IF T>0 THEN 360
310 PRINT \ PRINT "DO YOU HAVE A BILE STANDARDS FILE"; \ INPUT B$ \ PRINT
320 T=1 \ D$="BL"
330 IF B$="Y" THEN 70
340 IF B$="N" THEN 360
350 GO TO 310
360 PRINT \ PRINT \ PRINT \ CHAIN "DATOUT.SV"
100 DIM A$(10),Y(270),N(60),U(60)
20 UDEFDIS(X,Y,M$)
30 PRINT TAB(20);"##### DATA OUTPUT PROGRAM ######" \ PRINT
40 PRINT \ PRINT "DO YOU WANT TODAY'S DATA (Y/N)"; \ INPUT B$ \ PRINT
50 IF B$="Y" THEN 70 \ IF B$="N" THEN 60 \ GO TO 40
60 PRINT "ENTER EXPERIMENT DATE"; \ INPUT D$ \ GO TO 80
70 DS=DATS(X)
80 PRINT TAB(31);SEG$(D$,1,5)&"/83" \ PRINT \ PRINT
90 CS=SEGS(D$,2,2)&SEG$(D$,4,5) \ A$="LBD"&CS&".DA" \ FILEN#2:A$ \ INPUT
#2:SO,10
100 CLOSE #2 \ A$="LBL"&CS&".DA" \ FILEN#3:A$ \ INPUT #3:S1,I1 \ CLOSE #3
110 PRINT "SLOPE/INTERCEPT DATA: BLOOD =";SO;IO;" BILE =";S1;I1 \ PRINT
120 A$="BB"&CS&".DA" \ FILEN#1:A$ \ INPUT #1:H3,H2 \ PRINT \ PRINT "ANIMAL WEIGHT =";H3;,"HEMATOCRIT =";H2
130 INPUT #1:1,T,V \ PRINT \ PRINT "THIS EXPERIMENT RAN";I;"MINUTES"
140 PRINT \ PRINT "IT HAD";T;"DROP TOTALS AND";V;"BILE SAMPLES"
150 FOR J=1 TO I \ INPUT #1:Y \ Y(J)=(Y-I0)/S0 \ IF Y(J)>=0 THEN 180 \ Y(J)=0
160 NEXT J \ PRINT \ PRINT 
170 PRINT "BLOOD DYE DATA IS NOW LOADED" \ PRINT \ PRINT "ENTER X-Y SCALE FACTORS"; \ INPUT X1,Y1 \ PRINT
180 PRINT "PLOTTING BLOOD DYE DATA NOW..." \ PRINT
190 PRINT "ENTER FINAL TIME <";I; \ INPUT Z \ IF Z>I THEN 210 \ PRINT
200 D=DIS(0,-1,"") \ FOR J=1 TO Z \ D=DIS(J*X1,Y(J)*Y1,"") \ NEXT J
210 PRINT "IS THIS ACCEPTABLE"; \ INPUT B$ \ PRINT \ IF B$="Y" THEN 250
220 IF B$="N" THEN 190 \ GO TO 230
230 PRINT "***** OPEN PDP-11 FILE 'BLD"CS&".DAT' NOW *****"
240 INPUT B$ \ PRINT \ PRINT "PRESS RETURN FOR BLOOD VALUES" \ INPUT B$
250 FOR J=1 TO Z \ INPUT J,Y \ PRINT J,Y(J)
260 PRINT 
270 FOR J=1 TO T \ INPUT N(J)=N/35 \ IF N(J)>=0 THEN 300 \ N(J)=0
280 NEXT J \ PRINT "ENTER X-Y SCALE FACTORS"; \ INPUT X1,Y1 \ PRINT
290 PRINT "PLOTTING BILE DROP DATA NOW..." \ PRINT \ D=DIS(0,-1,"")
300 FOR J=5 TO T\5 STEP 5 \ D=DIS(J*X1/5,N(J/5)*Y1,"") \ NEXT J
310 PRINT "IS THIS ACCEPTABLE"; \ INPUT B$ \ PRINT \ IF B$="Y" THEN 360
320 IF B$="N" THEN 310 \ GO TO 340
330 PRINT "***** OPEN PDP-11 FILE 'DPS"CS&".DAT' NOW *****"
340 INPUT B$ \ PRINT \ PRINT "PRESS RETURN FOR DROP VOLUMES" \ INPUT B$
350 FOR J=5 TO T\5 STEP 5 \ INPUT J,N(J/5) \ NEXT J
360 PRINT "***** CLOSE PDP-11 FILE *****" \ INPUT B$
370 PRINT "***** OPEN PDP-11 FILE 'BIL"CS&".DAT' NOW *****"
380 INPUT B$ \ PRINT \ PRINT "PLOTTING BILE DYE DATA NOW..." \ PRINT \ D=DIS(0,-1,"")
390 PRINT "IS THIS ACCEPTABLE"; \ INPUT B$ \ PRINT \ IF B$="Y" THEN 450
400 IF B$="N" THEN 390 \ GO TO 450
410 NEXT J \ PRINT \ PRINT "BILE DYE DATA IS NOW LOADED" \ PRINT \ PRINT "ENTER X-Y SCALE FACTORS"; \ INPUT X1,Y1 \ PRINT
420 PRINT "PLOTTING BILE DYE DATA NOW..." \ PRINT \ D=DIS(0,-1,"")
430 FOR J=5 TO V\5 STEP 5 \ D=DIS(J*X1/5,U(J/5)*Y1,"") \ NEXT J
440 PRINT "IS THIS ACCEPTABLE"; \ INPUT B$ \ PRINT \ IF B$="Y" THEN 470
450 IF B$="N" THEN 440 \ GO TO 450
460 PRINT "***** OPEN PDP-11 FILE 'BIL"CS&".DAT' NOW *****"
480 INPUT B$ \ PRINT \ PRINT "PRESS RETURN FOR BILE VALUES " \ INPUT B$
490 FOR J=5 TO V*5 STEP 5 \ PRINT J,U(J/5) \ NEXT J
500 PRINT \ PRINT "***** CLOSE PDP-11 FILE *****" \ INPUT B$ \ CLOSE #1
510 PRINT \ PRINT \ PRINT \ CHAIN "CRDOUT.SV"
10 DIM A$(10), Y(1600)
20 UDEFDIS(X,Y,M$)
30 PRINT TAB(15); "### CARDIAC OUTPUT DATA OUTPUT PROGRAM ###";
PRINT
40 PRINT "DO YOU WANT TODAY'S DATA (Y/N)"; 
INPUT B$ 
50 IF B$="Y" THEN 70 
IF B$="N" THEN 60 
GO TO 40
60 PRINT "ENTER EXPERIMENT DATE"; 
INPUT D$ 
PRINT 
GO TO 80
70 D$=DAT$(X)
80 PRINT TAB(34); SEG$(D$, l, 5) & "/83" 
PRINT 
PRINT
90 C$=SEG$(D$, 2, 2)&SEG$(D$, 4, 5) 
A$="LBD"&C$&".DA" 
FILEN#2:A$
100 INPUT #2:S,I 
CLOSE #2 
PRINT "BLOOD DATA SLOPE ="; S; " INTERCEPT ="; I 
110 A$="HRT"&C$&".DA" 
FILEN#1:A$
120 PRINT 
PRINT 
PRINT TAB(10); "*** OPENING FILE ";A$; " FOR DATA INPUT ***" 
130 PRINT 
INPUT #1:G(0), A, Q 
H=30*INT(G(0)-A+.5)
140 IF H>=0 THEN 150 
H=0
150 PRINT G(0), A, Q, H 
PRINT 
160 Q=Q+H 
PRINT "DYE CURVE BEGAN";G(0)-A; "SECONDS AFTER INJECTION" 
PRINT
170 FOR J=H TO Q 
INPUT #1:Y 
Y(J)=(Y-I)/S 
IF Y(J)>=0 THEN 180 
Y(J)=0
180 IF J=H THEN 200 
IF Y(J)<=MO THEN 200 
IF Y(J) >512 THEN 200
190 MO=Y(J) 
TO=J
200 NEXT J 
PRINT 
CLOSE #1
210 PRINT 
PRINT "MAXIMUM VALUE ="; MO; "AT TIME"; TO/30; "SECONDS" 
PRINT
220 PRINT "ENTER X, Y-SPACING FACTORS"; 
INPUT V, U
230 PRINT "PLOTTING AT AN EFFECTIVE SAMPLE RATE OF"; 30/V; ";-Hz" 
240 D=DIS(0,-1,""
250 FOR J=0 TO Q STEP V 
DO=DIS(J/V, Y(J)*U,"") 
NEXT J
260 PRINT 
PRINT "DO YOU WANT TO SEE ANOTHER DATA PLOT (Y/N)"; 
INPUT B$
270 IF B$="Y" THEN 220
280 IF B$="N" THEN 300
290 GO TO 260
300 PRINT 
PRINT "***** LINEAR REGRESSION ANALYSIS *****" 
Z=.93 
W=.6
310 PRINT 
PRINT "INITIAL LIMITS FOR DECAY CURVE: UPPER ="; Z; " LOWER ="; W
320 PRINT 
A=0 
B=0 
C=0 
D=0 
E=0 
N=0
330 FOR J=TO TO Q 
IF Y(J)<=Z*MO THEN 340 
NEXT J
340 X0=INT(J/V+.5)*V 
FOR J=X0 TO Q 
IF Y(J)<=W*MO THEN 360
350 GOSUB 530 
NEXT J
360 X1=INT(J/V+.5)*V 
L=C-A*E/N 
K=B-A2/N 
M=D-E2/N
370 S=L/K 
I=(E-S*A)/N 
R=L/SQR(K*M)
380 PRINT "REGRESSION COMPLETE FOR RANGE";X0/30; "TO";X1/30; "SECONDS" 
390 PRINT 
PRINT TAB(10); "SLOPE =" ; S 
PRINT TAB(10); "INTERCEPT =" ; I 
400 PRINT TAB(10); "CORRELATION COEFFICIENT =" ; R 
PRINT
410 PRINT "NOW INDICATING RANGE TESTED FOR LINEARITY" 
PRINT
420 DO=DIS(X0/V,Y(X0)*U,"""
430 PRINT "IS THIS ACCEPTABLE"; 
INPUT B$ IF B$="Y" THEN 460
440 IF B$="N" THEN 450 
GO TO 430
450 PRINT 
PRINT "ENTER NEW UPPER/LOWER LIMITS"; 
INPUT Z,W 
GO TO 320
460 PRINT 
PRINT "REPLOTTING DATA AT EFFECTIVE SAMPLE RATE OF"; 30/V; ";-Hz"
103

470 DO=DIS(0,-1,""), FOR J=0 TO Q STEP V DO=DIS(J/V,Y(J)*U,""), NEXT J
480 DO=DIS(X0/V,Y(X0)*U,""--"), DO=DIS(X1/V,Y(X1)*U,""--"")
490 FOR J=X0 TO Q Y(J)=EXP(I)*EXP(S*J/30) IF Y(J)>=0 THEN 500 Y(J)=0
500 NEXT J
510 FOR J=X0 TO Q STEP V Y(J)=EXP(I)*EXP(S*J/30) NEXT J PRINT
520 PRINT "PRESS RETURN TO CONTINUE"; INPUT B$ PRINT GO TO 560
530 X=J/30 Y=LOG(Y(J)) N=N+1
540 A=A+X B=B+X2 C=C+X*Y D=D+Y2 E=E+Y
550 RETURN
560 A$="BB"&C$&".DA" FILEN#2:A$ INPUT #2:H3 CLOSE #2
570 PRINT "CALCULATIONS TO FOLLOW:" X=0 Y=0 Y1=0
580 FOR J=0 TO Q X=X+(J/30)*Y(J) Y=Y+Y(J) Y1=Y1+(Y(J)/30) NEXT J
590 Y2=EXP(I)*EXP(S*J/30) IF Y2>=0 THEN 600 Y2=0
600 X=X+(J/30)*Y2 Y=Y+Y2 Y1=Y1+(Y2/30) IF Y2<=2.47875E-03*MO THEN 620
610 J=J+1 GO TO 590
620 T1=X/Y C0=H3*60/Y1 C1=C0*1000*T1/60
630 PRINT "FINAL TIME CALCULATED =";J/30;"SECONDS"
640 PRINT "TOTAL DYE INJECTED =";H3;"MG"
650 PRINT "AREA UNDER CURVE =";Y1;"MG-S/L" PRINT
660 PRINT "CARDIAC OUTPUT =";C0;"L/MIN"
670 PRINT "MEAN TRANSIT TIME =";T1;"SECONDS"
680 PRINT "CENTRAL BLOOD VOLUME =";C1;"ML"
690 PRINT "OPEN PDP-11 FILE 'CAR"&C$&".DAT' NOW
700 INPUT B$ PRINT "PRESS RETURN FOR DATA" INPUT B$ PRINT
710 PRINT J/30,H3,Y1 C0,T1,C1 PRINT PRINT
720 FOR J=0 TO Q PRINT Y(J) NEXT J PRINT
730 PRINT "CLOSE PDP-11 FILE " INPUT B$ PRINT
740 PRINT "## PROGRAM SEQUENCE COMPLETED ##" END
APPENDIX D: SURGICAL PROCEDURE

The animal was prepared for midline laparotomy and femoral cutdown and then placed on the surgical table in dorsal recumbancy. Body temperature was maintained using a heating pad placed underneath the animal and was monitored using an esophageal thermistor and a YSI tele-thermometer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH). Needle electrodes were placed in the extremities such that a Lead II ECG could be obtained.

After application of surgical drapes, a midline incision was made using an electrosurgical device (Cameron-Miller Surgical Instrument Co., Chicago, IL). Entrance into the peritoneal cavity was expedited by the use of electrocautery to cut and seal all tiny blood vessels and results in greatly reducing any subsequent hemorrhage later when the animal will be given heparin.

Upon completion of the abdominal opening and insertion of a Balfour retractor, the right lateral, right central, and caudate lobes of the liver are gently raised and reflected cranially using moistened sponges held on sponge forceps. This results in adequate exposure of the gall bladder and cystic duct to allow for ligation of the cystic duct. Exposure is further enhanced by placing a ribbon retractor wrapped in a saline-moistened lap sponge over the exterior of the duodenum and pyloric antrum and then applying gentle pressure caudally. A small tunnel under the cystic duct medial to the body of the gall bladder and the entrance to the common bile duct is then made using bile duct forceps. A length of umbilical tape is passed around the cystic duct and the duct is then
constricted by placing a plastic retainer over the free ends of the tape, passing the retainer down to the duct, then securing the retainer with a hemostat while applying backward pressure on the tape. This results in a constriction that will remain in place for the duration of the experiment (Figure 13). Care must be taken to avoid impaling the hepatic capsule during the ligation of the cystic duct. However, when this occurred the wound was cauterized carefully and covered with a strip of Gelfoam sponge (The Upjohn Company, Kalamazoo, MI) when the procedure had been completed.

The ribbon retractor is withdrawn slightly more caudal to expose the area of the common bile duct for cannulation. The surrounding connective tissue is carefully removed from the vicinity of the common duct using the bile duct forceps and employing cautery when needed to control hemorrhage in the fatty deposits of the surrounding tissue. Two lengths of 3-0 silk suture are then placed, but not tied, around the duct to be used as stay sutures (Figure 14). Immediately prior to cannulation, the caudal stay suture is tied as close to the duodenum as possible, then a small cut is made in the common duct with iris scissors. A cannula of 1.57 mm ID Silastic tubing (Cat. #602-205, Dow Corning, Inc., Midland, MI) with a 2.0 cm plastic tubing connector of similar diameter on the duct end is inserted into the duct using a catheter introducer. Once in place, the cranial stay suture is tied over the connector in the duct to secure the cannula (Figure 15). Bile usually begins to flow immediately once the cranial stay suture has been tied.

The procedure for portal-caval shunt (Eck fistula) has been described previously (Antol, 1980; Markowitz et al., 1964). The surgical approach
in these experiments was still made from the midline incision rather than
the more complex abdominal incision described by Markowitz et al. Follow­
ing completion of the shunt, the vessels were wrapped in Gelfoam sponge to
assist in hemostasis of the suture lines before the spleen and intestines
were replaced in the abdominal cavity prior to closing.

In those experiments requiring cannulation of the hepatic vein, the
left common hepatic vein was cannulated according to Shoemaker et al.
(1959) using a length of PE-190 tubing previously fitted with an 18 ga.
luer hub. The cannula was anchored to the abdominal wall with 3-0 silk
suture to prevent accidental withdrawal of the cannula from the insertion
site.

The femoral cutdown to allow introduction of arterial and venous
cannulas was performed in the usual manner with the femoral artery and
vein being exposed from a point distal to the deep inguinal ring to a
point proximal to the knee. When the surrounding connective tissue had
been cleared, a cannula of 1.57 mm ID Silastic tubing was introduced into
the femoral artery and advanced into the abdominal and thoracic aorta
until coming to rest near the aortic valve, as indicated by the arterial
blood pressure waveform. In a similar manner, a cannula of 3.35 mm ID
Silastic tubing (Cat. #601-335) was introduced into the femoral vein and
advanced into the inferior vena cava until coming to rest near the right
atrium, as indicated by the venous blood pressure waveform. Both cannulas
were then secured with 3-0 silk suture.

Upon completion of both procedures, the incisions were drawn together
and closed with towel clamps. A circulating warm water pad was placed
over the animal and the pad was covered with two layers of surgical drapes. In the latter experiments involving the portal-caval shunt procedure, the bladder of each animal was catheterized using a 50 cm 5F cannula with the urine being channeled through rubber tubing into a floor drain.
Figure 13. Photograph of cystic duct ligation showing gall bladder (G), umbilical tape (U), plastic retainer (R), and restraining hemostat (H)
Figure 14. Photograph of bile duct before cannulation showing stay sutures (S), bile duct (B), and duodenum (D)
Figure 15. Photograph of bile duct after cannulation showing bile duct (B), duodenum (D), plastic tubing connector (T) tied in place, and the cannula (C)
APPENDIX E: RAW DATA FROM ANIMAL STUDIES WITH MEANS AND STANDARD ERRORS OF THE MEAN
Table 8. Body weights, hematocrits, and cardiac outputs for both animal groups

<table>
<thead>
<tr>
<th>Animal #</th>
<th>Type</th>
<th>BW (kg)</th>
<th>Hematocrit (%)</th>
<th>Cardiac output (L min⁻¹ kg⁻¹)</th>
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<tbody>
<tr>
<td>502</td>
<td>Normal</td>
<td>17.2</td>
<td>36</td>
<td>...</td>
</tr>
<tr>
<td>504</td>
<td>Normal</td>
<td>17.2</td>
<td>42</td>
<td>0.130</td>
</tr>
<tr>
<td>518</td>
<td>Normal</td>
<td>17.2</td>
<td>35</td>
<td>0.240</td>
</tr>
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<td>520</td>
<td>Normal</td>
<td>22.7</td>
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<td>0.173</td>
</tr>
<tr>
<td>523</td>
<td>Normal</td>
<td>19.1</td>
<td>51</td>
<td>0.162</td>
</tr>
<tr>
<td>525</td>
<td>Normal</td>
<td>20.0</td>
<td>36</td>
<td>0.171</td>
</tr>
<tr>
<td>527</td>
<td>Normal</td>
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<td>0.138</td>
</tr>
<tr>
<td>Mean</td>
<td>Normal</td>
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<td>(SEM)</td>
<td>(0.7)</td>
<td>(2)</td>
<td>(0.014)</td>
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<tr>
<td>805</td>
<td>Shunt</td>
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<tr>
<td>810</td>
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<td>0.080</td>
</tr>
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<td>Shunt</td>
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<td>Group 1</td>
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<td>Normal</td>
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<td>0.216</td>
</tr>
<tr>
<td>822</td>
<td>Normal</td>
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<td>50</td>
<td>0.124</td>
</tr>
<tr>
<td>Mean</td>
<td>Shunt</td>
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<tr>
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<td>(3)</td>
<td>(0.034)</td>
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<td>831</td>
<td>Shunt</td>
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<tr>
<td>907</td>
<td>Shunt</td>
<td>19.8</td>
<td>50</td>
<td>0.176</td>
</tr>
<tr>
<td>Mean</td>
<td>Shunt</td>
<td>19.8</td>
<td>50</td>
<td>0.176</td>
</tr>
<tr>
<td>(SEM)</td>
<td>(0.2)</td>
<td>(2)</td>
<td>(0.016)</td>
<td></td>
</tr>
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</table>

^SEM = standard error of the mean.

* p < 0.01.
Table 9. Blood results for hepatic uptake of ICG in normal and shunted animals in Group 1

<table>
<thead>
<tr>
<th>Animal #</th>
<th>ln (CBO)</th>
<th>BV (L)</th>
<th>BV/BW (L kg⁻¹)</th>
<th>K_B (min⁻¹)</th>
<th>Cl (L min⁻¹)</th>
<th>AUC_B (mg min L⁻¹)</th>
<th>T_BCO (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>502</td>
<td>2.735</td>
<td>1.74</td>
<td>0.101</td>
<td>-0.0964</td>
<td>-0.168</td>
<td>1121</td>
<td>25</td>
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<tr>
<td>504</td>
<td>2.338</td>
<td>2.86</td>
<td>0.166</td>
<td>-0.0827</td>
<td>-0.236</td>
<td>897</td>
<td>25</td>
</tr>
<tr>
<td>518</td>
<td>2.396</td>
<td>2.41</td>
<td>0.140</td>
<td>-0.0717</td>
<td>-0.173</td>
<td>1313</td>
<td>30</td>
</tr>
<tr>
<td>520</td>
<td>2.790</td>
<td>2.91</td>
<td>0.128</td>
<td>-0.0861</td>
<td>-0.250</td>
<td>1561</td>
<td>30</td>
</tr>
<tr>
<td>523</td>
<td>2.500</td>
<td>3.20</td>
<td>0.168</td>
<td>-0.0723</td>
<td>-0.231</td>
<td>1622</td>
<td>35</td>
</tr>
<tr>
<td>525</td>
<td>2.631</td>
<td>2.25</td>
<td>0.112</td>
<td>-0.1345</td>
<td>-0.302</td>
<td>546</td>
<td>20</td>
</tr>
<tr>
<td>527</td>
<td>3.024</td>
<td>1.69</td>
<td>0.087</td>
<td>-0.0725</td>
<td>-0.123</td>
<td>2774</td>
<td>40</td>
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<td>601</td>
<td>2.821</td>
<td>1.52</td>
<td>0.093</td>
<td>-0.1041</td>
<td>-0.158</td>
<td>1104</td>
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<td>603</td>
<td>2.432</td>
<td>2.68</td>
<td>0.144</td>
<td>-0.0801</td>
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<td>30</td>
</tr>
<tr>
<td>Mean</td>
<td>2.630</td>
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<td>-0.206</td>
<td>1347</td>
<td>29</td>
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<tr>
<td>(SEM)a</td>
<td>(0.077)</td>
<td>(0.20)</td>
<td>(0.010)</td>
<td>(0.0068)</td>
<td>(0.018)</td>
<td>(209)</td>
<td>(2)</td>
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<td>805</td>
<td>2.917</td>
<td>2.12</td>
<td>0.104</td>
<td>-0.0745</td>
<td>-0.158</td>
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<td>810</td>
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<td>2.89</td>
<td>0.155</td>
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<td>2241</td>
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<tr>
<td>Mean</td>
<td>2.736</td>
<td>2.51</td>
<td>0.130</td>
<td>-0.0690*</td>
<td>-0.171</td>
<td>2421**</td>
<td>40**</td>
</tr>
<tr>
<td>(SEM)</td>
<td>(0.181)</td>
<td>(0.38)</td>
<td>(0.026)</td>
<td>(0.0055)</td>
<td>(0.013)</td>
<td>(180)</td>
<td>(0)</td>
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*aSEM = standard error of the mean.

*p < 0.05.

**p < 0.01.
Table 10. Bile results for hepatic excretion of ICG in normal and shunted animals in Group 1

<table>
<thead>
<tr>
<th>Animal #</th>
<th>$Q_{LB}$ (ml min$^{-1}$)</th>
<th>$P_T$ (min)</th>
<th>$P_C$ (mg L$^{-1}$)</th>
<th>$AUC_{LB}$ (mg min L$^{-1}$)</th>
<th>$AUC_{LBM}$ (mg min)</th>
<th>$AUC_{LBU}$ (mg min L$^{-1}$)</th>
</tr>
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<tbody>
<tr>
<td>502</td>
<td>0.185</td>
<td>41</td>
<td>1155</td>
<td>1.07</td>
<td>1092</td>
<td>120242</td>
</tr>
<tr>
<td>504</td>
<td>0.167</td>
<td>40</td>
<td>748</td>
<td>1.30</td>
<td>1092</td>
<td>23739</td>
</tr>
<tr>
<td>518</td>
<td>0.134</td>
<td>33</td>
<td>1680</td>
<td>1.47</td>
<td>941</td>
<td>118651</td>
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<tr>
<td>520</td>
<td>0.111</td>
<td>35</td>
<td>1656</td>
<td>2.58</td>
<td>1443</td>
<td>58993</td>
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<tr>
<td>523</td>
<td>0.148</td>
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<td>893</td>
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<td>17815</td>
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<tr>
<td>525</td>
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<td>64</td>
<td>1941</td>
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<td>95201</td>
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<td>(SEM)$^a$</td>
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<td>(147)</td>
<td>(0.32)</td>
<td>(92)</td>
<td>(13615)</td>
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<td>805</td>
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<td>33</td>
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<td>1490</td>
<td>1.62</td>
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<td>Mean</td>
<td>0.082*</td>
<td>39</td>
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<tr>
<td>(SEM)</td>
<td>(0.031)</td>
<td>(6)</td>
<td>(392)</td>
<td>(0.59)</td>
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<td>(75101)</td>
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$^a$SEM = standard error of the mean.

* p < 0.05.
<table>
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<tr>
<th>AUC_{LBMU} (mg min)</th>
<th>K_{LB} (min⁻¹)</th>
<th>K_{LB} (min⁻¹)</th>
<th>ln (C_{LBO})</th>
<th>T_{LBCO} (min)</th>
<th>ZR (min)</th>
<th>T_F (min)</th>
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<tr>
<td>26.8</td>
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<td>43.9</td>
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<td>(9)</td>
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<td>(0.0029)</td>
<td>(0.025)</td>
<td>(105)</td>
<td>(10.7)</td>
<td>(—)</td>
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Table 11. Blood results for hepatic uptake of ICG in normal and shunted animals in Group 2

<table>
<thead>
<tr>
<th>Animal #</th>
<th>ln(CBG) (L)</th>
<th>BV (L)</th>
<th>BV/BW (L kg⁻¹)</th>
<th>K (min⁻¹)</th>
<th>Cl (L min⁻¹)</th>
<th>AUC₂ (mg min L⁻¹)</th>
<th>TBCO (min)</th>
<th>ER (-)</th>
<th>EHBF (ml min⁻¹)</th>
<th>EHBF/BW (ml min⁻¹ kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>817</td>
<td>2.853</td>
<td>2.39</td>
<td>0.101</td>
<td>-0.0554</td>
<td>-0.132</td>
<td>4243</td>
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<td>819</td>
<td>2.586</td>
<td>2.84</td>
<td>0.128</td>
<td>-0.0944</td>
<td>-0.268</td>
<td>983</td>
<td>25</td>
<td>34.4</td>
<td>13</td>
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<tr>
<td>822</td>
<td>2.610</td>
<td>3.21</td>
<td>0.147</td>
<td>-0.1228</td>
<td>-0.394</td>
<td>606</td>
<td>20</td>
<td>14.9</td>
<td>53</td>
<td>2.4</td>
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<tr>
<td>Mean</td>
<td>2.683</td>
<td>2.81</td>
<td>0.125</td>
<td>-0.0909</td>
<td>-0.265</td>
<td>1944</td>
<td>32</td>
<td>19.2</td>
<td>31</td>
<td>1.4</td>
</tr>
<tr>
<td>(SEM)a</td>
<td>(0.085)</td>
<td>(0.24)</td>
<td>(0.013)</td>
<td>(0.0195)</td>
<td>(0.076)</td>
<td>(1155)</td>
<td>(9.3)</td>
<td>(7.8)</td>
<td>(12)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>831</td>
<td>2.580</td>
<td>2.86</td>
<td>0.143</td>
<td>-0.1328</td>
<td>-0.380</td>
<td>527</td>
<td>20</td>
<td>17.6</td>
<td>41</td>
<td>2.1</td>
</tr>
<tr>
<td>907</td>
<td>2.718</td>
<td>2.68</td>
<td>0.138</td>
<td>-0.1123</td>
<td>-0.301</td>
<td>890</td>
<td>25</td>
<td>51.8</td>
<td>12</td>
<td>0.6</td>
</tr>
<tr>
<td>Mean</td>
<td>2.649</td>
<td>2.77</td>
<td>0.141</td>
<td>-0.1226</td>
<td>-0.341</td>
<td>709</td>
<td>23</td>
<td>34.7</td>
<td>27</td>
<td>1.4</td>
</tr>
<tr>
<td>(SEM)</td>
<td>(0.069)</td>
<td>(0.09)</td>
<td>(0.002)</td>
<td>(0.0103)</td>
<td>(0.040)</td>
<td>(182)</td>
<td>(2.5)</td>
<td>(17.1)</td>
<td>(15)</td>
<td>(0.8)</td>
</tr>
</tbody>
</table>

^SEM = standard error of the mean.
Table 12. Bile results for hepatic excretion of ICG in normal and shunted animals in Group 2

<table>
<thead>
<tr>
<th>Animal #</th>
<th>( Q_{LB} ) (ml min(^{-1} ))</th>
<th>( P_T ) (min)</th>
<th>( P_C ) (mg L(^{-1} ))</th>
<th>( \text{AUCL}_{LB} ) (mg min L(^{-1} \times 10^6 ))</th>
<th>( \text{AUCL}_{BM} ) (mg min)</th>
<th>( \text{AUCL}_{BU} ) (mg min L(^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>817</td>
<td>0.041</td>
<td>56</td>
<td>2325</td>
<td>6.29</td>
<td>1253</td>
<td>304726</td>
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<tr>
<td>819</td>
<td>0.271</td>
<td>44</td>
<td>910</td>
<td>1.29</td>
<td>1518</td>
<td>32031</td>
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<tr>
<td>822</td>
<td>0.177</td>
<td>35</td>
<td>1061</td>
<td>1.59</td>
<td>1324</td>
<td>32325</td>
</tr>
<tr>
<td>Mean</td>
<td>0.163</td>
<td>45</td>
<td>1432</td>
<td>3.06</td>
<td>1365</td>
<td>123027</td>
</tr>
<tr>
<td>(SEM)(^a)</td>
<td>(0.067)</td>
<td>(6)</td>
<td>(449)</td>
<td>(1.62)</td>
<td>(79)</td>
<td>(90849)</td>
</tr>
<tr>
<td>831</td>
<td>0.105</td>
<td>64</td>
<td>1244</td>
<td>2.54</td>
<td>1280</td>
<td>15764</td>
</tr>
<tr>
<td>907</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

\(^a\)SEM = standard error of the mean.
<table>
<thead>
<tr>
<th>AUC_{LBMU} (mg min)</th>
<th>K_{LBU} (min^{-1})</th>
<th>K_{LBD} (min^{-1})</th>
<th>ln (C_{LBO})</th>
<th>T_{LBCO} (min)</th>
<th>%R</th>
<th>T_F (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>55.6</td>
<td>0.0640</td>
<td>-0.0070</td>
<td>8.176</td>
<td>1166</td>
<td>51.8</td>
<td>206</td>
</tr>
<tr>
<td>46.2</td>
<td>0.1443</td>
<td>-0.0047</td>
<td>6.251</td>
<td>1336</td>
<td>92.5</td>
<td>174</td>
</tr>
<tr>
<td>32.5</td>
<td>0.1682</td>
<td>-0.0097</td>
<td>7.098</td>
<td>730</td>
<td>82.2</td>
<td>191</td>
</tr>
<tr>
<td>44.8 (6.7)</td>
<td>0.1255 (0.0315)</td>
<td>-0.0071 (0.0014)</td>
<td>7.125 (0.557)</td>
<td>1077 (180)</td>
<td>75.5</td>
<td>190</td>
</tr>
<tr>
<td>9.8 (12.8)</td>
<td>0.1895 (12.2)</td>
<td>-0.0109 (9)</td>
<td>7.742 (12.2)</td>
<td>709 (9)</td>
<td>59.4</td>
<td>162</td>
</tr>
<tr>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
APPENDIX F: BASIC SOURCE LISTINGS OF DATA ANALYSIS

PROGRAMS FOR PDP-11/23/RT11OS COMPUTER
05 REM PROGRAM <KTEST.BAS> FOR EVALUATION OF BLOOD DATA
10 DIM Y(IO0)
20 PRINT "ENTER EXPERIMENT DATE (MDD) < Q=QUIT >"; \ INPUT A$
30 IF A$="Q" THEN 590
40 PRINT \ A$="DY:BLD"+A$
50 IF A$="DY:BLD" THEN 20
60 OPEN A$ FOR INPUT AS FILE #1
70 BS=SEG$(A$,7,7) \ GS=SEG$(A$,8,9)
80 FOR J=1 TO 100 \ IF END #1 THEN 130 \ INPUT #1,E$
90 X=VAL(TRM$(SEG$(E$,1,4))) \ Y=VAL(TRM$(SEG$(E$,4,LEN(E$))))
100 IF Y<1 THEN Y=1
110 Y(X)=LOG(Y)
120 NEXT J
130 CLOSE #1 \ F=J-1
140 A=0 \ B=0 \ C=0 \ D=0 \ E=0 \ N=0
150 PRINT "ENTER RANGE TO EVALUATE :"; \ INPUT P,Q
160 FOR J=P TO Q
170 A=A+J \ B=B+J2 \ C=C+J*Y(J) \ D=D+Y(J)2 \ E=E+Y(J) \ N=N+1
180 NEXT J
190 L=C-A*E/N \ K=B-A2/N \ M=D-E2/N \ S=L/K \ I=(E-S*A)/N 
200 PRINT "SLOPE=";S ; ; ;"INTERCEPT=";I ; ; ;"CORR. COEFF. =";R
210 OPEN "BDT"+BS+GS+.DAT" FOR OUTPUT AS FILE #3
220 M0=0
230 FOR J=0 TO F
240 PRINT #3,J,Y(J),S*J+I
250 IF S*J+I<=0 THEN 280
260 MO=MO+EXP(I)*EXP(S*J)*J
270 NEXT J
280 CLOSE #3
282 IF S*J+I<=0 THEN 296
284 J=J+1 \ GO TO 282
296 J0=INT(J/5)*5
298 FOR Z=J0 TO J \ M0=M0-EXP(I)*EXP(S*J)*J \ NEXT Z
300 OPEN "KVL"+BS+GS+.DAT" FOR OUTPUT AS FILE #2
310 PRINT #2,"EXPERIMENT DATE: ";
320 PRINT #2,"SLOPE=";S ; ; ;"INTERCEPT=";I ; ; ;"CORR. COEFF. =";R
330 PRINT #2,"ENTER DYE DOSE"; \ INPUT DO \ PRINT #2,"DYE DOSE=";DO
340 PRINT \ PRINT #2
350 PRINT "ENTER HEMATOCRIT"; \ INPUT HO \ PRINT #2,"HEMATOCRIT=";HO
360 PRINT \ PRINT #2
370 BO=DO/EXP(I)/(1-HO/100)
380 PRINT "DETERMINANT RANGE OF TIME FOR REGRESSION =";P;"TO";Q; "MINUTES"
390 PRINT #2,"DETERMINANT RANGE OF TIME FOR REGRESSION =";P;"TO";Q; "MINUTES"
400 PRINT \ PRINT #2
410 PRINT "INTERCEPT=";I,"CORR. COEFF.=";R
420 PRINT #2,"INTERCEPT=";I,"CORR. COEFF.=";R
430 PRINT \ PRINT #2
440 PRINT "K-VALUE=";S,"BLOOD VOLUME=";BO;"LITERS"
450 PRINT #2,"K-VALUE=";S,"BLOOD VOLUME=";BO;"LITERS"
460 PRINT \ PRINT #2
470 PRINT TAB(17)"CLEARANCE=";S*B0;"L/MIN" \ PRINT#2,TAB(17)
   "CLEARANCE=";S*B0;"L/MIN"
480 PRINT \ PRINT #2
490 PRINT "K-VALUE/BODY WEIGHT=";S/D0;" /KG" \ PRINT
500 PRINT "BLOOD VOLUME/BODY WEIGHT=";B0/D0;"L/KG" \ PRINT
510 PRINT #2,"K-VALUE/BODY WEIGHT=";S/D0;"/KG","BLOOD VOLUME/BODY
  WEIGHT=";B0/D0;"L/KG"
520 PRINT #2
530 PRINT "AREA UNDER BLOOD CONCENTRATION CURVE=";M0;"MG-MIN/L" \ PRINT
540 PRINT #2,"AREA UNDER BLOOD CONCENTRATION CURVE=";M0;"MG-MIN/L"
545 PRINT#2
550 PRINT "TIME OF ZERO BLOOD CONCENTRATION=";J0;"MINUTES (NEAREST 5TH)"
560 PRINT #2,"TIME OF ZERO BLOOD CONCENTRATION=";J0;"MINUTES (NEAREST
  5TH)"
570 PRINT \ PRINT #2 \ PRINT \ PRINT #2
580 CLOSE #2 \ GO TO 10
590 END
REM PROGRAM <BILTES.BAS> FOR EVALUATION OF BILE DATA

DIM V(100),C(100),P(100),T(100),L(100),K(100)

X=0 \ Z=0

PRINT "ENTER EXPERIMENT DATE (MDD) < Q=QUIT> ": INPUT A$ \ PRINT
35 PRINT
40 IF A$="Q" THEN 880
50 OPEN "DY:BIL"+A$+.DAT" FOR INPUT AS FILE #2 \ M1=0 \ M0=9999
60 FOR J=1 TO 100 \ IF END #2 THEN 150
70 INPUT #2,C$
80 T=VAL(TRM$(SEG$(C$,1,5))) \ C=VAL(TRM$(SEG$(C$,6,LEN(C$))))
90 IF C<=0 THEN 140
100 T(J)=T \ C(J)=C
110 IF C(J)>M1 THEN M1=C(J) \ F=J
120 IF J<M0 THEN M0=J
130 IF T(J)=100 THEN U0=J
140 NEXT J
150 CLOSE #2
160 IF C(F+1)=M1 THEN F=F+.5
170 Q=J-1
180 OPEN "DY:DPS"+A$+.DAT" FOR INPUT AS FILE #1
190 FOR J=1 TO Q
200 INPUT #1,B$
210 IF VAL(TRM$(SEG$(B$,1,4)))=0 THEN 200
220 IF J<M0 THEN 260
230 V(J)=VAL(TRM$(SEG$(B$,6,LEN(B$))))/1000
240 X=X+V(J)*1000
250 P(J)=V(J)*C(J)
260 NEXT J \ CLOSE #1
270 V=X/(Q*5) \ PRINT "FINAL TIME =";Q*5,"MEAN FLOW =";V \ PRINT
275 IF A$="NOR" THEN 320 \ IF A$="SHN" THEN 320
280 R=1.6/V \ PRINT "BILE CANNULA DELAY TIME =";R;"MINUTES" \ PRINT
290 FOR J=M0 TO Q \ IF T(J)-R<0 THEN M0=M0+1
300 T(J)=T(J)-R
310 NEXT J \ CLOSE #1
320 OPEN "DMS"+A$+.DAT" FOR OUTPUT AS FILE #3
330 OPEN "PAC"+A$+.DAT" FOR OUTPUT AS FILE #4
340 PRINT #3,Z,Z \ PRINT #4,Z,Z
350 Y=0 \ PRINT "ENTER DYE DOSE:"; \ INPUT D \ PRINT \ PRINT
360 PRINT "ENTER CUT-OFF TIME FOR UPSLOPE "; \ INPUT T0 \ PRINT TO OUTPUT #3
370 FOR J=M0 TO Q
380 PRINT #3,T(J),P(J)
390 Y=Y+P(J)/D*100 \ PRINT #4,T(J),Y
400 L(J)=LOG(C(J))
410 NEXT J \ CLOSE #3 \ CLOSE #4
415 FOR J=M0 TO Q \ IF INT(T(J))>T0 THEN 417 \ NEXT J
417 H0=J-1
420 PRINT "PERFORMING REGRESSION FOR UP-SLOPE RANGE",INT(T(M0));"TO"; INT(T(H0));"MINUTES" \ PRINT
430 A0=0 \ B0=0 \ C0=0 \ D0=0 \ E0=0 \ N=0
440 FOR J=M0 TO H0
450 A0=A0+T(J) \ B0=B0+T(J)^2 \ C0=C0+T(J)*L(J) \ D0=D0+L(J)^2 \ E0=E0+L(J)^2
N=N+1
460 NEXT J
470 L=CO-AO*E0/N \ K=B0-A02/N \ M=DO-E02/N
480 S1=L/K \ I1=(E0-S1*A0)/N \ R1=L/SQR(K*M)
490 PRINT "UP-SLOPE=";S1;;;
"INTERCEPT=";I1;;;
"CORR. COEFF. =";R1 \ PRINT
500 A=0 \ B=0 \ Z0=0 \ Z1=0
510 FOR J=M0 TO Q
520 A=A+C(J)*T(J) \ B=B+P(J)*T(J)
530 IF J>H0 THEN 545
540 Z0=Z0+EXP(L(J))*T(J)
543 Z1=Z1+P(J)*T(J)
545 NEXT J
550 GO=(T(H0)+T(H0+1))/5 \ G1=T0-INT(T(H0))
553 G2=(L(H0)+L(H0+1))/5
555 FOR J=1 TO G1 \ Z0=Z0+EXP(G2)*(T(H0)+GO) \ NEXT J
560 U1=INT(T(U0))
570 PRINT "PERFORMING REGRESSION FOR DOWN-SLOPE RANGE";U1;
"TO";INT(T(Q));
"MINUTES"
580 PRINT \ A0=0 \ B0=0 \ CO=0 \ DO=0 \ EO=0 \ N=0
590 FOR J=U0 TO Q
600 AO=AO+T(J) \ B0=B0+T(J)2 \ C0=C0+T(J)*L(J) \ D0=D0+L(J)2 \ E0=E0+L(J)
605 N=N+1
610 NEXT J
620 L=CO-AO*E0/N \ K=B0-A02/N \ M=DO-E02/N
630 S2=L/K \ I2=(E0-S2*A0)/N \ R2=L/SQR(K*M)
640 PRINT "DOWN-SLOPE=";S2;;;
"INTERCEPT=";I2;;;
"CORR. COEFF. =";R2 \ PRINT
650 OPEN "BLG";A$+.DAT" FOR OUTPUT AS FILE #6
660 FOR J=M0 TO Q \ W1=Sl*T(J)+Il \ W2=S2*T(J)+I2
670 IF J>F THEN W1=Sl*T(F)+Il
680 PRINT #6,T(J),L(J),W1,W2
690 NEXT J
700 CLOSE #6
710 OPEN "BIN";A$+.DAT" FOR OUTPUT AS FILE #5
720 PRINT #5,EXPERIMENT DATE : "+SEG$(A$,l,1)+"/"+SEG$(A$,2,3)+"/83"
725 PRINT #5
730 PRINT #5,FINAL EXPERIMENTAL TIME : ";INT(T(Q));"MINUTES" \ PRINT #5
740 PRINT #5,MEAN BILE FLOW : ";V;" ML/MINUTE" \ PRINT #5
750 PRINT #5,BILE CANNULA DELAY TIME : ";R;"MINUTES" \ PRINT #5
760 PRINT #5,PEAK TIME : ";INT(T(F));"MINUTES" \ PRINT #5
770 PRINT #5,PEAK CONCENTRATION : ";M1;"MG/L" \ PRINT #5
780 PRINT #5,AREA UNDER BILE CONCENTRATION CURVE : ";A;"MG-MIN/L" \ PRINT#5
790 PRINT #5,AREA UNDER BILE MASS CURVE : ";B;"MG-MIN" \ PRINT #5 \ PRINT#5
795 PRINT #5,AREA UNDER BILE MASS CURVE TO";INT(T(HO))+G1;"MINUTES ";Z1;
"MG-MIN" \ PRINT #5
800 PRINT #5,AREA UNDER UP-SLOPE CURVE TO";INT(T(HO))+G1;"MINUTES ";Z0;
"MG-MIN/L" \ PRINT #5
810 PRINT #5,UP-SLOPE INTERCEPT CORR. COEFF. FROM TO (MINUTES)
820 PRINT #5,------- -------- ---------------- ---- --"
830 PRINT #5, S1; TAB(11); I1; TAB(27); R1; TAB(41); INT(T(M0)); TAB(49); TO
835 PRINT #5
840 PRINT #5, "DOWN-SLOPE INTERCEPT CORR. COEFF. FROM TO"
850 PRINT #5, "---------- ---------- ------------ ----- --"
860 PRINT #5, S2; TAB(18); I2; TAB(34); R2; TAB(48); U1; TAB(54); INT(T(Q))
870 PRINT #5 \ PRINT #5 \ PRINT #5, CHR$(12) \ CLOSE #5 \ GO TO 10
880 END