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THE USE OF TECHNETIUM-99M DISOFENIN CLEARANCE AS A TEST FOR HEPATIC FUNCTION

The Ohio State University

Ph.D. 1985

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THE USE OF TECHNETIUM-99m DISOFENIN CLEARANCE AS A TEST FOR HEPATIC FUNCTION

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of the Ohio State University

By
James E. Love, Jr., B.S., M.S., M.B.A.

* * * * *

The Ohio State University
1985

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To Sondra, Jaime, and Jonathan
ACKNOWLEDGEMENTS

My deepest appreciation and thanks go to Dr. Peter Fabri for his optimism, Dr. John Lott for his realism, and Dr. Al Staubus for his mechanisms.

I am also indebted to Dr. John Olsen, Dr. Phil Shaffer, and the fine staff of technologists and pharmacists of the Division of Nuclear Medicine, without whom this work would not have been possible.

A special acknowledgment is due my God and Saviour who "hast delivered my soul [and this project] from death" (Psalms 56:13).
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Love, J.E., Computerized interpretive reporting of acid-
base data: a review and comment. J. Med. Tech. 1:113-116


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INTRODUCTION

Measuring the ability of the liver to remove metabolites or foreign substances from the circulation is considered the "truest" test of liver function. The sensitivity and reliability of this type of procedure is difficult to match with any single enzyme or protein marker of liver damage. Unlike other biochemical markers, clearance tests evaluate the functional capacity of the liver and are capable of detecting early phase or subclinical hepatic disease. They can also be used to monitor the progress of healing after damage has occurred.

A number of processes are involved in the removal of molecules from the blood and the subsequent formation of bile. Uptake into the hepatocyte occurs through membrane bound carrier-mediated transport pathways that accommodate four major classes of molecules: organic anions, organic cations, neutral organic compounds, and bile salts. Once in the hepatocyte, substances are bound to cytoplasmic proteins, excreted unchanged, or undergo further metabolic processes prior to excretion into the bile canaliculi. The exquisite sensitivity of clearance tests are based on the fact that any disease or injury that interrupts this
sequence of events can alter one or more of the parameters of clearance.

Because the formation of bile occurs in such a complex system, criteria for the perfect liver function test are many. Thus, a number of exogenous and endogenous substances have been tried. Anion dyes such as sulfobromophthalein (BSP), indocyanine green (ICG) and rose bengal (RB) have received the greatest clinical use. Testing with these dyes usually involves an intravenous injection, followed by a single blood sample at some specified point in time to determine the fraction of dye remaining. A more accurate assessment is obtained by measuring the rate of disappearance from several blood samples at different intervals. In Despite some good results, all of these tests have limitations that hinder their widespread acceptance and day-to-day use. Other substances, such as antipyrine, [14C-] aminopyrine, and galactose have been used but will not be reviewed, because they are used to evaluate primarily specific hepatic metabolic capacities.

The first radioisotopically-labeled agent used to study liver function was iodine-131-rose bengal (RIRB). By combining the known clearance characteristics of this dye with the ability to trace its excretion, both hepatocyte function and patency of the biliary tract could be assessed. By using over the liver or probe scintillation counters, rates of blood clearance and liver uptake and excretion
could be determined.

Extending the usefulness of the traditional rose bengal test opened up a whole new era of investigation in the development and use of hepatobiliary imaging agents; radioactive labeled compounds that permitted the visualization and photography of the liver and associated structures. Additionally, the actual pathway of bile flow could be traced. Despite the vast improvement in diagnoses afforded by RIRB, the need for even better agents has led to the development of other radiolabeled products.

Technetium (Tc)-99m disofenin (DISIDA) is one of the newest of several radioactive labeled iminodiacetic acid derivatives used for liver scans. It is cleared rapidly from the circulation under normal conditions, and visualization of the gallbladder usually occurs within 30 to 45 minutes after injection. Delayed gallbladder visualization, or nonvisualization is commonly associated with cholecystitis or biliary tract obstruction. A few reports have appeared where delayed visualization has occurred in the presence of hepatocellular disease. The significance of these observations have not been fully explored, and no study has focused specifically on the use of disofenin as a test of hepatocyte function.
LITERATURE REVIEW

Organic Anions as Liver Function Tests

Sulfof bromophthalein
Bromsulphalein (phenoltetrabromphthalein disulphonate sodium, BSP, mol. wt. 838 Da) was the first widely accepted exogenous dye used to detect impaired liver function. There are several physio-chemical properties of this dye that are important in its use for hepatic studies. Although a great deal is unknown about the factors which cause a dye to be excreted by the liver and not the kidney, molecular weight plays a role. A molecule must generally have a molecular mass of more than 500 daltons for extensive biliary excretion (Abdel 1971, Millburn 1967). With compounds of 300 to 500 Da, the extent of biliary excretion will vary according to species. BSP is readily soluble in water, relatively stable, and has a absorption maximum at 560 to 585 nm (Leevy 1963, Seligson 1957). Upon intravenous injection, BSP binds to both albumin (Baker 1966a) and alpha(1)-lipoprotein (Baker 1966b). The dye is taken up by liver parenchymal cells through a carrier-mediated mechanism (Hunton 1961), at a rate that can exceed the maximum hepatic excretory rate, Tm. When this occurs, bromsulphalein
accumulates in the liver, and can be measured by a parameter called the hepatic storage capacity, S (Baker 1966b). Within the hepatocyte, BSP is bound to cytoplasmic protein fractions designated as Y and Z proteins (Levi 1969), until it is conjugated with glutathione and excreted into the bile (Combes 1960). Some degree of extrahepatic uptake (Ingelfinger 1948, Leevy 1963), renal excretion (Cohn 1947, Leevy 1963), and enterohepatic circulation can be found (Wheeler 1958).

Rowntree et al. (1913) was the first to suggest the excretion of a phthalein dye as an index of liver function. They observed that phenoltetrachlorphthalein was specifically excreted by the liver after its intravenous administration; elimination was analogous to the way the kidney excreted phenolsulphonephthalein. Limited clinical studies showed a decreased clearance of dye in liver disease. An undesirable feature of the test was that the dye was measured in feces collected over a 48 hour period.

Rosenthal (1922) developed a method for estimating the removal of dyes from the blood; together with White (1924), they determined the superiority of bromsulphalein over other phthalein dyes for investigating hepatic function. Subsequently, they showed that in liver disease, the retention of BSP in the blood 30 minutes after its injection varied from 3 to 99 per cent of the original dose. They further concluded that the percentage of dye present in the
serum at 30 minutes reflected the degree of impaired function, since a 2 mg/kg of body weight injection of dye is normally undetectable in the blood at that time (Rosenthal 1925). The percent of bromsulphalein present was determined by making the serum alkaline and comparing the purple color that developed to a series of bromsulphalein standards in a comparator box.

The 30 minute retention of BSP was more sensitive to liver injury than phenoltetrachlorphthalein, and BSP consistently gave abnormal retention in moderate to advanced liver disease, but tended to be normal in minor grades of liver injury. BSP could not detect early liver disease or show variations from normal in cases where residual liver damage was suspected after the patient had recovered. This demonstrated the tremendous reserve capacity of the liver. An abnormal liver, if given time, could do the same work as a normal one.

Several investigators (Deutsch 1941, MacDonald 1938, Rosenthal 1928) then explored the value of serial percent retention determinations, or what was known as a fractional BSP test. They discovered that when graphs were plotted of percent retention versus time, curves were generated that distinguished normal and abnormal function, even when the thirty minute retention was normal. These early studies were the first designed to consider the rate of dye removal as an important tool in providing additional information on
hepatic function.

The fractional BSP test never received widespread use because a simpler procedure was developed by Mateer et al. (1943). In a careful comparative evaluation of 2 mg vs. 5 mg doses with serial determinations, Mateer et al. found that a 5 mg/kg single 45 minute specimen provided sufficient sensitivity to uncover even slight functional impairment. They recommended that the test be interpreted as normal or abnormal, depending on whether the dye had or had not completely disappeared from the blood in the 45 minute specimen. Although the basic dosing procedure has remained intact, 4 to 5 percent retention at 45 minutes is now considered normal (Mateer 1947, Zieve 1955).

Although the 5 mg/kg dose was more useful in demonstrating mild liver dysfunction, some patients experienced transient reactions to this larger dose. The extremely irritating effects of BSP when injected subcutaneously had been known (Rosenthal 1924). Headaches, feelings of faintness, and chills were now being reported (Mateer 1943). In addition, the 45 minute retention test would be occasionally normal in the presence of known liver disease. False negative results were subsequently investigated and found to result from miscalculations of the initial dye concentration (Mendenhall 1961). When a 5 mg/kg dose was injected, it was assumed that the plasma volume was equal to 50 mL/kg of body weight, and the zero-time concentration of
BSP was 10 mg/dL. The percentage of BSP remaining in the plasma was obtained by dividing the concentration at a given time by the assumed zero-time plasma concentration (10 mg/dL) times 100. In liver disease, the extracellular fluid volume may be expanded, yielding a falsely low percent retention at 45 minutes. Dosing on the basis of lean body mass (Broholt 1967) or surface area (Ingelfinger 1948) would appear to have advantages, but this did not correct for errors in estimating plasma volume. The only recommended way to identify a false-negative BSP test of this type was to evaluate the plasma decay pattern and determine the percentage disappearance rate of the dye which was calculated from the following formula:

\[ R = 1 - d \] (1)

\[ C(2) - \log C(1) \]

\[ \log d = \frac{C(2) - \log C(1)}{t(2) - t(1)} \] (2)

where \( R \) is rate of decay per minute, \( d \) is fraction retained per minute, \( C(2) \) is concentration of bromsulphalein at time \( t(2) \), and \( C(1) \) the concentration at \( t(1) \). Despite the clinical potential shown by the fractional BSP test, introduction of the concept of percentage disappearance rate (PDR) occurred, not as a modification to the standard BSP test, but only to obtain more information concerning the
rate and character of bromsulphalein disappearance. Ingelfinger et al. (1948) found that in most normal subjects, a constant proportion of the dye disappeared per minute following a single injection. Using a 10 minute interval within the first 30 min, their normal range for percentage disappearance rate was between 10 and 16% per minute. In another application of this concept, Lavers et al. (1949), in a study of patients about to undergo elective cholecystectomy, designated the slope in this formula as the "clearance coefficient". They found a significant difference between the clearance coefficients of normal controls and patients with liver disease. The latter had coefficients of less than 0.037, while 90% of controls had values greater than 0.037, with an average of 0.082. Their results indicated that measuring the rate of dye removal was a better index of liver function than studying dye retention. The clearance coefficient could distinguish controls from patients who generally had minimal liver damage owing to chronic cholecystitis. They were also among the first investigators to make the assumption that BSP uptake and excretion were separate, biphasic mechanisms after observing that the semi-logarithmic plot did not always remain linear beyond the 20 minute interval.

Goodman (1952) extended the clearance coefficient of Laver et al. into the more formally defined concept of clearance, i.e. the volume of plasma cleared of a substance
per unit of time. He plotted the logarithm of plasma dye concentration versus time for four samples collected during a 20 minute interval, and then extrapolated the line to the intercept with the ordinate. This provided an estimate of the initial dye concentration. Since the quantity of injected dye was known, the apparent volume of distribution, \( V(d) \), and the extent of dye distribution in the body could be calculated. Goodman felt that it was safe to assume this volume represented plasma, because BSP was plasma-protein bound. By multiplying the clearance coefficient, (a term he called "fractional clearance") by the estimated plasma volume, the clearance of BSP in mL/min could be obtained. In a later clinical study the use of clearance provided a definite separation between controls and those with liver disease (Goodman 1953). The control group had a mean value of 5.33 ± 0.75 mL/min/kg; patients with liver disease had a mean value of 2.06 ± 0.30 mL/min/kg with no values above 3 mL/min/kg being found.

Further investigation into the disappearance rate of BSP from plasma has shown that the semi-logarithmic plot of the decay curve can be separated into two phases when the test was extended beyond 20 minutes. The initial exponential phase was principally owing to hepatic uptake, and the terminal, decelerated phase was attributed to biliary excretion (Leevy 1963, Winkler 1965). This curve can be represented as the following biexponential equation:
where \( C(p) \) is plasma concentration, alpha (\( a \)) and beta (\( b \)) are exponential coefficients (rate constants) for the initial and terminal phases respectively, and the constants A and B are intercepts on the y axis for each exponential segment of the curve.

Hacki et al. (1976), in order to improve the clinical usefulness of the BSP plasma disappearance curve, examined both the alpha and beta rate constants as well as two other clearance parameters, the transport maximum, Tm, and storage capacity, S. They found that the uncorrected alpha disappearance rate had the greatest clinical sensitivity in liver disease. A statistically significant difference was found between the beta phase results in patients and controls, however, the ability of the beta phase to discriminate was appreciably less.

The quantitation of Tm and S requires a prolonged infusion of BSP, and has limited clinical usefulness. This method has been used successfully and is perhaps the procedure of choice for distinguishing between the Dubin-Johnson and Rotor syndromes. In the Dubin-Johnson syndrome, the Tm is close to zero (Dollinger 1967, Shani 1970). In Rotor's syndrome S is markedly reduced (Schiff 1959, Wolpert 1977).

It is important to discuss briefly models, because they provide the basis for all of the kinetic parameters. The

\[
C(p) = Ae^{-at} + Be^{-bt}
\]
body can be represented as a series of compartments. In an attempt to explain the shape of the BSP plasma disappearance curve in terms of what is occurring within and between body compartments, models have been used. These models not only give a visual representation to the rate processes, but they enable equations to be written with sufficient numbers of rate constants to describe the process. Two-compartment models (Quarfordt 1971) representing extrahepatic and hepatic compartments have been widely used. But three-compartment (Barber 1961, Evans 1953, Richards 1959, Winkler 1961), four-compartment (Braver 1963) and six-compartment models (Combes 1965) have been derived. They are useful in gaining a better understanding of how the dye is handled.

Bromosulphalein is believed to be the most sensitive of the available dyes in detecting liver dysfunction (Leevy 1961). A decreased rate of removal can be demonstrated in acute hepatitis (Deutsch 1941, Ingelfinger 1948, Hacki 1976, Quarfordt 1971), even before other tests are abnormal (Neefe et al., 1946). There is also decreased clearance in chronic hepatitis (Mackay 1968), cirrhosis (Deutsch 1941, Goodman 1953, Hacki 1976, Quarfordt 1971, Mateer 1943, Rosenthal 1925), carcinomas (Ingelfinger 1948, Mateer 1943,) and biliary obstructions (Deutsch 1941, Quarfordt 1971). Impaired BSP excretion may also be caused by certain non-hepatic conditions such congestive heart failure
(Evans 1953), fever (Blaschke 1973, Hicks 1948), obesity (Frestin 1967), and a number of drugs (Jablonski 1969). BSP has not proved helpful in discriminating between hepatocellular and obstructive jaundice (Jablonski 1969).

The most important limitation in the use of BSP has been the rare, but severe systemic reactions causing skin eruptions, anaphylactic shock and death (Chambers 1948, Iber 1965, Katz 1964, Walker 1957)

**Indocyanine Green**

Indocyanine green (ICG), is a tricarbocyanine dye that was introduced by Fox et al. (1957), for the measurement of blood flow and the detection of cardiac malformations. It was later discovered that approximately 97% of an administered dose could be recovered in an apparently unaltered form in bile (Wheeler 1958), and that it might be useful in the study of hepatic function.

Like BSP, the molecular weight of ICG of 775 Da. favors biliary excretion. But ICG is less soluble and less stable in aqueous solution than BSP, and ICG solutions must be freshly prepared before use (Paumgartner 1975). The absorption maximum of ICG in plasma is between 800 and 815 nm (Leevy 1963), and there is less interference from hemolysis and bilirubin at these wavelengths than in BSP determinations. ICG is rapidly bound to plasma proteins with a higher affinity for alpha(1)-lipoproteins than BSP (Baker
1966b), a property believed to give ICG a more predictable volume of distribution (Kaplowitz 1982). ICG apparently passes through the hepatocyte and is excreted unchanged into bile. (Cherrick 1960). However, Thiessen (1984), using high-pressure liquid chromatography has uncovered an unknown ICG metabolite in rabbits. ICG is bound to the acceptor proteins Y and Z within the hepatocyte, but unlike BSP, ICG shows negligible extrahepatic uptake (Caesar 1961, Cherrick 1960, Rapaport 1959), renal excretion (Cherrick 1960, Leevy 1959, Rapaport 1959, Wheeler 1958), or enterohepatic circulation (Wheeler 1958). Because clearance is only through the liver, there is a more consistent relationship between plasma concentrations of ICG and hepatic uptake (Caesar 1961), theoretically making ICG a better relative index of liver function because of limited alternate means of removal. ICG is not irritating when introduced subcutaneously (Cherrick 1960) and no significant toxic effects have been observed in humans (Leevy 1967). Toxicity of the dye to rat liver mitochondria has been reported (Laperche 1977) and most recently a report "of the deterioration in the biological state" of rabbits that suggested a cumulative toxicity effect from repeated administration of ICG (Thiessen 1984).

The first studies of human plasma disappearance rates of ICG were reported by Rapaport (1959) and Leevy (1959). Their studies confirmed the exponential removal of ICG that
had been observed in dogs (Wheeler 1958) and established the potential usefulness of ICG in evaluating liver function. The plasma disappearance half-life, as determined from the equation:

\[ T_{1/2} = \frac{0.693}{k} \]  

(4)

and reported by these two investigators (4.1 minutes and less than 5 minutes respectively) agrees well with the 3.4 ± 0.65 (Wiegand 1960) found in normal subjects by others who gave doses that ranged from 0.5 - 1.0 mg/kg. The 20% /min plasma disappearance rate found in normal subjects by Leevy et al. (1959) also agrees well with reports of 18.5% ± 3.1 (Cherrick 1960), 18.6% ± 2.8 (Wiegand 1960), 23.2% ± 6.5 (Reemtsma 1960), and 20.7% ± 5.2 (Caesar 1961). Hunton et al. (1960a) reported an average percentage disappearance rate on normal subjects of 25.9% /min using a dose of 0.25 mg/kg. This value did not compare well with those of other studies. But when examined in light of earlier animal studies; it was concluded that increased doses give reduced removal rates (Hunton 1960b).

Although the value of using parameters such as percentage disappearance rate and half-life, in contrast to percent retention had been demonstrated with BSP, Cherrick et al. (1960) found ICG removal to be so rapid that the percentage retained at 20 minutes was selected for patient
studies. The mean value in the normal group was 3.85% ± 1.0 which compared favorably with the 3.1% ± 1.2 determined by Caesar (1961). Even today, a percent retention of less than 4% at 20 minutes, a percentage disappearance rate of less than 16%/min, or a half-life of less than 5 minutes are still considered normal by some authors for a 0.5 mg/kg dose (Leevy 1961, Kaplowitz 1982). A recent study cited significant differences in the percentage disappearance rates between normal men and women. When given a 0.5 mg/kg dose, a 20.5% ± 0.005 rate is observed for men and a 24.3% ± 0.006 rate is observed for women. (Martin 1975).

The clearance of ICG has been found comparable to that of BSP in distinguishing normal subjects from patients who have a significant degree of liver disease (Cherrick 1960, Leevy 1963). A major disadvantage of ICG was that at doses conventionally used (ca. 0.5 mg/kg) it was less sensitive than BSP in detecting mild liver dysfunction (Leevy 1963). Leevy et al. (1967) found that this limitation could be eliminated with a sufficiently high dose of the dye. In a two-step clearance study, where each patient was given first a low dose and then a high dose of ICG, Leevy found that patients with mild liver disease (e.g., fatty liver, mild hepatitis, early-phase cholestasis) had normal fractional disappearance rates at the 0.5 mg/kg dose and abnormal fractional disappearance rates at the 5.0 mg/kg dose. Patients with severe liver disease had an abnormal
removal of both doses. This study also supported an ear­lier finding of an inverse relationship between dose and removal rate (Hunton 1960b, 1961).

For reasons that are not fully understood, there are several clinical situations where BSP and ICG results may differ. An abnormal retention of BSP has been seen in newborns (Obrinsky 1952, Yudkin 1949) and in febrile patients (Blaschke 1973, Hicks 1948). In contrast, ICG retention is normal in newborns (Leevy 1963) and its removal is unaffected by fever (Hunton 1960a). The administration of norethandrolone, a synthetic androgenic steroid, or glucagon reduces BSP removal but not ICG (Leevy 1963). In patients with an established chronic liver disease, BSP retention at 45 minutes may be normal but ICG at 20 minutes may be abnormal (Mendanhall 1961).

In the development of the BSP test, the rate of clearance parameters were believed to provide better and more sensitive indices of liver function than the percent retention. With ICG, the question has been raised as to which measure of clearance gave the most diagnostic information. Wiegand et al. (1961) preferred half-life, because it clearly separated normal subjects from those with hepatic disease, and it was easy to calculate. They simply plotted the absorbance readings of several samples on semi-logarithmic paper against time and determined the half-life of the decay from the slope. On the basis of extensive
kinetic studies of ICG (Martin 1975, Paumgartner 1970), Brody and Leichter (1979) recommended determining the maximum removal rate, \( V_{\text{max}} \), as a more accurate index to quantify liver function. This procedure requires serial doses, and does not appear to be practical for routine use. Martin et al. (1975) found the calculation of clearance to be subject to considerable statistical uncertainty owing to incomplete mixing of the dye in the plasma space, and extrapolation errors in the calculation of volume of distribution upon which the clearance calculations were based. They preferred the initial disappearance rate constant. A more cogent argument in favor of using clearance is presented by Gilmore et al. (1982). They reasoned that while errors owing to changes in the volume of distribution alter the slope and half-life, the area under the curve remains unchanged. Clearance was therefore the better parameter.

Spectrophotometry has been the most widely used analytical method for the determination of ICG concentration. Leevy et al. (1967), however, introduced and evaluated bichromatic ear densitometry as an alternative approach in liver studies. Because of the high molar absorptivity of ICG at physiological pH, external recordings of the approximate blood concentrations could be obtained. Their results showed good correlation between the ear densitometer and spectrophotometric methods. Even though it does not appear to be a popular alternative, its use can be found
occasionally. (Pollack et al. 1979). Recent studies have shown that ICG results from spectrophotometry and HPLC can be notably different in rabbits, and that spectrophotometric methods give ambiguous results when doses greater than 2.5 mg/kg are used. Rapaport (1982) and Thiessen (1984) believe that ICG is metabolized and the higher results they obtained by spectrophotometer are owing to a dose dependent formation of an ICG metabolite.

**Rose Bengal**

Rose bengal (Tetraiodotetrachlorfluorescein, RB) like BSP is an anionic phthalein dye with a molecular weight of 1,050 Daltons. It is deep pink in color with a maximum absorbance at 530 nm in aqueous solution that shifts to 550 nm in plasma (Cohen 1953). Its uptake and excretion by the liver is not as rapid as BSP (Rosenthal 1924), but it appears to be excreted unchanged (Kubin 1960). Like ICG, RB undergoes negligible extrahepatic removal (Sapirstein 1955), but unlike ICG, the disappearance rate of RB from plasma is independent of plasma concentration when doses below 60 to 120 mg are used (Sapirstein 1955). No evidence of toxicity has been reported, but RB is photodynamically active, and in the presence of direct sunlight the dye will hemolyze red blood cells (Kerr 1925).

The discovery of RB as a potential dye for testing hepatic function was the result of a deliberate examination
of over 300 dyes for their specificity for liver excretion (Delprat 1931). Following preliminary tests in dogs, Delprat et al. injected 100 mg of into subjects with liver disease, without liver disease, and with chronic cholecystitis. Through a complex mathematical calculation, they determined the percent of dye in the plasma at four and eight minutes after injection. The concentration of dye at four and eight minutes post injection was considerably higher in the group with liver disease than in the group without liver disease. Results from the patients with chronic cholecystitis were borderline (Delprat 1923, 1924).

Further studies by Kerr et al. (1925) confirmed Delprat's results and cited the specificity of RB for elimination by the liver as a definite advantage over BSP. BSP can be found in the urine in amounts up to 20% when significant dye retention occurs in the blood. Epstein et al. (1927) introduced modifications to the test that included the elimination of the four minute sample and a prolongation of the test to 16 minutes. They also simplified the calculation and established normal ranges as 42 to 52% retention at eight minutes, and 23 to 26% retention at 16 minutes. Within four years after the initial human study over 500 patients had been tested, and RB had become the most clinically applied dye removal test of liver function at that time. As a result of this vast experience it was found that only trace amounts of dye were present in the
plasma of healthy individuals at 16 minutes, and they rarely presented with more than 25% of the injected amount at 16 minutes. A delay in dye elimination was always present in obstructive jaundice, infectious hepatitis, and cirrhosis. The test was of particular value in the differential diagnosis when ascites was present. In cirrhosis, elimination of the dye was delayed, but in liver unassociated ascites, elimination of RB was usually normal. In chronic cholecystitis and chronic passive congestion of the liver, results were usually within normal limits.

Certain problems with the RB test were difficult to surmount. Because of the hemolytic action of RB samples had to be protected from sunlight until the cells were removed. Rose bengal also had a photosensitizing effect on tissue and patients were advised to keep out of direct sunlight for a few hours after injection.

With continued experience, another modification was introduced that expressed percentage of normal liver function to give a better quantitative correlation between the results of the test and the extent of liver cell damage (Delprat 1931, Stowe 1933).

Despite improvements in the RB test, it was Soffer's (1935) opinion that results obtained from this test were essentially similar to those of other dye methods. It was most effective when liver damage was extensive and diffuse and not as effective when liver damage was
comparatively slight.

The rose bengal test as introduced by Delprat was all but abandoned with the introduction of radioactive I-131 tagged rose bengal by Tapin (1955a). This ushered in a whole new era in the study of hepatic diseases.

**Radiolabeled Agents as Liver Function Tests**

**Iodinated Rose Bengal**

The substitution of the stable iodine atoms in rose bengal with radioactive gamma emitting iodine-131, gave Taplin et al. (1955a) a dynamic record of the liver's capacity to remove and excrete the dye. Following an intravenous injection, they could monitor the rate of radioactive dye uptake and elimination into the bile with scintillation counting equipment placed over the liver. In their initial experiments, they found that normal rabbits had a rapid and high uptake followed by a rapid excretion. In rabbits with hepatocellular damage induced by carbon tetrachloride, the uptake was not as rapid or high, and the excretion was much slower. Rabbits with liver damage were also tested with BSP, and the radioactive rose bengal (RIRB) curves were abnormal before BSP indicated functional impairment. In preliminary clinical studies, patients with various liver diseases had prolonged uptake slopes and sometimes delayed excretion. The researchers reported a
number of advantages over nonradioactive dye tests, which included its safety in the presence of obstruction, greater sensitivity, and its potential for distinguishing hepatocellular dysfunction from obstructive biliary disease (Taplin 1955b).

Taplin's method of interpreting RIRB curves was based on the visual comparison of each curve with a group of normal curves, and was therefore semiquantitative at best. Lowenstein (1956) introduced a quantitative analysis that is similar to the present day method for curve-fitting a biexponential decay function. He plotted the counts per minute of liver activity on semi-logarithmic graph paper, and drew a line along the descending portion of the curve to the ordinate. This line represented the entire biliary excretion component. Points for the uptake curve were obtained by subtracting values on the rising portion of the curve from corresponding values along the excretion curve, and then plotting these new points. A line drawn through these points represented the uptake component. From these two lines, an uptake half-time and excretion half-time could be derived. After analyzing Taplin's published curves along with data obtained from his own clinic, Lowenstein concluded that hepatocellular damage was manifested by delayed uptake and excretion, whereas obstructive processes primarily caused delayed excretion.

Following the presentation of a quantitative method
for interpreting uptake-excretion curves, many investigators studied the clinical application of RIRB. Results from studies by Snell (1956), Wood (1951), Blahd (1957) and Nordyke (1959) supported many of Taplin's findings. Nordyke and Blahd's approach was somewhat novel in that they measured the rate of disappearance of RIRB from blood by using a scintillation probe placed against the right lateral part of the head centered at the ear. By their account, this procedure provided smoother, more reproducible disappearance curves than those obtained by external liver counting. Factors such as body and organ movement made the reproducibility of external liver counting difficult. The rate of dye disappearance was quantitated by expressing the count rate at 20 minutes as a percentage of the count rate at five minutes. In their initial results they found that RIRB and BSP tests were equivalent in detecting borderline and moderate liver dysfunction without jaundice. In the differential diagnosis of jaundice, the RIRB had definite but limited usefulness. By adding an injection of cholecystokinin and an abdominal scintillation probe to the test protocol, Nordyke determined that the most important use of RIRB was its ability to rule out surgical jaundice by indicating biliary tract patency in the presence of jaundice (1965). Two more recent investigators, using a gamma scintillation camera with a digital image processing system capable of quantifying activity
in selected liver regions of interest, have found RIRB useful as a function test. Both Gamlen et al. (1975) and Verow and Wisbey (1975) reported delayed hepatic uptake in the presence of hepatocellular disease. They disagreed, however, on the ability of the test to distinguish intra- and extrahepatic jaundice.

Not all reports on RIRB as a function test have been favorable. Brown and Glasser (1956) found that the uptake curves in patients with cirrhosis, obstructive jaundice and other liver diseases were too similar to be differentially diagnostic, and that the inability of the test to differentiate hepatic from obstructive jaundice lessened its value. Cohn et al. (1957), and Moertel et al. (1958) were equally unimpressed with results from their investigations. Garcia et al. (1959) found that the test was not as good as BSP in liver disease without jaundice, and Davies et al. (1976) reported that blood clearance of RIRB was not helpful in discriminating hepatic from obstructive jaundice.

Radiolabeled rose bengal has facilitated scintiphography as a method of determining liver size, shape, and the presence of foreign masses (Fee 1960). This radiopharmaceutical has also been particularly useful in the difficult diagnosis of neonatal jaundice (Sharp 1967, Silverberg 1973). But inconsistent results in the use of RIRB, and the relatively high radiation dose for I-131, have limited its continued use as a radiopharmaceutical.
99m-Technetium Iminodiacetic Acid Derivatives

The Tc-99m labeled N-acetanilido-iminodiacetic acids are a family of hepatobiliary imaging agents in which a chelating group (iminodiacetic acid, IDA) capable of binding a gamma-emitting radiometal, are attached to an analog of lidocaine. They are believed to exist as dimers with two molecules of IDA forming a stable complex with one molecule of Tc-99m (Fonda 1978, Fritzberg 1980, Loberg 1976, 1977). Monomer and perhaps trimer complexes may also be present (Cox 1979, 1981a). Derivatives are formed by substituting different side chains on the benzene ring (Figure 1, Table 1). Although the precise structure of IDA derivatives are unknown, Figure 2 represents the most likely structure of a typical IDA-derivative.

Following intravenous injection, IDA-derivatives bind to plasma albumin (Popescu 1979), and are rapidly taken up by the hepatocytes. Rates of uptake may vary according to the lipophilicity of the molecule (Subramanian 1977), molecular weight (Cox 1979) or charge (Burns 1977). As organic anions, they share a common excretion pathway with bilirubin, an observation based on impaired biliary visualization in jaundiced patients (Rosenthal 1978, Ryan 1977) and competitive clearance studies performed with BSP (Harvey 1979). However, Janshoit (1979) observed minimal competition between bilirubin and IDA-derivatives for plasma protein binding sites and no decrease in liver uptake in
Figure 1. N. acetanilido-iminodiacetic acid

Table 1

IDA Substituents Most Commonly Used As Hepatobiliary Imaging Agents

<table>
<thead>
<tr>
<th>Derivative</th>
<th>( R_1 )</th>
<th>( R_2 )</th>
<th>( R_3 )</th>
<th>Mol. Wt.</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl-IDA</td>
<td>( \text{CH}_3 )</td>
<td>H</td>
<td>( \text{CH}_3 )</td>
<td>687.6</td>
<td>HIDA</td>
</tr>
<tr>
<td>Trimethyl-IDA</td>
<td>( \text{CH}_3 )</td>
<td>( \text{CH}_3 )</td>
<td>( \text{CH}_3 )</td>
<td>715.7</td>
<td>HIDA</td>
</tr>
<tr>
<td>Diethyl-IDA</td>
<td>( \text{C}_2\text{H}_5 )</td>
<td>H</td>
<td>( \text{C}_2\text{H}_5 )</td>
<td>763.7</td>
<td>DE-IDA</td>
</tr>
<tr>
<td>( p )-Isopropyl-IDA</td>
<td>H</td>
<td>( \text{C}_3\text{H}_7 )</td>
<td>H</td>
<td>715.7</td>
<td>PIPIDA</td>
</tr>
<tr>
<td>Diisopropyl-IDA</td>
<td>( \text{C}_3\text{H}_7 )</td>
<td>H</td>
<td>( \text{C}_3\text{H}_7 )</td>
<td>799.8</td>
<td>DISIDA</td>
</tr>
<tr>
<td>( p-n )-Butyl-IDA</td>
<td>( \text{C}_4\text{H}_9 )</td>
<td>H</td>
<td>H</td>
<td>763.7</td>
<td>BIDA</td>
</tr>
</tbody>
</table>
Figure 2. The Most Likely Structural Formula of a Typical Iminodiacetic Acid (Diethy-IDA).
induced hyperbilirubinemia. It may be that other mechanisms are responsible for the delayed uptake and excretion found in hepatocellular and obstructive jaundice. IDA-derivatives are excreted unconjugated (Loberg 1976, Wistow 1977), but there is evidence that they bind to bile salts (Cox 1981b) and may undergo some form of hydrolysis, oxidation or reduction (Popescu 1979). Differences among the derivatives are also found in their rates of secretion into bile (Fueger 1981). Renal clearance, which varies, is usually greater with the less lipophilic molecules (Subramanian 1977); increased radioactivity in urine has been associated with decreased blood clearance for certain derivatives (Cox 1981c). Intestinal reabsorption has not been reported.

The success of technetium-IDA derivatives over RIRB has been due to their clear superiority as hepatobiliary imaging agents. The physical properties of Tc-99m result in a better quality of image, and IDA-derivatives show a consistently higher concentration in bile than RIRB (Wistow 1978). Despite the popularity of IDA-derivatives and frequent references to their possible use for assessing liver parenchymal cell function, few studies have been reported.

Diethyl-IDA, one of the first derivatives widely used for imaging, was examined in dogs with galactosamine-induced parenchymal damage. Delays in time of maximum liver accumulation of diethyl-IDA (Tmax) and excretion
half-time were found (Biersack 1981). Erjavec et al. (1979) conducted a similar experiment in dogs using carbon tetrachloride to induce liver damage. They observed a more rapid decrease in IDA-accumulation and excretion than that found by Biersack. Popescu et al. (1979) also used carbon tetrachloride to create liver cell damage in rabbits. In addition, they examined the elimination kinetics of several agents including dimethyl-, diethyl-, p-isopropyl-, trimethyl-, diisopropyl-, and p-butyl-IDA. Following a dose of carbon tetrachloride, the biliary elimination of all IDA-derivatives were decreased, with trimethyl-IDA showing the largest change. Cox et al. (1981c) used diethyl-IDA to study 38 patients with jaundice owing to various parenchymal diseases. Using computer generated Tmax, T1/2 and functional images, they concluded that the diagnosis of parenchymal disease could be determined most accurately: 1) when the typical patterns of time-activity curves of obstructed patients were absent; 2) when both time activity curves and functional images were disturbed; 3) in patients with normal time-activity curves and disturbed functional images. Neither time-activity curves or functional imaging alone provided consistently accurate information. Dietrich et al. (1979) examined the biological behavior of trimethyl-, diethyl-, and p-butyl-IDA in five healthy persons and five patients with hepatobiliary diseases. They determined that p-butyl-IDA was the most
useful derivative for hepatic clearance studies, primarily because of its low, relatively constant urinary excretion, even in the presence of bilirubinemia. Other results have been less favorable. Taavitsainen et al. (1980) studied the mean body disappearance rate and liver mean transit times, a measure of liver excretory function, with diethyl-IDA in 98 patients. They found considerable overlap in body disappearance rates between normal subjects and patients with liver disease. They also found poor correlation between the mean transit time and more routine liver function tests such as serum bilirubin and AST.

Tc-99m diisopropyl-IDA, (N-[2,6-diisopropylphenylcarbamoylmethyl] iminodiacetic acid), commercially known as disofenin, (tradename, Hepatolite), is in current regular use as an hepatobiliary imaging agent in the Division of Nuclear Medicine, The Ohio State University Hospital. Disofenin is present in the highest concentration in bile during the first 60 minutes post-injection as compared to other IDA-derivatives (Winstow 1978). It has also been found superior in its ability to visualize the biliary system in the presence of abnormal serum bilirubin concentrations (Green 1982, Hernandez 1980, Weissmann 1981). Studies with baboons (Wistow 1978) and humans (Green 1982, Stadelnik 1981, Wistow 1978) have shown that disofenin is cleared rapidly from the circulation under normal conditions with about 8 to 10% of the injected activity
remaining in the blood 30 minutes post-injection. It has a low urinary excretion rate (10% of dose in urine at 2 hours), and is almost exclusively cleared by the liver. Visualization of the gallbladder usually occurs within 30-45 minutes after injection. Delayed gallbladder visualization or nonvisualization is commonly associated with acute cholecystitis or biliary tract obstruction. A finding incidental with delayed or nonvisualization has been the presence of hepatocellular disease. In a study of 20 patients with primary hepatocellular disease, Green (1982) found that all but two had decreased hepatic uptake and/or abnormal hepatic retention. Shaffer (1982) has observed poor uptake of disofenin with disturbed hepatocellular function, and Stadalnik (1981) has reported that the time of gastrointestinal uptake varied with the severity of liver disease. No study, however, has reported the specific quantitative use of disofenin in humans as a test of hepatocyte function.
OBJECTIVES OF STUDY

The purpose of this study was to examine the clearance properties of disofenin and to determine if it could be used to assess liver function. Studies were undertaken to describe the pharmacokinetics of disofenin; to investigate its ability to distinguish disease from non-disease states; and to determine the association between the clearance of disofenin and enzyme markers of hepatic dysfunction. Studies were also done on a selected group of patients receiving total parenteral nutrition to examine for the presence of cholestasis.
MATERIALS AND METHODS

Equipment

A Compac 120 gamma counting system (Picker, Northford, CT 06472) was used to count the radioactivity in blood samples. The imaging device used was a Dyna Camera 4/15 scintillation camera (Picker, Northford, CT 06472) fitted with an ultra-fine, parallel-hole collimator. The X and Y positional output signals from the camera were fed into an A2 Dual Station computer imaging system (Medical Data Systems, Ann Arbor, MI 48105) where the signals were converted to a digital form, and the resulting image was displayed as a 64 x 64 matrix. Serial images were made and stored on tape at the rate of 2 frames per minute for 90 minutes. The system has region-of-interest capabilities allowing the user to select a particular area of the target organ for study and data analysis. All biochemical determinations were done on the ASTRA 8 Analyzer (Beckman Instruments, Brea CA 92621).

Preparation of Radiopharmaceutical

Tc-99m disofenin was prepared by the Nuclear Pharmacy from a Technetium Tc-99m Disofenin Kit (Hepatolite, New England Nuclear, North Billerica, MA 01862) according to
the manufacturer's instructions. Sodium pertechnetate, obtained from a Technetium Tc-99m Generator (Cintichem, Inc., Tuxedo, NY 10987) was added to a vial containing disofenin and stannous chloride. Tin reduces technetium from a +7 to a +3 oxidation state where it then forms a technetium-disofenin complex.

Patients

This study was approved by the Human Subjects Review Committee of The Ohio State University, and informed consent was obtained from each subject after the nature of the procedure was fully explained. A total of 30 patients were studied; 16 men, ages 26 to 88 and 14 women, ages 23 to 67. The control group consisted of six apparently healthy volunteers and three patients who were referred to the Nuclear Medicine Division but who presented no evidence of cholecystitis or liver dysfunction after evaluation of clinical, laboratory, and hepatobiliary scanning results. One of the apparently healthy volunteers had a bilirubin transport deficiency (possibly Gilbert's syndrome), but no other disturbance of liver function. The test group consisted of 20 patients with various primary and secondary liver dysfunctions. The final diagnosis was determined by laparotomy, biopsy, clinical, laboratory, or radiological review. A second test group consisted of four patients currently receiving total parenteral
nutrition (TPN) who had suspected cholestasis as indicated by an increase in alkaline phosphatase or gamma-glutamyl transpeptidase activity of at least 100 U/L over a previous determination. TPN patients with a history or evidence of significant overt hepatic disease (total bilirubin greater than 2.0 mg/dL) or renal disease (BUN greater than 30 mg/dL or creatinine greater than 1.5 mg/dL) were considered on a case by case basis. Table 2 summarizes the clinical and laboratory data for all subjects.

**Procedures**

All subjects fasted for at least two hours before the test. The standard 6.1 to 6.5 mCi dose of disofenin was administered by rapid intravenous injection into one arm with subjects in a supine position under the scintillation camera. Using a three-way stopcock, venous blood samples were drawn from the other arm through an indwelling-needle and heparinized saline line. A background count sample and timed samples were collected into 2 mL, 10 x 50 mm EDTA tubes at 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 70, 80, and 90 minutes after the dose of disofenin was given. To avoid saline dilution approximately 1 mL of blood was collected and discarded just prior to each timed sample, then 1 mL of blood was transferred to a 16 x 100 mm test tube and the radioactivity was measured. Simultaneous external scintillation counting directly over the liver
## Table 2
Clinical and Laboratory Data for All Subjects

<table>
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<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>TBIL (mg/dL)</th>
<th>ALP (U/L)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>LD (U/L)</th>
</tr>
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<td>Controls</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>1 AS</td>
<td>26</td>
<td>M</td>
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<td>0.4</td>
<td>92</td>
<td>31</td>
<td>13</td>
<td>94</td>
</tr>
<tr>
<td>2 IF</td>
<td>37</td>
<td>M</td>
<td>Normal</td>
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<td>64</td>
<td>37</td>
<td>38</td>
<td>152</td>
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<tr>
<td>3 JO</td>
<td>42</td>
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<td>70</td>
<td>23</td>
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<td>138</td>
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<tr>
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<td>17</td>
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<td>165</td>
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<td>F</td>
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<td>M</td>
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<tr>
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<td>M</td>
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<tr>
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<td>16 BS</td>
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<td>Hepatitis</td>
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<td>167</td>
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<td>17 HH</td>
<td>46</td>
<td>M</td>
<td>Intestinal infarct</td>
<td>Myeloid metaplasia</td>
<td>14.0</td>
<td>233</td>
<td>114</td>
<td>32</td>
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Table 2 (continued)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>TBIL (mg/dL)</th>
<th>ALP (U/L)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>LD (U/L)</th>
</tr>
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<tbody>
<tr>
<td>Liver/Biliary Disease</td>
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<tr>
<td>18 WK</td>
<td>60</td>
<td>M</td>
<td>Chronic myelogenous leukemia</td>
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<td></td>
<td></td>
<td></td>
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<tr>
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<td>3.1</td>
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<td>652</td>
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<td></td>
</tr>
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<td>19 AW</td>
<td>32</td>
<td>F</td>
<td>Cholelithiasis</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Choledocholithiasis</td>
<td>1.4</td>
<td>393</td>
<td>437</td>
<td>438</td>
<td>-</td>
</tr>
<tr>
<td>20 DG</td>
<td>44</td>
<td>M</td>
<td>Chronic lymphocytic leukemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.2</td>
<td>467</td>
<td>221</td>
<td>217</td>
<td>478</td>
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<tr>
<td>21 HC</td>
<td>67</td>
<td>F</td>
<td>Duodenal diverticulum</td>
<td>0.6</td>
<td>328</td>
<td>36</td>
<td>47</td>
<td>-</td>
</tr>
<tr>
<td>22 JC</td>
<td>58</td>
<td>F</td>
<td>Ovarian cancer</td>
<td>1.2</td>
<td>138</td>
<td>50</td>
<td>28</td>
<td>224</td>
</tr>
<tr>
<td>23 JN</td>
<td>33</td>
<td>M</td>
<td>Chronic choledocystitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.4</td>
<td>108</td>
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<td>-</td>
</tr>
<tr>
<td>24 RA</td>
<td>23</td>
<td>F</td>
<td>Afferent loop syndrome</td>
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<td>120</td>
<td>38</td>
<td>28</td>
<td>154</td>
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<tr>
<td>25 VH</td>
<td>33</td>
<td>F</td>
<td>Chronic pancreatitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Irritable bowel syndrome</td>
<td>0.4</td>
<td>194</td>
<td>38</td>
<td>17</td>
<td>184</td>
</tr>
<tr>
<td>26 LP</td>
<td>38</td>
<td>F</td>
<td>Chronic cholangitis</td>
<td>0.4</td>
<td>217</td>
<td>70</td>
<td>36</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2 (continued)

Clinical and Laboratory Data for All Subjects

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>TBIL (mg/dL)</th>
<th>ALP (U/L)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>GGT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Parenteral Nutrition (TPN)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27 DB</td>
<td>36</td>
<td>M</td>
<td>Crohn's disease</td>
<td>0.4</td>
<td>50</td>
<td>28</td>
<td>24</td>
<td>101</td>
</tr>
<tr>
<td>28 KS</td>
<td>32</td>
<td>F</td>
<td>Crohn's disease</td>
<td>0.6</td>
<td>308</td>
<td>--</td>
<td>209</td>
<td>---</td>
</tr>
<tr>
<td>29 MA</td>
<td>38</td>
<td>F</td>
<td>Cervical cancer</td>
<td>2.6</td>
<td>417</td>
<td>91</td>
<td>81</td>
<td>667</td>
</tr>
<tr>
<td>30 SR</td>
<td>42</td>
<td>F</td>
<td>Crohn's disease</td>
<td>0.3</td>
<td>294</td>
<td>40</td>
<td>56</td>
<td>255</td>
</tr>
</tbody>
</table>

Reference Ranges: TBIL, up to 1.5 mg/dL; ALP, up to 105 U/L (age & sex dependent); AST, up to 60 U/L; ALT, up to 40 U/L; LD, up to 220 U/L; GGT, 5-85 U/L.
occurred during the 90 minutes to gather data on liver uptake and excretion. An additional 10 mL blood sample was drawn from all control subjects for the following liver and renal function test: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase, (LD), total bilirubin (TBIL), urea nitrogen and creatinine. Some of the laboratory test results on test or control patients were obtained from their medical records. The same analytical methods and quality control had been used for these patients.

Data Analysis

The logarithm of the disofenin counts per minutes (cpm) were plotted against time for plasma disappearance and liver uptake and excretion. Liver counts / 30 sec frame were acquired from the right lobe. The plasma data were analyzed on an IBM 4331 with NONLIN 74, a package of computer programs for pharmacokinetic modeling. These programs which were developed by Metzler et al. (1974), allows the user to write subroutines that calculate pharmacokinetic parameters based on the model suggested by the experimental data. Tables 3 and 4 are two of several output pages from the subroutine in Appendix A. The elimination of disofenin from the plasma followed a biexponential decay as shown in Figure 3. The line in this figure represents the computer
Table 3
Sample Page of NONLIN Output Showing
Observed and Calculated Data

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>(CONTROL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FUNCTION 1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>X</th>
<th>OBS. Y</th>
<th>CALC. Y</th>
<th>OBS-CALC</th>
<th>% DEVIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0000</td>
<td>0.2106E+06</td>
<td>0.2134E+06</td>
<td>-2834.4</td>
<td>-1.35</td>
</tr>
<tr>
<td>4.0000</td>
<td>0.1863E+06</td>
<td>0.1801E+06</td>
<td>6117.4</td>
<td>3.28</td>
</tr>
<tr>
<td>6.0000</td>
<td>0.1567E+06</td>
<td>0.1555E+06</td>
<td>1246.6</td>
<td>0.80</td>
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<tr>
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<td>0.1351E+06</td>
<td>0.1371E+06</td>
<td>-2028.2</td>
<td>-1.50</td>
</tr>
<tr>
<td>10.0000</td>
<td>0.1188E+06</td>
<td>0.1234E+06</td>
<td>-4594.4</td>
<td>-3.87</td>
</tr>
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<td>30.0000</td>
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</tr>
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<td>72018.</td>
<td>73047.</td>
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<td>-1.43</td>
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<td>50.0000</td>
<td>67614.</td>
<td>68015.</td>
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<td>-0.59</td>
</tr>
<tr>
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<td>63495.</td>
<td>-1461.8</td>
<td>-2.36</td>
</tr>
<tr>
<td>70.0000</td>
<td>59713.</td>
<td>59310.</td>
<td>403.33</td>
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<tr>
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</tr>
<tr>
<td>90.0000</td>
<td>51964.</td>
<td>51764.</td>
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<td>0.38</td>
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</tbody>
</table>

CORRECTED SUM OF SQUARED OBSERVATIONS = 0.3389481E+11
SUM OF SQUARED DEVIATIONS = 0.8690708E+08
SUM OF WEIGHTED SQUARE DEVIATIONS = 0.3215384E+08
R-SQUARED = 0.999  COR= 0.999
S = 1793.1491004 WITH 10 D.F.
Table 4

Sample Page of NONLIN Output Showing
Calculated Clearance Parameters

PATIENT (CONTROL)

AFTER 5 ITERATIONS THE ESTIMATES AND THEIR VARIABILITY ARE:

<table>
<thead>
<tr>
<th>NO.</th>
<th>ESTIMATE</th>
<th>STD. DEV.</th>
<th>95% CONFIDENCE LIMITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>162954.</td>
<td>7004.27</td>
<td>147348. - 178561.</td>
</tr>
<tr>
<td>2</td>
<td>0.156120</td>
<td>0.104065E-01</td>
<td>0.132933 - 0.179307</td>
</tr>
<tr>
<td>3</td>
<td>95471.5</td>
<td>2735.06</td>
<td>89377.3 - 101566.</td>
</tr>
<tr>
<td>4</td>
<td>0.680149E-02</td>
<td>0.447021E-03</td>
<td>0.580546E-02 - 0.779752E-02</td>
</tr>
</tbody>
</table>

K1 = 0.156122
K2 = 0.00680
A = 162954.14
B = 95471.49
HALF-LIFE OF DRUG = 4.440 MIN
HALF-LIFE OF SECOND COMPONENT = 101.911 MIN

APPARENT TOTAL BODY CLEARANCE = 793.734 ML/MIN
APPARENT CENTRAL COMPARTMENT VOLUME = 46.319 L
Figure 3. Plasma Time-Activity Decay Curve of Disofenin in a Control Subject.
fit to the data. Data from scintillation counts over the liver were analyzed on an IBM PC with a program that uses the method of Lowenstein (1956) for the quantitative analysis of an uptake-excretion curve (see Appendix B). The program plots a time-activity curve for the data points. The user prints the curve and draws a line along the descending (excretion) limb of the curve to obtain the time, in sec, that represent the beginning and ending points of the most linear portion of this limb. The time of the first data point up to the time just preceding the beginning point of the descending limb represents the ascending (uptake) limb. These four times are interactively entered into the program. With this information, the regression line for the excretion phase is extrapolated to the y axis. Points from the uptake portion of the curve are subtracted from the corresponding values along the extrapolated excretion limb. These points when plotted characterizes the uptake limb. The half-life of liver uptake, T1/2(U), is the period during which the liver takes up one half of the circulating radioactivity from the blood. The half-life of liver excretion, T1/2(E), is the period during which one half of the radioactivity is excreted by the liver (Figures 4 and 5). A third phase seemed present towards the tail of the curve on the liver uptake and excretion plots of several subjects. This was not a consistent finding, and it was often located in a region of the curve where there was
Figure 4. Liver Uptake and Excretion Curve of Disofenin in a Control Subject.
Patient Control

\[ Y = B \times (10^M)^X \]

\[
\alpha = 3.31E+04 \times (10^{-7.23E-02})^X \\
T1/2(U) = 4.2 \text{ min}
\]

\[
\beta = 3.47E+04 \times (10^{-1.82E-02})^X \\
T1/2(E) = 16.5 \text{ min}
\]

Figure 5. Determining The Half-Lives of Liver Uptake and Excretion.
considerable scatter of data points. Because our study was limited to 90 minutes, a biexponential approach to data analysis was used.

Owing to the nongaussian distribution of the data for several parameters, the Wilcoxon two-sample rank sum test was used for comparing control and patient groups. Spearman rank correlations were calculated to determine the relationship between clearance parameters and laboratory markers of liver dysfunction.
RESULTS

Pharmacokinetics of Disofenin

Figures 6 through 8 are different compartment models used to examine the plasma disappearance and liver uptake data. The biexponential appearance of the data when plotted on semi-logarithmic graph paper indicated that the drug was distributed in more than one compartment. A two-compartment model, as seen in Figure 6 provided the best fit for analyzing the plasma data. In this model, a bolus intravenous dose of disofenin enters the central compartment, C(1), which represents the blood, extracellular fluid and highly perfused tissues. An equilibrium is established between the central compartment and the more slowly perfused tissue compartment, C(2). Elimination of the drug occurs from the central compartment. Because the drug is excreted unchanged by the liver, this model is also appropriate for examining the liver data. C(1) represents the hepatic sinusoids and space of Disse. The hepatocyte is represented by C(2). A difference in using this model for the liver is that excretion into the bile occurs from C(2). Theoretically, for a homogeneous drug it should be possible to fit the plasma and liver data simultaneously with the
Figure 6. Two-Compartment Model for the Analysis of Plasma Data.

Figure 7. Two-Compartment Model With Liver for the Simultaneous Analysis of Plasma and Liver Data.
model represented in Figure 8. In fact, the data from plasma and liver counts did not follow the same kinetics. A third compartment to represent portal tissue circulation as shown in Figure 8 did not improve the fit. The half-life of the elimination (beta) phase in the blood was always longer than expected. It appeared as though disofenin contained a "second" fraction that was not taken up as rapidly by the liver.

Two sets of animal experiments conducted by Dr. Ian Fraser and Dr. Phillip Shaffer provided some support for this hypothesis. In the first experiment a catheter was inserted into the jugular vein and placed as peripherally as possible in a hepatic vein. The opposite jugular was also catherized. Disofenin was injected through a peripheral vein and timed samples were collected simultaneously from the jugular and hepatic vein. The extraction ratio was calculated for each paired sample using the formula:

\[
\text{Extraction Ratio} = \frac{\text{jugular count} - \text{hepatic artery count}}{\text{jugular count}}
\]

(5)

The data for this experiment is shown in Table 5. The extraction ratio began at 0.76 but only averaged
Figure 8. Three-Compartment Model With Liver and a Delayed Portal Zone for the Simultaneous Analysis of Plasma and Liver Data.
Table 5
Extraction Ratios From a Healthy Dog Injected with Disofenin

<table>
<thead>
<tr>
<th>Time Following Injection (min)</th>
<th>Jugular Activity (cpm)</th>
<th>Venous Activity (cpm)</th>
<th>Extraction Ratio</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>85013</td>
<td>20585</td>
<td>0.76</td>
</tr>
<tr>
<td>2</td>
<td>48120</td>
<td>12633</td>
<td>0.74</td>
</tr>
<tr>
<td>3</td>
<td>33983</td>
<td>13759</td>
<td>0.60</td>
</tr>
<tr>
<td>4</td>
<td>25184</td>
<td>10880</td>
<td>0.57</td>
</tr>
<tr>
<td>5</td>
<td>25033</td>
<td>9601</td>
<td>0.62</td>
</tr>
<tr>
<td>6</td>
<td>19503</td>
<td>9736</td>
<td>0.50</td>
</tr>
<tr>
<td>8</td>
<td>16585</td>
<td>8936</td>
<td>0.46</td>
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<tr>
<td>10</td>
<td>16432</td>
<td>8154</td>
<td>0.50</td>
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<td>15</td>
<td>11216</td>
<td>7539</td>
<td>0.33</td>
</tr>
<tr>
<td>20</td>
<td>9612</td>
<td>6862</td>
<td>0.29</td>
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<td>25</td>
<td>8739</td>
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<td>50</td>
<td>5436</td>
<td>4472</td>
<td>0.18</td>
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<td>60</td>
<td>4762</td>
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<td>70</td>
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</tr>
<tr>
<td>80</td>
<td>4118</td>
<td>3319</td>
<td>0.19</td>
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<tr>
<td>90</td>
<td>3612</td>
<td>3170</td>
<td>0.12</td>
</tr>
</tbody>
</table>
0.23 during the next 80 minutes. The dose was far below saturation levels, and a change in extraction was not expected for a homogeneous substance.

For the next experiment, a dog was exsanguinated 35 minutes after receiving an injection of disofenin. The blood was centrifuged and the plasma reinjected into a second dog, with timed samples collected during a 90 minute period. As noted in Figure 9 the decay is not like the expected disofenin curve (Figure 10). It is primarily the "second" fraction after the rapidly removed disofenin has been taken up by the liver of the exsanginated dog.

This has led to the model suggested in Figure 11 which represents two single-compartment models. It assumes that the drug contains both fast and slow removed fractions. The available experimental data is insufficient to complete the details of this model. However, the two-compartment model is valid for the data we have, and it is this model that will be used for our analysis and presentation of the data.

**Patient Studies**

Individual patient values for clearance parameters and a summary of group means, standard error of the means, and standard deviations are in Tables 6 and 7 respectively. Patients with liver or biliary dysfunction and elevated enzyme activities were classified as icteric if the
Figure 9. Decay Curve in a Dog Injected With Plasma Containing Disofenin from an Exsanguinated Dog.

Figure 10. Expected Disofenin Decay Curve in a Dog.
Figure 11. Proposed Two Single-Compartment Model for the Analysis of Disofenin Decay.
Table 6

Disofenin Plasma and Liver Clearance Parameters

<table>
<thead>
<tr>
<th>Patient</th>
<th>k(alpha) (min⁻¹)</th>
<th>T₁₅(alpha) (min)</th>
<th>k(beta) (min⁻¹)</th>
<th>T₁₅(beta) (min)</th>
<th>Cl(T) (mL/min)</th>
<th>T₅(U) (min)</th>
<th>T₅(E) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.1561</td>
<td>4.4</td>
<td>0.0068</td>
<td>101.9</td>
<td>793.7</td>
<td>2.8</td>
<td>20.9</td>
</tr>
<tr>
<td>2</td>
<td>0.1933</td>
<td>3.6</td>
<td>0.0126</td>
<td>55.0</td>
<td>2492.3</td>
<td>2.2</td>
<td>13.2</td>
</tr>
<tr>
<td>3</td>
<td>0.1954</td>
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<td>0.0088</td>
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<td>1418.5</td>
<td>4.5</td>
<td>17.6</td>
</tr>
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<td>3.0</td>
<td>0.0090</td>
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<td>1006.7</td>
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<td>9.6</td>
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<td>0.0099</td>
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<td>1110.4</td>
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<td>11.2</td>
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<td>0.2372</td>
<td>2.9</td>
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<td>59.8</td>
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<tr>
<td>7</td>
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<td>0.0102</td>
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<td>1354.6</td>
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<td>20.7</td>
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<td>0.2060</td>
<td>3.4</td>
<td>0.0080</td>
<td>86.4</td>
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<td>5.1</td>
<td>0.0091</td>
<td>76.5</td>
<td>1729.6</td>
<td>4.2</td>
<td>16.5</td>
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<td>Liver/Biliary Disease</td>
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<tr>
<td>10</td>
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<td>81.9</td>
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<tr>
<td>11</td>
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<td>0.0070</td>
<td>98.4</td>
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<td>12</td>
<td>0.1769</td>
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<td>0.0038</td>
<td>184.0</td>
<td>237.5</td>
<td>4.3</td>
<td>143.6</td>
</tr>
<tr>
<td>13</td>
<td>0.1109</td>
<td>6.3</td>
<td>0.0035</td>
<td>196.8</td>
<td>455.0</td>
<td>5.8</td>
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<tr>
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<td>199.7</td>
<td>185.1</td>
<td>2.6</td>
<td>169.7</td>
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<tr>
<td>15</td>
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<td>5.5</td>
<td>0.0043</td>
<td>162.7</td>
<td>350.5</td>
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<td>0.0108</td>
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<tr>
<td>18</td>
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<td>0.0059</td>
<td>118.5</td>
<td>3246.8</td>
<td>2.5</td>
<td>91.5</td>
</tr>
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</table>
### Table 6 (continued)

**Disofenin Plasma and Liver Clearance Parameters**

<table>
<thead>
<tr>
<th>Patient</th>
<th>k(α) (min⁻¹)</th>
<th>T_α (min)</th>
<th>k(β) (min⁻¹)</th>
<th>T_β (min)</th>
<th>C1(T) (mL/min)</th>
<th>T_2(U) (min)</th>
<th>T_3(E) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver/Biliary Disease</td>
<td>Liver/Biliary Disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anicteric</td>
<td>Anicteric</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>0.5824</td>
<td>1.2</td>
<td>0.0097</td>
<td>71.3</td>
<td>316.3</td>
<td>2.3</td>
<td>30.7</td>
</tr>
<tr>
<td>20</td>
<td>0.3749</td>
<td>1.9</td>
<td>0.0057</td>
<td>122.7</td>
<td>499.9</td>
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<td>142.2</td>
</tr>
<tr>
<td>21</td>
<td>0.1463</td>
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<td>177.9</td>
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<td>4.0</td>
<td>20.9</td>
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<tr>
<td>22</td>
<td>0.3535</td>
<td>2.0</td>
<td>0.0128</td>
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<td>1462.5</td>
<td>3.8</td>
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<tr>
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<td>9.1</td>
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<tr>
<td>25</td>
<td>0.2453</td>
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<td>291.8</td>
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<tr>
<td>26</td>
<td>0.1059</td>
<td>6.5</td>
<td>0.0074</td>
<td>93.1</td>
<td>872.5</td>
<td>2.5</td>
<td>19.1</td>
</tr>
<tr>
<td>TPN</td>
<td>TPN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>0.3364</td>
<td>2.1</td>
<td>0.0076</td>
<td>91.2</td>
<td>294.3</td>
<td>3.1</td>
<td>25.4</td>
</tr>
<tr>
<td>28</td>
<td>0.3745</td>
<td>1.9</td>
<td>0.0078</td>
<td>88.5</td>
<td>529.9</td>
<td>2.1</td>
<td>38.2</td>
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<tr>
<td>29</td>
<td>0.2476</td>
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<td>0.0048</td>
<td>144.8</td>
<td>399.9</td>
<td>2.2</td>
<td>162.7</td>
</tr>
<tr>
<td>30</td>
<td>0.2206</td>
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<td>0.0091</td>
<td>76.2</td>
<td>1108.9</td>
<td>---</td>
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</tr>
</tbody>
</table>

--- = missing data
Table 7

Summary of Group Clearance Means, SEM's and SD's

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Plasma Disappearance</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$k(a)$ (min$^{-1}$)</td>
<td>$T_{5(a)}$ (min)</td>
<td>$k(b)$ (min$^{-1}$)</td>
</tr>
<tr>
<td>Controls (9)</td>
<td></td>
<td>0.2037±0.0255 (0.0765)</td>
<td>3.8±0.46 (1.38)</td>
<td>0.0096±0.0006 (0.0018)</td>
</tr>
<tr>
<td>Liver/Biliary Disease</td>
<td></td>
<td>0.2179±0.0376 (0.0995)</td>
<td>3.8±0.62 (1.64)</td>
<td>0.0055±0.0008 (0.0022)</td>
</tr>
<tr>
<td>Icteric (7)</td>
<td></td>
<td>0.2669±0.0569 (0.1609)</td>
<td>3.5±0.64 (1.81)</td>
<td>0.0082±0.0008 (0.0024)</td>
</tr>
<tr>
<td>Anicteric (8)</td>
<td></td>
<td>0.2948±0.0363 (0.0726)</td>
<td>2.5±0.30 (0.61)</td>
<td>0.0073±0.0009 (0.0018)</td>
</tr>
<tr>
<td>TPN (4)</td>
<td></td>
<td>0.3043±0.0363 (0.0726)</td>
<td>3.0±0.30 (0.61)</td>
<td>0.0073±0.0009 (0.0018)</td>
</tr>
</tbody>
</table>

Mean ± Standard Error of the Mean
( ) = Standard Deviation
### Summary of Group Clearance Means, SEM's and SD's

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma Disappearance</th>
<th>Liver Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cl(T) (mL/min)</td>
<td>T15(U) (min)</td>
</tr>
<tr>
<td>Controls</td>
<td>1233.5±203.80 (611.40)</td>
<td>3.2±0.42 (1.18)</td>
</tr>
<tr>
<td></td>
<td>n=9</td>
<td>n=8</td>
</tr>
<tr>
<td>Liver/Biliary Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Icteric</td>
<td>383.9±59.60 (157.70)</td>
<td>3.7±0.62 (1.38)</td>
</tr>
<tr>
<td></td>
<td>n=7</td>
<td>n=5</td>
</tr>
<tr>
<td>Anicteric</td>
<td>641.8±143.58 (406.10)</td>
<td>3.0±0.39 (1.09)</td>
</tr>
<tr>
<td></td>
<td>n=8</td>
<td>n=8</td>
</tr>
<tr>
<td>TPN</td>
<td>575.9±187.07 (374.15)</td>
<td>2.5±0.32 (0.55)</td>
</tr>
<tr>
<td></td>
<td>n=4</td>
<td>n=3</td>
</tr>
</tbody>
</table>
bilirubin was greater than 1.5 mg/dL, the upper limit of normal for our laboratory. This served as a somewhat arbitrary indicator of the severity of liver damage. Data for two patients were excluded from further calculations as being outliers. Patient 17 had a calculated central compartment volume of less than 0.3 liters. Patient 18 had a total body clearance of disofenin that was greater than the combined liver and kidney clearance capacities in health. A correlation of 0.98 (p < 0.001) was found between the uncorrected total body clearance and the total body clearance corrected for body surface area. The uncorrected total body clearance was therefore used throughout the study.

There is considerable overlap between the means of the control group and the liver/biliary disease groups for those values representing the initial removal of disofenin from the circulation. This is seen in both alpha parameters of plasma disappearance, \( k(a) \) and \( T1/2(a) \), and in the uptake phase of liver distribution, \( T1/2(U) \). A difference in the means between the control group and the TPN group is more evident for these same parameters. For \( k(a) \) and \( T1/2(a) \), the two-sample rank test revealed a statistically significant difference between the results of the control group and the TPN group, as shown in Table 8. The TPN patients had a faster rate of removal and shorter half-life than the controls. Differences between the mean
Table 8  
Wilcoxon Two-Sample Sum of Rank Probabilities Between  
Control and Patient Groups

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>k(a)</th>
<th>Plasma Disappearance</th>
<th>Liver Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>k(b)</td>
<td>T(2)</td>
</tr>
<tr>
<td>Icteric</td>
<td>0.918</td>
<td>0.918</td>
<td>0.002*</td>
</tr>
<tr>
<td>Anicteric</td>
<td>0.606</td>
<td>0.606</td>
<td>0.138</td>
</tr>
<tr>
<td>TPN</td>
<td>0.050*</td>
<td>0.050*</td>
<td>0.106</td>
</tr>
</tbody>
</table>

* Statistically significant
elimination rate constants and half-lives for the beta phase of disofenin clearance are more pronounced, especially between the control group \( (k = 0.0096 \pm 0.0006 \text{ min}^{-1}, T_{1/2} = 74.9 \pm 4.69 \text{ min}) \), and the icteric patients \( (k = 0.0055 \pm 0.0008 \text{ min}^{-1}, T_{1/2} = 145.0 \pm 19.83 \text{ min}) \). The differences between the results of \( k(b) \) and \( T_{1/2}(b) \) for the controls and the icteric patients is statistically significant \( (p = 0.002) \).

The greatest differences between the control group and other patient groups are observed in total body clearance, \( C_l(T) \), and in the half-life of liver excretion \( T_{1/2}(E) \). In total clearance the mean values for the icteric, anicteric, and TPN groups are 68%, 48%, 53% lower than the mean of the control group. These differences are magnified even greater in the half-life of liver excretion. The mean half-life for the icteric group (126.2 min) is considerably prolonged from the control group mean (16.0 min). Means for the anicteric (36.9 min) and TPN (75.4 min) groups are also considerably prolonged. For \( C_l(T) \) the two-sample rank test showed a statistically highly significant difference between control and icteric subjects \( (p = 0.002) \) and a statistically significant difference between control and anicteric subjects \( (p = 0.036) \). The difference between control and TPN groups just misses the level of significance at \( p = 0.076 \). The \( T_{1/2}(E) \) was also highly significant between the
control and icteric groups but it was not significant in the difference between control and anicteric groups. This suggest that Cl(T) is the most sensitive parameter for distinguishing healthy subjects from those with liver disease. It is interesting to note that T1/2(E) revealed the statistically significant difference between control and TPN groups that had been missed by Cl(T).

The separation of control subjects from the icteric patients is clearly evident in Figure 12. All controls subjects except one, have clearances above 700 mL/min. A clear distinction between the icteric patients and the control group is also present using half-life of liver excretion as shown in Figure 13.

Coefficients of rank correlations between clearance parameters and laboratory data are presented in Table 9. For the plasma disappearance of disofenin, a statistically significant but unexpected positive correlation existed between AST and k(a), and also between LD and k(a). All other statistically significant correlations occurred in the expected direction. TBIL, ALP, AST, and ALT are all negatively correlated with k(b) and Cl(T), and positively correlated with T1/2(b). The most significant associations (p < 0.001) between laboratory test and clearance parameters were found between ALP and k(b) (r = -0.647), T1/2(b) (r = 0.639), Cl(T) (r = 0.595) and T1/2(E) (r = 0.718). Highly statistically significant correlations were
Figure 12. Total Body Clearance of Disofenin in Control and Patient Groups.

Group 1 - Control
2 - Icteric
3 - Anicteric
4 - TPN
Figure 13. Half-Life of Liver Excretion of Disofenin in Control and Patient Groups.
Table 9

Spearman Rank Correlations Between Clearance Parameters and Laboratory Data

<table>
<thead>
<tr>
<th>Lab Tests</th>
<th>k(a)</th>
<th>Plasma Disappearance</th>
<th>Liver Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T^2_a</td>
<td>k(b)</td>
</tr>
<tr>
<td>TBIL</td>
<td>-0.010</td>
<td>0.011</td>
<td>-0.431*</td>
</tr>
<tr>
<td></td>
<td>n=28</td>
<td>n=28</td>
<td>n=28</td>
</tr>
<tr>
<td>ALP</td>
<td>0.064</td>
<td>-0.062</td>
<td>-0.647**</td>
</tr>
<tr>
<td></td>
<td>n=28</td>
<td>n=28</td>
<td>n=28</td>
</tr>
<tr>
<td>AST</td>
<td>0.414*</td>
<td>-0.414*</td>
<td>-0.414*</td>
</tr>
<tr>
<td></td>
<td>n=27</td>
<td>n=27</td>
<td>n=27</td>
</tr>
<tr>
<td>ALT</td>
<td>0.345</td>
<td>-0.345</td>
<td>-0.448*</td>
</tr>
<tr>
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<td>n=27</td>
<td>n=27</td>
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<tr>
<td>LD</td>
<td>0.569*</td>
<td>-0.569*</td>
<td>0.188</td>
</tr>
<tr>
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<td>n=18</td>
<td>n=18</td>
</tr>
</tbody>
</table>

* Significant at p .05

** Significant at p .001
also observed between T1/2(E) with TBIL, AST, and ALT. A weak but significant correlation was found between T1/2(U) with TBIL. In general, all of the correlation coefficients are weak to fair. The relatively low magnitude of these coefficients probably reflects the presence of other variables upon the associations examined here.

Table 10 shows the rank correlation coefficients among clearance parameters. Like the laboratory tests, these associations are not particularly strong. A weak but significant association existed between k(a) and T1/2(a) with T1/2(U). Statistically significant correlations were also found between T1/2(E) with k(b), T1/2(b), and Cl(T).
Table 10
Spearman Rank Correlations Among
Clearance Parameters

<table>
<thead>
<tr>
<th>Plasma Disappearance</th>
<th>Liver Distribution</th>
<th>$T_2(U)$</th>
<th>$T_2(E)$</th>
</tr>
</thead>
<tbody>
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<td>n=24</td>
<td>n=24</td>
<td></td>
</tr>
<tr>
<td>$k(a)$</td>
<td>-0.412*</td>
<td>0.306</td>
<td></td>
</tr>
<tr>
<td>$T_2(a)$</td>
<td>0.419*</td>
<td>-0.306</td>
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</tr>
<tr>
<td>$k(b)$</td>
<td>-0.133</td>
<td>-0.590*</td>
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</tr>
<tr>
<td>$T_2(b)$</td>
<td>0.114</td>
<td>0.604*</td>
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</tr>
<tr>
<td>$Cl(T)$</td>
<td>0.080</td>
<td>-0.562*</td>
<td></td>
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</table>

* Significant at p .05
** Significant at p .001
DISCUSSION

Pharmacokinetics of Disofenin

The principal finding in our present study of the pharmacokinetics of disofenin is the observation of an in-vivo "second" component. This finding is contrary to previous studies on the decay of labeled iminodiacetic acid derivatives (Loberg 1976, Ryan 1977). IDA-derivatives prepared by the aqueous reduction of the pertechnetate anion contain several possible impurities, i.e., free pertechnetate, reduced technetium hydroxide (which behaves as a colloid), and oxidized tin compounds that may react with technetium to form colloidal particles (Cox 1981). Our nuclear medicine pharmacy routinely assays for these impurities and has found that free pertechnetate averages 5% and reduced technetium approximately 4% of each dose. The remaining 91% is labeled disofenin. It is unlikely that the additional form could be reduced technetium hydroxide or other oxidized labeled compounds. Their colloidal behavior makes them prime candidates for removal by the reticuloendothelial system of the liver.

Another possibility is that we are seeing a monomer of disofenin. The biscomplex structure is believed to occur
through the initial formation of a monocomplex. The in-vitro presence of two components in DISIDA has been confirmed by HPLC. This second component was observed, however, only at pH 6 or below. When diethyl-IDA was prepared at pH 4 and added to plasma, its second component was rapidly converted into a single component within two minutes (Fritzberg 1980). This would suggest that the second species does not exist a physiological pH.

It is likely that disofenin or free pertechnetate has become tightly bound to selected plasma or tissue proteins, and is removed from the circulation more slowly than the more loosely bound, rapidly removed disofenin. Even though free pertechnetate concentrates in the thyroid, salivary glands, and gastric mucosa (Harper 1964), Hays (1977) has found evidence that free pertechnetate can bind tightly and possibly irreversibly to what is believed to be albumin. Strong plasma albumin binding can impair dissociation within the liver and subsequently delay uptake and biliary elimination (Popescu 1979).

**Patient Studies**

Disofenin, an hepatobiliary imaging agent, was developed primarily to provide anatomic and dynamic information concerning the biliary tract and gallbladder. Principal clinical indications for the use of disofenin include assessing cystic duct patency in patients with suspected
acute cholecystitis, detecting extrahepatic biliary obstructions, evaluating the pathway of biliary drainage and the presence of bile reflux. Our present studies indicate that selected parameters of disofenin clearance can be used to distinguish, quantitatively control subjects from patients with liver/biliary disease.

The inability to separate control and liver disease groups by using the alpha elimination rate, and half-life was not surprising. Gilmore et al. (1978) determined that the fast phase of 14-C-glycocholic acid clearance was not affected by liver disease. Hacki et al. (1976) found a significant positive correlation between hepatic blood flow and the initial phase of BSP decay. This relationship holds true for drugs with a high extraction ratio, which is what we believe is the case for a fraction of disofenin. Branch et al. (1976) also determined that liver disease results in a greater reduction in intrinsic hepatic clearance than in liver blood flow. Unless liver blood flow is severely reduced, k(a) and T1/2(a) would not be affected.

The beta elimination rate, and half-life adequately separated the control group from the icteric group; however, these parameters were unable to distinguish the control group from the group with milder liver disease. If our assumptions are correct concerning the two-fraction nature of disofenin, the slowly removed fraction represented by k(b) and T1/2(b) should provide a better measure of
liver function. In our dog experiment, we found that this fraction had an average extraction ratio of 0.23, which would make its removal less dependent on liver blood flow, and more a factor of hepatocyte function (Wilkinson 1975).

The total body clearance and the half-life of liver excretion provided the best discrimination between the control group and all the other patient groups. The Cl(T) was particularly sensitive to both severe and milder forms of liver/biliary disease. One reason for this greater sensitivity is that clearance is less influenced by changes in the volume of distribution than either the elimination rate or half-life. Half-life assumes elimination only and may not reflect changes in protein binding or drug displacement that would affect the volume of distribution. As the volume of distribution changes, the slope and therefore the half-life are altered, but the total area under the curve is not affected. Clearance therefore becomes a more precise index of liver dysfunction. Our results illustrate this point. The mean apparent volume of the central compartment for the anicteric patients (13.67L±3.23 S.E.M.) was 53% lower than the mean central compartment volume for the control group (29.11L±4.04 S.E.M.). This had the effect of spuriously shortening the beta half-lives of the anicteric group so that they were statistically indistinguishable from those of the control group. The two groups
were significantly different in clearance. The T1/2(E) was successful at discriminating the control group from two out of three patient groups; the icteric and TPN groups. The actual value of this parameter as a measure of liver function is uncertain. Different investigators have reached opposing conclusions about its effectiveness. Theoretically, as a direct measure of liver excretory capacity, it would appear to be good index. In this study the T1/2(E) was not sufficiently sensitive to detect liver/biliary dysfunction in the anicteric group.

Most investigators who have attempted to correlate clearance tests of liver function (dye or radionuclide tracers) with biochemical tests have found only poor to marginal associations. Gamlen et al. (1975) found a poor correlation between hepatic uptake half-time of I-131 rose bengal and conventional biochemical tests. He noted, however, that the uptake half-time was of better prognostic value than either serum albumin or prothrombin ratio. Taavitsainen et al. (1980) found a poor correlation between the liver mean transit time of diethyl-IDA with bilirubin and AST. As Leevy has stated..."It is difficult to compare dye-removal patterns with results of other biochemical tests of hepatic function since mechanisms for abnormality are often completely different" (1967). Our investigation does not contradict previous findings, but some interesting trends were observed.
The positive correlation between AST and LD with \( k(a) \) was unexpected and difficult to explain. It is plausible that this association is the result of a common cause. If it is assumed that the \( k(a) \) for disofenin is a function of hepatic blood flow, an increase in blood flow could simultaneously increase \( k(a) \) and the wash-out of these enzymes.

Among enzymes, the most significant correlations with clearance parameters occurred with ALP. This might suggest that the mechanisms which influence the clearance of disofenin are more sensitive to cholestasis than to hepatic necrosis. Another likely explanation is that the selection of the population sample was biased. Many of our subjects were tested with disofenin to rule out acute cholecystitis and extrahepatic obstruction.

The most significant laboratory tests correlations with among clearance parameters were observed with \( T1/2(E) \). The closer association of this parameter with laboratory test values might reflect its more direct measurement of hepatic dysfunction. Plasma disappearance parameters are indirect and are perhaps more subject to extraneous influences such as blood flow, protein binding, etc.

The association between \( k(a) \) and \( T1/2(a) \) with \( T1/2(U) \) was significant but not as strong as expected considering how close group means were for these parameters. Factors such as tissue distribution and renal clearance probably contribute to the low magnitude of this association.
Correlations between T1/2(E) with k(b), T1/2(b) and C1(T) were as anticipated. The more prolonged the half-life of liver excretion the lower the clearance.

The TPN patients represent a special group of subjects in this study. Total parenteral nutrition has been suspected of causing cholestasis (Allardyce 1978, Grant 1977). Our original intent was to investigate this occurrence with disofenin clearance. Problems finding and recruiting TPN patients who met our criteria to be included in this study yielded only four patients (with some compromise). Despite the fact that conclusions regarding this group must be interpreted cautiously, several interesting findings have emerged.

The TPN group had a significantly shorter T1/2(a) than the control group. In a recent study, TPN patients who received a high caloric load and whose parenteral solutions were exposed to light, showed a decrease in the half-life of sodium taurocholate clearance after one week on TPN. The reason for this is not readily understood. The T1/2(E) of the TPN group was significantly longer than that of the control group. If the T1/2(E) of disofenin can be shown to be a sensitive measure of excretory function, this might be the parameter of choice for studying cholestasis in TPN patients.

Although the C1(T) of disofenin in TPN patients was not statistically significant, the marked difference
between the mean values for controls and TPN groups is noteworthy. The medical histories of the patients in this study will not allow the conclusion that cholestasis is present as a result of TPN. The data confirms, however, the diminution of liver function as the result of other organ pathologies.

**Suggestions for Further Study**

A number of areas concerning disofenin and its use to evaluate liver function remain to be investigated. The study just completed should be expanded to include a variety of suspected and confirmed cases of hepatocellular dysfunction without extrahepatic obstruction. This would provide a more definitive assessment of its ability to detect solely hepatocyte disease. Many of these patients should be tested serially to determine if disofenin can be used to monitor changes in status.

One of the most pressing questions is the identity of the more slowly removed component in the decay curve. The substance represented in the initial rapid phase has a relatively high extraction ratio and could possibly serve as a measure of hepatic blood flow. Because the second component appears to have a low extraction ratio, it would qualify as a possible test of hepatocyte function. The identity, characteristics, and separation of this second component is important for a better understanding of
the pharmacokinetics and clinical usefulness of this drug.

Up to 10% of the administered activity of disofenin is excreted in the urine over the first 2 hours (Stadalnik 1981). The contribution and composition of renal activity to total body clearance in health and disease should be investigated.
APPENDIX A

A Mainframe Computer Subroutine for Biexponential Curve-Fitting

(Written by A. E. Staubus)
SUBROUTINE DFUNC(F,P,CON,VAL,X,I,J,ISPEC,XVEC,Y,W,NOBS)
IMPLICIT REAL*8(A-H,O-Z)
DIMENSION ISPEC(1),NOBS(1)
DOUBLE PRECISION P(1),VAL(1),F,CON(1),Y(1),W(1),X,XVEC(1)
DATA ICODE/O/
IFLAG=ISPEC(8)
NTOT=ISPEC(2)
IF (IFLAG.NE.-1) GO TO 15
15 ACOEFF=F(1)
BCOEFF=F(3)
RK1=F(2)
RK2=F(4)
DOSE=CON(1)
CONTINUE WITH EXPONENTIAL FUNCTION EVALUATION
C C CONTINUE
30 CONTINUE
XT=X
F1=0.0
IF(-RK1*XT.GT.-79.0)F1=ACoeff*DEXP(-RK1*XT)
F2=0.0
IF(-RK2*XT.GT.-79.0)F2=BCoeff*DEXP(-RK2*XT)
F=F1+F2
IF (ISPEC(8)-9)190,160,190
160 AUC1=ACoeff/RK1
CO=ACoeff+BCoeff
DOSE1=DOSE*(ACoeff/CO)
DOSE2=DOSE-DOSE1
AUC2=BCoeff/RK2
AUCT=AUC1+AUC2
CLT1=DOSE1/AUC1
CLT2=DOSE2/AUC2
THALF1=0.6931472/RK1
THALF2=0.6931472/RK2
FAUC1=AUC1*100/AUCT
FAUC2=AUC2*100/AUCT
PDOSE1=DOSE1*100/DOSE
PDOSE2=DOSE2*100/DOSE
CLT=DOSE/(AUC1+AUC2)
V1=DOSE/(CO*1000)
170 FORMAT(4F16.5)
171 FORMAT(15X)
182 FORMAT(4F16.5)
183 FORMAT(4F16.5)
184 FORMAT(4F16.5)
185 FORMAT(4F16.5)
186 FORMAT(4F16.5)
187 FORMAT(4F16.5)
188 FORMAT(4F16.5)
189 FORMAT(4F16.5)
211 FORMAT(4F16.5)
216 FORMAT(4F16.5)
217 FORMAT(4F16.5)
190 CONTINUE
RETURN
END
APPENDIX B

A Personal Computer Program for Biexponential Curve-Fitting
(Written by J. E. Love)
"A Curve Fitting Program" 

L = LEN(T$); T$ ' * centers title *

PRINT:"This program, using the method of residuals (also known as feathering) 

"fits a curve to the experimental data of a drug which demonstrates a 
two compartment model. A least-squares approximation is performed of 
a semi-logarithmic line where the abscissa is linear and the ordinate 
is logarithmic. The equation of the line is:

Y = B * (10^M)^X 

where the intercept(B) and slope(M) are calculated and graphed.

PRESS ANY KEY TO CONTINUE"

REM PRINT "Is This Data:

1 = Blood Disappearance  2 = Liver Uptake"

REM PRINT "SELECTION 

140   AS$ = INKEY$: IF AS$="" THEN 140
150   CLS:PRINT "Data must be entered from a file located on this disk."
160   PRINT:PRINT: PRINT "File Name 

170   PRINT :PRINT "Is This Data:

180   PRINT "1 = Blood Disappearance   2 = Liver Uptake"
190   PRINT :PRINT :INPUT "SELECTION 

200   PRINT:PRINT: PRINT "Patient's Initials 

210   PRINT:PRINT: PRINT "Reading data from file"
220   OPEN #1 FOR INPUT AS #1
230   DIM X(200), Y(200), SX(200), SY(200), YAt50, SXA(50), SYA(50)
240   INPUT #1,F$
250   N = 0: I = 1
260   IF EOF(1) THEN CLOSE: GOTO 320
270   INPUT #1, X(I>, Y(I)
280   N = N + I
290   I = I + 1
300 GOTO 260
310    YMAX = Y(I) ' * finds largest y value *
320   FOR I = 1 TO N
330   IF Y(I) > YMAX THEN YMAX = Y(I)
340   NEXT I
350 IF YMAX > 100000 THEN GOSUB 2210 ELSE GOSUB 1730
370 LOCATE 1,30 
380 ON YL GOTO 420,450 
390 LOCATE 11,3 
400 PRINT "CPM"
410 GOTO 420,450 
420 LOCATE 11,3 
430 PRINT "CPM"
440 GOTO 490 
450 LOCATE 11,3 
460 PRINT "Cts/

470 LOCATE 12,2 
480 PRINT "30sec"
490 M = 0: S = 0 ' * x labels *
500 FOR TM = 12 TO 57 STEP 5 
510 LOCATE 22,TM 
520 PRINT M 
530 M = M + 10 
540 LOCATE 25,TM 
550 PRINT S 
560 S = S + 600
83

570 NEXT TM
580 LOCATE 22,64
590 PRINT "Min"
600 LOCATE 23,64
610 PRINT "Sec"
620 IF INTERPO = 1 THEN GOTO 2690
630 AS = INKEY$: IF AS = "" THEN 630
640 CLS: PRINT "Select data points on alpha and beta curves to be included"
650 PRINT "in the regression analysis."
660 PRINT : PRINT "ALPHA CURVE (Initial)"
670 PRINT "Begin at (in seconds) " ; ABE
680 PRINT "End at (in seconds) " ; AED
690 IF ABE > AED OR AED > 5400 THEN PRINT "Try Again" : GOTO 670
700 PRINT : PRINT "BETA CURVE"
710 PRINT "Begin at (in seconds) " ; BBE
720 PRINT "End at (in seconds) " ; BED
730 PRINT "One moment please -- I'm cogitating!"
740 IF BBE > BED OR BED > 5400 THEN PRINT "Try Again" : GOTO 710
750 FOR I = 1 TO N
760 IF ABE = X(I) THEN U1 = I
770 IF AED = X(I) THEN U2 = I
780 IF BBE = X(I) THEN B1 = I
790 IF BED = X(I) THEN B2 = I
800 NEXT I
810 X1=0; Y1=0; X2=0; Y2=0; C=0: XY=0: C=0 ' * initial alpha regression *
820 FOR I = U1 TO U2
830 LY = LOG(Y1)/LOG(10)
840 XI = XI + X(I)
850 Y1 = Y1 + LY
860 X2 = X2 + X(I)^2
870 Y2 = Y2 + Y(I)^2
880 XY = XY + X(I) * LY
890 C = C + 1
900 NEXT I
910 D = C * X2 - X1^2
920 IF D<0 THEN 940
930 CLS: PRINT "No solution found-initial alpha":END
940 MU = (C * XY - X1 * Y1)/D
950 LBU = (Y1 * X2 - X1 * XY)/D
960 BU = 10^LBU
970 HLU = (LOG(2)/(2.303 * MU))/60
980 HLU = ABS(HLU)
990 CI = SQR(((C * X2) - X1^2) * ((C * Y2) - X1^2))
1000 RU = ((C * XY) - (X1 * Y1))/CI
1010 RUA = EXP(RU)
1020 IF C < 3 THEN GOTO 1060
1030 SEU = ABS((X2 - (BU * Y1) - (MU * XY))/(C-2))
1040 SEU = SQR(SEU)
1050 SEU = EXP(SEU)
1060 X1=0; Y1=0; X2=0; Y2=0; C=0: XY=0: C=0 ' * beta regression *
1070 FOR I = B1 TO B2
1080 LY = LOG(Y(I))/LOG(10)
1090 XI = XI + X(I)
1100 Y1 = Y1 + LY
1110 X2 = X2 + X(I)^2
1120 Y2 = Y2 + Y(I)^2
1130 XY = XY + X(I) * LY
1140 C = C + 1
1150 NEXT I
1160 D = C * X2 - X1^2
1170 IF D<0 THEN 1190
1180 CLS:PRINT :PRINT "No solution found-beta":END
1190 MB = (C * XY - XI * Y1)/D
1200 LBB = (Y1 * X2 - X1 * XY)/D
1210 BB = 10^LBB
1220 HLB = (LOG(2)/(2.303 * MB))/60
1230 HLB = ABS(HLB)
1240 CB = SQR(((C * X2) - X1^2) * ((C * Y2) - X1^2))
1250 SD = 0
1260 FOR I = 1 TO N
1270 SD » SD + LOG( Y(I)>/(LOG(10) - MB * X(I) - LBB)^2
1280 SD = INT(SD * 100! + .5)/100!
1290 RB = ((C * XY) - (XI * Y1))/CB
1300 RBB = EXP(RB)
1310 RBB = ABS(RBB)
1320 IF C < 3 THEN GOTO 1360
1330 SEB = ABS((X2 - (BB * Y1) - (MB * XY))/(C-2))
1340 SEB = SQR(SEB)
1350 SEB = EXP(SEB)
1360 K = 0
1370 IF YMAX > 100000! THEN P = 1000000! ELSE P = 100000!
1380 IF YMAX > 100000! THEN Q = 300/LOG(1000000!) ELSE Q = 250/LOG(100000!)
1390 FOR I = U1 TO U2
1400 YA(I) = Y(I) - (BB * (10^MB)^(X(I)))
1410 YA(I) = ABS(YA(I))
1420 SYA(I) = CINT((LOG(P) - LOG(YA(I)))*Q + 10)
1430 SXA(I) = CINT(X(I) * RATIORANGE + 100)
1440 IF SYA(I) < 10 OR SYA(I) > 160 THEN GOTO 1470
1450 K = K + 1
1460 NEXT I
1470 X1=0;Y1=0;X2=0;Y2=0;XY=0;C=0
1480 FOR I = U1 TO U2
1490 LY » LOG(YA(I))/LOG(10)
1500 X1 = X1 + X(I)
1510 Y1 = Y1 + LY
1520 X2 = X2 + X(I)^2
1530 Y2 = Y2 + YA(I)^2
1540 XY = XY + X(I) * LY
1550 C = C + 1
1560 NEXT I
1570 D = C * X2 - X1^2
1580 IF D<0 THEN 1600
1590 CLS:PRINT :PRINT "No solution found-alpha":END
1600 MA = (C * XY - XI * Y1)/D
1610 LBA = (Y1 * X2 - X1 * XY)/D
1620 BA = 10^LBA
1630 HLA = (LOG(2)/(2.303 * MA))/60
1640 HLA = ABS(HLA)
1650 CA = SQR(((C * X2) - X1^2) * ((C * Y2) - X1^2))
1660 RA = ((C * XY) - (XI * Y1))/CA
1670 RAA = EXP(RA)
1680 IF C < 3 GOTO 1720
1690 SE = ABS((X2 - (BA * Y1) - (MA * XY))/(C-2))
1700 SE = SQR(SE)
1710 SE = EXP(SE)
1720 INTERPO = I:GOTO 320:END
1730 CLS:SCREEN 2:KEY OFF
1740 DRAW "bm100,10; dl50; r360" ' plots axes
1750 LINE (BB,60) - (96,60)
1760 LINE (101,10) -(101,159)
1770 SRANGERATIO = 250/LOG(100000!) 'y hash marks
1780 FOR TA = 100000! TO 20000! STEP -10000!
1790 YTICKS = CINT((LOG(100000!)-LOG(TA)) * SRANGERATIO + 10)
1800 LINE (97,YTICKS) - (104,YTICKS)
1810 NEXT TA
1820 LINE (88,10) - (96,10)
1830 FOR TB = 10000! TO 20000! STEP -1000!
1840 YTICKS = CINT((LOG(100000!)-LOG(TB)) * SRANGERATIO + 10)
1850 LINE (97,YTICKS) - (104,YTICKS)
1860 NEXT TB
1870 FOR TC = 1000! TO 100! STEP -100!
1880 YTICKS = CINT((LOG(100000!)-LOG(TC)) * SRANGERATIO + 10)
1890 LINE (97,YTICKS) - (104,YTICKS)
1900 NEXT TC
1910 LINE (88,110) - (96,110)
1920 LINE (88,160) - (96,160)
1930 FOR TD = 100 TO 460 STEP 40 'x hash marks'
1940 LINE (97,YTICKS) - (104,YTICKS)
1950 NEXT TD
1960 FOR TE = 104 TO 460 STEP 4
1970 LINE (TE,158) - (TE,160)
1980 NEXT TE
1990 FOR CPM = 2 TO 20 STEP 6
2000 LOCATE CPM,8 'y labels'
2010 PRINT "10"
2020 NEXT CPM
2030 EXPONENT = 5
2040 FOR POWER = 1 TO 19 STEP 6
2050 LOCATE POWER,9
2060 PRINT EXPONENT
2070 EXPONENT = EXPONENT + 1
2080 NEXT POWER
2090 FOR I = 1 TO N 'scales y values'
2100 SY(I) = CINT((LOG(100000!)-LOG(Y(I))) * SRANGERATIO + 10)
2110 IF SY(I) < 10 OR SY(I) > 160 THEN OUTRANGE = I AND GOTO 2130
2120 NEXT I
2130 RATIORANGE = 360/5400 'scales x values and plots x & y values'
2140 FOR I = 1 TO N
2150 SX(I) = CINT(X(I) * RATIORANGE + 100)
2160 IF I = OUTRANGE THEN GOTO 2200
2170 PSET (SX(I), SY(I))
2180 DRAW "m+0,-1; m-2,+2; m+4,0; m-2,-2" 'draws triangular points'
2190 NEXT I
2200 RETURN
2210 CLS;SCREEN 2;KEY OFF
2220 DRAW "bmi00,10; d150; r360" 'plots axes'
2230 LINE (101,10)-(101,159)
2240 LRANGERATIO = 300/LOG(1000000!) 'y hash marks'
2250 FOR TA = 1000000! TO 200000! STEP -1000000!
2260 YTICKS = CINT((LOG(1000000!)-LOG(TA)) * LRANGERATIO + 10)
2270 LINE (97,YTICKS) - (104,YTICKS)
2280 NEXT TA
2290 LINE (88,10) - (96,10)
2300 FOR TB = 100000! TO 200000! STEP -100000!
2310 YTICKS = CINT((LOG(1000000!)-LOG(TB)) * LRANGERATIO + 10)
2320 LINE (97,YTICKS) - (104,YTICKS)
2330 NEXT TB
2340 LINE (88,60) - (96,60)
2350 FOR TC = 1000! TO 100! STEP -1000!
2360 YTICKS = CINT((LOG(100000!)-LOG(TC)) * LRANGERATIO + 10)
2370 LINE (97,YTICKS) - (104,YTICKS)  
2380 NEXT TC  
2390 LINE (88,110) - (96,110)  
2400 LINE (88,160) - (96,160)  
2410 FOR TD = 100 TO 460 STEP 40 ' * x hash marks *  
2420 LINE (TD,136) - (TD,164)  
2430 NEXT TD  
2440 FOR TE = 104 TO 460 STEP 4  
2450 LINE (TE,158) - (TE,160)  
2460 NEXT TE  
2470 FOR CPU = 2 TO 20 STEP 6  
2480 LOCATE CPU,8 ' * y labels •  
2490 PRINT "10"  
2500 NEXT CPU  
2510 EXPONENT = 6  
2520 FOR POWER = 1 TO 19 STEP 6  
2530 LOCATE POWER,9  
2540 PRINT EXPONENT  
2550 NEXT POWER  
2560 FOR I = 1 TO N ' * scales y values *  
2570 SY(I) = CINT((LOG(1000000!) - LOG(Y(I)))) * LRANGERATIO + 10  
2580 IF SY(I) < 10 OR SY(I) > 160 THEN OUTRANGE = 1 AND GOTO 2610  
2590 NEXT I  
2600 RATIO RANGE = 360/5400 ' * scales x values and plots x & y values *  
2610 FOR I = 1 TO N  
2620 SX(I) = CINT(X(I) * RATIO RANGE + 100)  
2630 IF SX(I) = OUTRANGE THEN GOTO 2680  
2640 NEXT I  
2650 SX(I) = SX(I), SY(I))  
2660 DRAW "m+0,-l; m+2,+2; m+4,0; m-2,-2" ' * draws triangular points *  
2670 NEXT I  
2680 RETURN  
2690 FOR I = UI TO U2 ' * plots alpha points *  
2700 PSET (SXA(I),SYA(I))  
2710 LINE (SXA(I)-2,SYA(I)) - (SX A(I)+2,SYA(I))  
2720 LINE (SX A(I),SYA(I)-2) - (SX A(I),SYA(I)+2)  
2730 NEXT I  
2740 YB1 = INT((LOG(P) - LOG(BB)) * Q + 10) ' * beta regression line *  
2750 YBL = BB * (10^MB) - 5400  
2760 YB2 = INT((LOG(P) - LOG(YB2)) * Q + 10)  
2770 LINE (100,YB1) - (460,YB2)  
2780 YA1 = CINT((LOG(P) - LOG(YA1)) * Q + 10) ' * alpha regression line *  
2790 IF P = 10000000 THEN YMIN = 1000 ELSE YMIN = 100!  
2800 XA1 = (LOG(YMIN) - LOG(BA)) / (LOG(10) - MA)  
2810 XA2 = CINT(XA1) * RATIO RANGE + 100  
2820 LINE (100,YA1) - (X A2,160)  
2830 LINE (101,YA1) - (X A2+1,160) ' * thickens alpha line *  
2840 LOCATE 2,40  
2850 PRINT "Y = B * (10^M)^X"  
2860 LOCATE 4,40  
2870 PRINT USING "alpha =###.####":BA  
2880 MA = MA+60  
2890 LOCATE 4,56  
2900 PRINT USING " * (10**###.###)":MA  
2910 LOCATE 4,74  
2920 PRINT ""  
2930 LOCATE 5,40  
2940 REM PRINT USING "r = ###.###":RAA  
2950 LOCATE 6,40  
2960 PRINT USING "Tl/2 = ###.# min":HLA
2970 LOCATE 17,40
2980 PRINT USING "b#ta =###.#####";BB
2990 MB = MB*60
3000 LOCATE 17,56
3010 PRINT USING "* (10###.#####)";MB
3020 LOCATE 17,74
3030 PRINT "X"
3040 LOCATE 18,40
3050 REM PRINT USING "r = #.#####";RR
3060 REM PRINT "Sum of Dev^2 = "SD
3070 REM PRINT USING "SEE = #.#####";SEB
3080 LOCATE 19,40
3090 PRINT USING "T/2 = ###.# min";HLB
3100 A$ = INKEY$; IF A$ = "" THEN 3100
3110 END
BIBLIOGRAPHY


