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Hepatic blood flow: accuracy of estimation from infusions of indocyanine green in anaesthetized cats

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1 Experiments were performed to determine the accuracy of the estimation of hepatic blood flow from infusions of indocyanine green (ICG) in anaesthetized cats.

2 The estimated flows were compared to hepatic blood flows measured directly in a hepatic venous long-circuit preparation. This preparation allowed direct measurement and alteration of hepatic blood flows, and collection of arterial and mixed hepatic venous blood samples without depletion of blood volume in the animal.

3 Mean hepatic plasma flows estimated during infusions of 3.22 and 6.44 nmol $kg^{-1} min^{-1}$ were reliable indicators of true hepatic flow at three different flow levels, provided that a sufficiently long time (> 30 min) was allowed for distribution equilibrium and that data from several animals were pooled to reduce random variability. Variability arose through subtraction of plasma arterial and hepatic venous levels to obtain the arteriovenous difference.

4 Estimations of hepatic plasma flow by intravenous infusions of ICG were more accurate and reliable than estimations from bolus injections of ICG, or intravenous infusions of galactose studied previously.

5 The kinetics of hepatic uptake of ICG are complex. Extraction and clearance of ICG fell steadily with time during the infusions and constant plasma ICG levels were not attained during 150 min infusions. This is attributed to the effects of accumulation of ICG within the liver cells since hepatic uptake substantially exceeded biliary excretion rate.

6 Total ICG concentrations in sinusoid and liver cells increased in parallel. The concentration in the liver cell was 88 (60–115) times the concentration in the sinusoid but we have no data on whether or not the free concentrations in plasma and cell were in equilibrium.

Introduction

Estimation of hepatic blood flow in man by non-invasive procedures has been an important goal of investigators for many years (Bradley, 1963). Clearance calculations after a bolus dose or during a constant intravenous infusion of several xenobiotics have been employed and in recent years indocyanine green (ICG) has been widely used. ICG is believed to be confined to the plasma, not subjected to extra-hepatic circulation, non-toxic and not metabolized or degraded (Wheeler *et al.*, 1958; Banaszak *et al.*, 1960; Hunton *et al.*, 1960; Stekiel *et al.*, 1960; Wiegand *et al.*, 1960; Caesar *et al.*, 1961; Leevy *et al.*, 1962; Paumgartner *et al.*, 1970).

In a recent study in anaesthetized cats, we investigated the accuracy of measurements of hepatic blood flow from the clearance of ICG after administration of bolus doses at different levels of hepatic blood flow (Burczynski *et al.*, 1987). Significant problems in the use of this method were shown to exist and unless proven otherwise, these problems seem likely to occur during use of the method in humans. Errors in the estimation of flow arose primarily from extrahepatic distribution of ICG and from errors in the estimation of hepatic extraction, due to the unknown transit times of blood through the portal and hepatic vasculatures. Errors due to these causes should be minimal if ICG is given by slow intravenous infusion, since extrahepatic distribution should reach equilibrium and transit times become unimportant when arterial and hepatic venous concentrations are at steady-state. Therefore it seemed likely that the intravenous infusion method

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would give better estimates of hepatic blood flow than bolus injections of ICG. The intravenous infusion method, developed from the original method of Caesar *et al.* (1961), has been widely used in recent clinical studies (Leaman *et al.*, 1978; Nowak & Wennmalm, 1978; Sonnenberg *et al.*, 1981; Escourrou *et al.*, 1982; Rowell *et al.*, 1984; Braillon *et al.*, 1985; Chauvin *et al.*, 1985; Gasic *et al.*, 1985). In these studies, steady-state was assumed to be present after infusion of ICG for 12–35 min.

During an infusion of ICG, estimated hepatic plasma flow (EHPF) can be calculated from the equation:

$$EHPF = U/(C_a - C_v)$$

where U is the hepatic uptake rate and C_a and C_v are respectively the arterial and hepatic venous plasma ICG concentrations. If ICG uptake by organs other than the liver is zero, if distribution of ICG has reached equilibrium, and if arterial and hepatic venous plasma ICG concentrations are constant, then the infusion rate will equal the hepatic uptake rate. This was assumed to occur at the time measurements were made in the above cited clinical studies and EHPF was calculated from the equation:

$$EHPF = ko/(C_a - C_v)$$

where ko is the intravenous infusion rate. This method has not been subjected to a critical analysis in animal experiments, where hepatic blood flow is simultaneously measured by an independent method and varied over the normal range by the investigator. The validity of the assumption of steady-state after 12–35 min, and the possible influence of accumulation of ICG within the liver have not been studied. We therefore examined these factors in anaesthetized cats using the hepatic venous long-circuit technique (Burczynski *et al.*, 1987). This technique allows continuous direct and unequivocal measurement of total hepatic blood flow, frequent sampling of arterial and hepatic venous blood without depletion of the animal's blood volume, and deliberate alteration of cardiac output and hence hepatic blood flow over a wide range.

Methods

Cats of either sex (2.2–3.1 kg body weight; mean 2.8 ± 0.1 kg; $n = 9$) were anaesthetized by intraperitoneal injection of sodium pentobarbitone (120 μmol kg^{-1}). Supplementary doses of pentobarbitone (24 μmol) were given intravenously when reflex swallowing movements had returned in response to gentle traction of the tongue. Arterial pressure was recorded from the right femoral artery using a Beckman 4-327-C pressure transducer on a Beckman Type RM Dynograph. Artificial respiration was maintained by a Harvard respirator and adjusted to maintain blood gases and pH within normal limits (pH 7.40 $\pm$ 0.007; P_{CO_2} 25.2 $\pm$ 0.4; P_{O_2} 91.3 $\pm$ 2.4 mmHg; means $\pm$ s.e.). Blood gases and pH were analysed with an IL 1302 pH/blood gas analyser (Instrumentation Laboratory Inc.). Rectal temperature was maintained between 37 and 38°C.

The methods have been described in detail in a previous paper (Burczynski *et al.*, 1987). An extracorporeal venous long-circuit technique was used for direct measurement of hepatic blood flow and to allow repeated sampling of arterial and mixed hepatic venous blood without depletion of the animal's blood volume. Blood from the lower part of the inferior vena cava and blood from the hepatic segment of the inferior vena cava were separately drained to an extracorporeal reservoir and returned to the animal through a jugular vein. Hepatic blood flow was measured continuously by an electromagnetic flowmeter on the hepatic outflow pipe and intermittently by direct timing of a measured volume of hepatic outflow. Mixed hepatic venous blood was sampled from this outflow tube. A left femoral arteriovenous shunt was prepared to allow sampling of arterial blood directly with a needle and syringe without deadspace problems. The extracorporeal reservoir (50 ml) and associated tubing (30 ml) were primed with donor cat blood. The reservoir, and not the animal, was depleted as samples were taken throughout the experiments. Arterial and mixed hepatic venous blood was sampled every 10 min throughout the experiments. The bile duct was cannulated after ligation of the gall bladder and bile was collected throughout the experiments.

ICG (Cardiogreen; Hynson, Westcott and Dunning, Inc.) was freshly prepared for each experiment. Each dose was dissolved in distilled water containing bovine serum albumin (100 mg ml^{-1}; BSA fraction V, Sigma Chemical Co.) and administered into the brachial vein as an intravenous infusion. Plasma and bile samples were immediately refrigerated at 4°C until analysed within 48 h. ICG used for the preparation of all standards was taken from the same vial that was reconstituted for administration to the animal. All plasma and bile samples were analysed by a high pressure liquid chromatographic method (h.p.l.c.) described in detail in the previous paper (Burczynski *et al.*, 1987).

Data are presented as means $\pm$ s.e.mean. Student's *t* test for paired data, or blocked analysis of variance with multiple comparisons by Duncan's multiple range test was used to determine significant differences ($P < 0.05$; Steel & Torrie, 1960).

Results

In Series 1 (4 cats), hepatic blood flow was maintained

at the control rate. ICG was administered as a constant infusion of 3.22 nmol $min^{-1} kg^{-1}$ body weight (2.5 $\mu g\, min^{-1} kg^{-1}$) for 150 min followed by 6.44 nmol $min^{-1} kg^{-1}$ (5 $\mu g\, min^{-1} kg^{-1}$) for an additional 90 min. In Series 2 (5 cats), ICG was administered as a constant infusion of 6.44 nmol $min^{-1} kg^{-1}$ body weight (5 $\mu g\, min^{-1} kg^{-1}$) for 210 min. During the first 90 min, hepatic blood flow was maintained at the control level. During the next 60 min period, hepatic blood flow was increased to 150% of the control by infusing blood into the animal from the reservoir. Hepatic blood flow was then reduced to 50% control, by draining blood from the animal into the reservoir, for a final 60 min period.

Since hepatic blood flow was varied by changing cardiac output, significant changes in arterial pressure accompanied the changes in flow. Mean arterial pressures in Series 1 were 109 ± 11, 145 ± 18 and 143 ± 18 mmHg and hepatic blood flows were 137 ± 30, 133 ± 34 and 130 ± 33 ml min^{-1} 100 g^{-1} liver at the beginning, end of the first infusion and end of the second infusion, respectively. Mean liver weight excluding the gall bladder was 19 ± 1 g kg^{-1} body weight. Mean haematocrit was 41 ± 3% and this did not change by more than 1% throughout each experiment. Mean arterial pressures in Series 2 were 87 and 95 ± 7 mmHg at the start and end of the control flow, 127 and 138 ± 3 mmHg at the start and end of the raised flow and 80 and 85 ± 10 mmHg at the start and end of the reduced flow period. Hepatic blood flows were 120 ± 8 ml min^{-1} 100 g^{-1} liver during the control period, 190 ± 12 ml min^{-1} 100 g^{-1} during the raised flow and 64 ± 4 ml min^{-1} 100 g^{-1} during the low flow period. Mean liver weight excluding the gall bladder was 21 ± 2.1 g kg^{-1} body weight. Mean haematocrit was 35 ± 1% and this did not change by more than 1% throughout the experiments.

Plasma indocyanine green measurements and extraction

Figure 1a shows the arterial and hepatic venous plasma ICG concentrations during the two infusions of ICG (Series 1). Examination of the complete data suggests that constant plasma concentrations were not achieved at either infusion rate, and that both arterial and venous concentrations were slowly but steadily rising. However, if only two or three data points are examined for the low infusion rate, it might appear that constant plasma levels were reached by about 30 min after the start of the infusion rate.

Plasma arterial and hepatic venous ICG concentrations for Series 2 (altered flow), excluding the initial 30 min equilibration period, are shown in Figure 1b. Arterial and venous concentrations tended to rise during the period of control flow. When flow was increased, arterial and venous concentrations did not change significantly. When flow was decreased, both arterial and hepatic venous concentrations increased significantly and continuously until the end of the 60 min period.

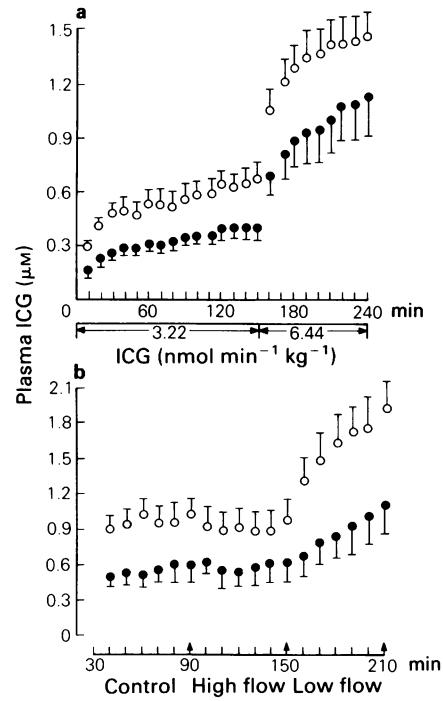

Figure 1 Arterial (○) and hepatic venous (●) plasma indocyanine green (ICG) levels during infusion of ICG at two rates in Series 1 (a, $n = 4$) and during constant infusion of ICG at 3 different flows in Series 2 (b, $n = 5$). Each point represents the mean and vertical lines show s.e.means.

Systemic indocyanine green clearance and estimated flow

Systemic clearance of ICG was calculated as infusion rate divided by arterial concentration at each time and estimated hepatic plasma flow was calculated as infusion rate divided by the arteriovenous difference for ICG as described in the Introduction. The results are shown in Figure 2a for Series 1. Early in the infusion period, EHPF exceeded measured plasma flow but from 40–150 min EHPF, although variable, was not significantly different from measured flow. When the infusion rate was increased, EHPF exceeded the measured flow for the duration of the infusion. After an initial rapid fall, systemic clearance of ICG declined slowly throughout the infusions.

Systemic clearances, measured plasma flows and

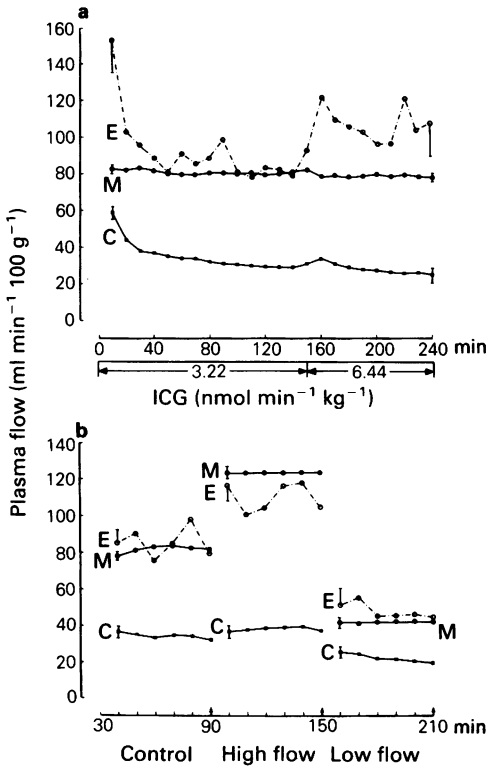

Figure 2 Measured hepatic plasma flows (M), systemic indocyanine green (ICG) clearances (C) and estimated hepatic plasma flows (E) for each time period during the two infusions in Series 1 (a, $n = 4$) and during constant infusion at different flow in Series 2 (b, $n = 5$). The points represent mean values with the pooled s.e.means from blocked analysis of variance, to remove variability between cats.

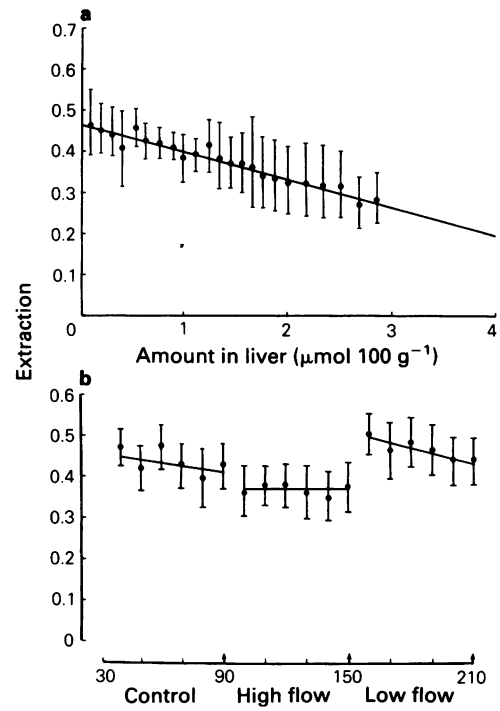

Figure 3 Extraction plotted against the amount of indocyanine green (ICG) accumulated in the liver during two infusions in Series 1 (a, $n = 4$) and extraction plotted against time during constant infusion at different flows in Series 2 (b, $n = 5$). Each point represents the mean with vertical lines showing s.e.mean.

estimated plasma flows for Series 2 (altered flows) are shown in Figure 2b. In this series the initial 30 min stabilization period was not measured. Mean EHPFs were not significantly different from the mean measured flows. However, the variability of the EHPF was large.

Kinetics of indocyanine green removal by the liver

Extraction of ICG was calculated as the arteriovenous difference divided by the arterial plasma concentration $((C_a - C_v)/C_a)$ for each paired arterial and hepatic venous plasma samples. There was a small but highly significant decrease ($P < 0.005$) in extraction with time and this was greater at the higher dose than at the lower dose. Initial extraction was 0.47 ± 0.08 and this decreased by 0.00075 min^{-1} at the low dose and by 0.0011 min^{-1} at the high dose. It seemed possible that these decreases in extraction were consequences of accumulation of ICG within the hepatocytes. We therefore estimated the amounts of ICG which had accumulated in the liver by addition of the hepatic uptake (measured flow times arteriovenous difference) and subtraction of the measured excretion of ICG in the bile. Direct measurement of liver tissue ICG content had confirmed this calculation in our previous study (Burczynski *et al.*, 1987). When extraction was plotted against the amount of ICG in the liver (Figure 3a), a linear relation was found throughout the two infusions. Extraction declined from the initial value of 0.47 with a slope of 0.070 for each μmol ICG accumulated per 100 g liver ($P < 0.01$).

The extractions in Series 2, plotted against time, are shown in Figure 3b. Initial extraction (0.46 ± 0.05) was similar to that in Series 1 and it decreased at a similar rate during the control flow period (0.00078 min^{-1}). However, extraction remained constant during the period of high flow and decreased at a faster rate (0.0013 min^{-1}) during the period of low flow. In this series, there was no obvious relationship

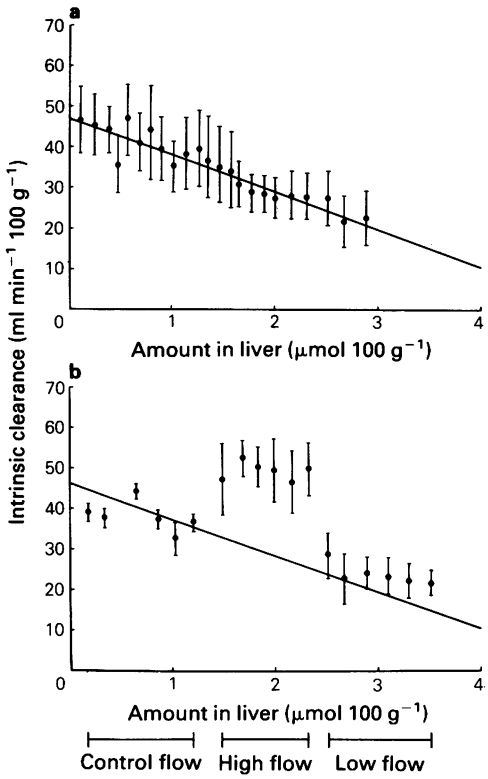

Figure 4 Intrinsic clearances plotted against the amount of indocyanine green (ICG) accumulated in the liver during two infusions in Series 1 (a) and during constant infusion of ICG at different flow rates in Series 2 (b). Each point represents the mean with vertical lines showing s.e.mean. The regression line shown in the lower panel is that derived from Series 1 (a).

between extraction and amount of ICG in the liver.

Intrinsic clearances (see Discussion) were calculated as:

$$CL_{int} = F \times \log_e (C_a/C_v)$$

where F is the measured flow per 100 g liver and C_a and C_v are respectively the arterial and hepatic venous concentrations, giving intrinsic clearance (CL_{int}) in ml min^{-1} 100 g^{-1} liver. The relationships between intrinsic clearances and the amounts of ICG accumulated in the liver for the two series of experiments are shown in Figure 4. For Series 1, intrinsic clearance was 46.2 ± 8.1 initially and it decreased by 8.7 ml min^{-1} 100 g^{-1} for each μmol ICG accumulated in the liver ($P < 0.01$). Since intrinsic clearance is predicted to be independent of flow (see Discussion), the relationships between intrinsic clearance and amount in the liver in Series 2 should be similar to that in Series 1. To allow this comparison the regression line from Series 1 is plotted again in Figure 4b. For Series 2 the values during the control and low flow periods appeared to lie close to the regression line obtained in Series 1, but the values during the high flow period were clearly above this line.

Bile flows and biliary excretion rates for the two series of experiments are shown in Figure 5. Bile flows and excretion rates were very variable between animals. In Series 1, bile flow declined while ICG excretion rate increased. However, in Series 2, bile flow was well maintained during the control and high flow periods, but declined markedly during the period of low flow ($P < 0.05$ by blocked analysis of variance). Biliary excretion rate rose steadily with time during the period of control flow. It appeared to increase during the period of high flow and decrease during the period of low flow but these changes were not statistically significant.

Discussion

In the Introduction, it was suggested that estimates of hepatic plasma flow during constant intravenous infusions of ICG might be more accurate than those from bolus injections, because extrahepatic distribution should reach equilibrium and transit time errors should be negligible when arterial and hepatic venous concentrations become constant. Our data support this view but indicate that at least 30 min is required for extrahepatic distribution to reach equilibrium. Before this time, estimated plasma flow overestimates the measured flow because the infusion rate exceeds hepatic uptake (Burczynski *et al.*, 1987). Thus the equilibration time of less than 30 min allowed in some clinical studies seems to be rather short and more accurate results might be obtained with a longer period.

In our study, the estimates of hepatic flow were quite variable even though the means were not significantly different from the measured flows after 30 min. This is perhaps not surprising. The infusion rate can be determined with accuracy but the arteriovenous concentration difference involves subtraction of two measured concentrations. When extraction is as low as 30–50%, as in our experiments, considerable variability is introduced by this subtraction given a coefficient of variation of 3–10% with the h.p.l.c. analytical method (Burczynski *et al.*, 1987). In clinical studies, extraction is usually higher, at least in individuals with healthy livers, but it varies widely from 40–75% (Caesar *et al.*, 1961; Leevy *et al.*, 1962; Rowell *et al.*, 1984; Chauvin *et al.*, 1985; Braillon *et al.*, 1985). The spectrophotometric method is usually used and this had a higher coefficient of variation at low concentrations (Burczynski *et al.*, 1987). Thus the

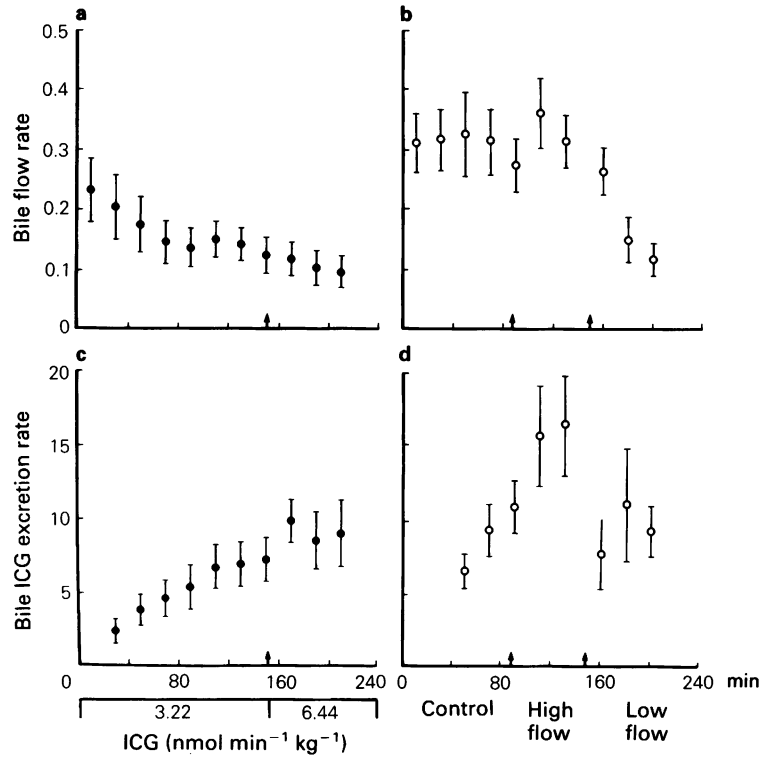

Figure 5 Bile flow rates (ml min^{-1} 100 g^{-1}) and biliary indocyanine green (ICG) excretion rates (nmol min^{-1} 100 g^{-1}) for Series 1 (a and c) and Series 2 (b and d) plotted against time. Each point represents the mean with vertical lines showing s.e.mean.

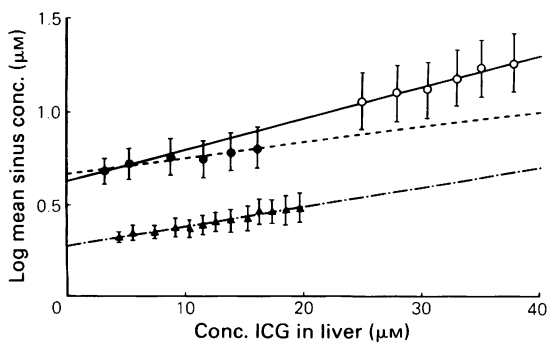

Figure 6 The relationship between log mean plasma indocyanine green (ICG) concentration in the sinusoids and ICG concentration in the liver cells for the three periods of infusions at control blood flow. (▲) Low dose Series 1; (○) high dose Series 1; (●) high dose at control flow Series 2. Each point represents the mean with vertical lines showing s.e.mean.

measurement of the arterial and hepatic venous concentrations and hence the calculation of arteriovenous difference of ICG appears to be the major determinant of variability of estimates of hepatic plasma flow in ICG infusion studies.

These studies show that even during prolonged infusions of 150 min, constant plasma arterial and hepatic venous levels of ICG are not attained. A similar phenomenon was demonstrated many years ago for ICG in man (Leevy *et al.*, 1962) and for sulphobromophthalein infusions (Wheeler *et al.*, 1960). The failure to reach constant plasma levels does not appear to invalidate this method of estimating hepatic flow. Since the volume of distribution of ICG is only slightly larger than plasma volume (Burczynski *et al.*, 1987), very little ICG is required to change the plasma levels and the infusion rate is still almost equal to the hepatic uptake. However, when the infusion rate was increased late in the experiment, EHPF continued to overestimate the measured flow for 90 min (Figure

2). For the best estimates of hepatic plasma flow, the lowest possible infusion rate of ICG should be used to minimize hepatic accumulation, provided arterial and hepatic venous levels can be accurately measured. This appears to make the use of the much more sensitive h.p.l.c. method mandatory for the best results.

Within the range of doses and plasma levels of ICG which are normally studied, uptake of ICG by the liver very substantially exceeds the biliary excretion rate resulting in progressive accumulation of ICG within the liver (Burczynski *et al.*, 1987). In these experiments, biliary excretion rates (Figure 5) were substantially lower than the infusion rates expressed per 100 g liver. The accumulation of ICG within the liver results in the progressively slower removal of repeated bolus doses (Burczynski *et al.*, 1987) and in the constantly rising plasma ICG levels during infusion of ICG. At a constant blood flow, extraction appears to be inversely related to the amount of ICG in the liver (Figure 3). However, in our previous study using bolus doses (Burczynski *et al.*, 1987), mean extraction declined with repeated bolus doses with a slope of 0.013 for each μmol ICG accumulated per 100 g liver, while in these experiments with infusions of ICG, mean extraction declined with a slope of 0.070. Thus accumulation appeared to reduce extraction five times more rapidly during an infusion than after a bolus dose. The reasons for this are not clear and it is not at present possible to eliminate a time-dependent component in the decline in extraction of ICG, since accumulation and time always go hand-in-hand. When hepatic blood flow was changed, extraction changed in a complex way (Figure 3) and for at least an hour, a high flow appeared to eliminate the effects of hepatic accumulation on extraction.

ICG has been found to show dose-dependent (Michaelis-Menten) kinetics (Hunton *et al.*, 1960; Paumgartner *et al.*, 1970), but there are difficulties with this interpretation (Stoeckel *et al.*, 1980; Burczynski *et al.*, 1987). Although the early slope of a log plasma concentration-time plot declines with increasing dose, these curves are not convex to the abscissae as expected for Michaelis-Menten kinetics. This decline in the early slope with increasing dose could be the result of increasing hepatic accumulation of ICG during the first minutes after administration. It is extremely difficult to study Michaelis-Menten kinetics in the presence of an accumulation effect. In addition, ICG kinetics do not appear to follow the predictions of either of the major models for hepatic elimination of drugs — the 'parallel-tube model' and the 'equilibrium model'. These models, the respective definitions of intrinsic clearance and the predicted effects of flow have been summarized by Keiding (1976) and Keiding & Andreasen (1979). Since extraction was quite low in our experiments, the logarithmic mean sinusoidal concentration and the hepatic venous concentration tended to change in parallel. The intrinsic clearances calculated for the two models were therefore numerically different but followed a similar pattern. Since the 'parallel-tube model' takes into account the decreasing concentration along the sinusoid and is therefore inherently more physiological, we have shown the data for this model in Figure 4. During constant control flow, intrinsic clearance declines with accumulation of ICG in the liver. The model predicts that intrinsic clearance is independent of blood flow and we therefore expected a similar relationship during the changes in hepatic flow in Series 2. As Figure 4 shows, the data during control and reduced flow appear to fit this line reasonably well. However intrinsic clearance is clearly greater during the increased flow period and is not independent of blood flow as the model predicts. These results are in agreement with the data but not the interpretation of Krarup & Larsen (1976). They observed an increase in 'true clearance' (calculated from arithmetic rather than logarithmic mean sinusoidal concentration) when hepatic flow was increased above normal, but attributed this to the presence of Macrodex (dextran plasma protein substitute) which was used to increase the flow. Our data suggest the effect is truly a consequence of the increased blood flow and ICG does not appear to follow the predictions of the model when flow is increased. One important factor not considered in our experiments is the role of binding of ICG to albumin and α_1-lipoproteins in plasma and to ligandin within the hepatocytes. However, it is not obvious why increasing hepatic flow should alter protein binding of ICG in a way that increases hepatic uptake.

The mechanism by which accumulation of ICG in the liver reduces net hepatic ICG uptake is not clear. It might be that uptake is unchanged but back-diffusion from the liver cells into the plasma increases. This is incorporated in the model of Stoeckel *et al.* (1980). However, we believe this is unlikely (Burczynski *et al.*, 1987). To compare what is happening to the overall concentration gradient between sinusoid and hepatocyte, we have plotted logarithmic mean sinusoidal concentration against hepatocyte concentration assuming 80% of liver weight is hepatocyte volume (Blouin *et al.*, 1977). These data are shown in Figure 6 for all measurements made at control blood flow. It appears that the logarithmic mean sinusoidal concentration increases linearly ($P < 0.01$) as liver concentration increases. By extrapolation, the sinusoidal concentration at zero accumulation is 0.3 μM for the low dose and 0.6 μM for the high dose. Thus doubling the infusion rate, doubles the logarithmic mean sinusoidal concentration when the effect of hepatic accumulation of ICG is eliminated and there is no indication of dose-related kinetics at these doses. The slopes of the relationships are quite variable but suggest that the concentration of ICG within the

hepatocytes is 60–115 (mean 88) times greater than the mean sinusoidal plasma concentration. This may reflect the very high degree of intracellular binding of ICG within the hepatocyte but we have no data on whether or not the free concentrations of ICG in plasma and hepatocytes are in equilibrium.

Bile flow rates and biliary ICG excretion rates showed extreme variability between animals and the reasons for this are not clear. During constant blood flow, biliary flow declined during ICG infusion in Series 1 but it did not decline when ICG was infused at double the rate in Series 2. This does not support a dose-dependent effect of ICG on bile flow (Klaassen & Plaa, 1969). However, effects of ICG on bile flow cannot be assessed in the absence of measurements of plasma bile acid levels since the decline in bile flow may reflect bile acid depletion. Although the data (Figure 5) provide some further indication that changes in hepatic blood flow may influence biliary ICG excretion rates, the effects were not statistically significant and require further study.

In summary, our data support the validity and accuracy of the estimation of hepatic blood flow during infusions of ICG, provided that at least 30 min are allowed for equilibration of extrahepatic distribution. This method appears to be more accurate than methods based on bolus injections of ICG (Burczynski *et al.*, 1987) or infusions of galactose (Burczynski & Greenway, 1986). However the kinetics of hepatic ICG uptake are complex. The steady decline in extraction and rise in plasma levels of ICG during a constant infusion seems to be related to accumulation of ICG within the liver, but we cannot yet provide a satisfactory quantitative explanation for these effects of accumulation which can be applied to both these experiments and our previous data with bolus doses of ICG.

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References

BANASZAK, E.F., STEKIEL, W.J., GRACE, R.A. & SMITH, J.J. (1960). Estimation of hepatic blood flow using a single injection dye clearance method. *Am. J. Physiol.*, **198**, 877–880.

BLOUIN, A., BOLENDER, R.P. & WEIBEL, E.R. (1977). Distribution of organelles and membranes between hepatocytes and nonhepatocytes in the rat liver parenchyma. *J. cell Biol.*, **72**, 441–455.

BRADLEY, S.E. (1963). The hepatic circulation. In *Handbook of Physiology*. Sect. 2, Vol. 2, pp. 1387–1438. Bethesda, M.D.: Am. Physiol. Soc.

BRAILLON, A., JIRON, M.I., VALLA, D., CALES, P. & LEBREC, D. (1985). Effect of propranolol on hepatic blood flow in patients with cirrhosis. *Clin. Pharmac. Ther.*, **37**, 376–380.

BURCZYNSKI, F.J. & GREENWAY, C.V. (1986). Hepatic blood flow: accuracy of estimation from galactose clearances in cats. *Can. J. Physiol. Pharmac.*, **64**, 1310–1315.

BURCZYNSKI, F.J., PUSHKA, K.L., SITAR, D.S. & GREENWAY, C.V. (1987). Hepatic blood flow: Accuracy of estimation from bolus injections of ICG. *Am. J. Physiol.*, (in press).

CAESAR, J., SHALDON, S., CHIANDUSSI, L., GUEVARA, L. & SHERLOCK, S. (1961). The use of indocyanine green in the measurement of hepatic blood flow and as a test of hepatic function. *Clin. Sci.*, **21**, 43–57.

CHAUVIN, M., BONNET, F., MONTEMBAULT, C., LAFAY, M., CURET, P. & VIARS, P. (1985). Hepatic plasma flow during sodium nitroprusside induced hypotension in humans. *Anesthesiology*, **63**, 287–293.

ESCOURROU, P., FREUND, P.R., ROWELL, L.B. & JOHNSON, D.G. (1982). Splanchnic vasoconstriction in heat-stressed men: role of renin-angiotensin system. *J. appl. Physiol.*, **52**, 1438–1443.

GASIC, S., EICHLER, H.G. & KORN, A. (1985). Effect of ketanserin on phenylephrine-dependent changes in splanchnic hemodynamics and systemic blood pressure in healthy subjects. *J. cardiovasc. Pharmac.*, **7**, 219–223.

HUNTON, D.B., BOLLMAN, J.L. & HOFFMAN, H.N. (1960). Studies of hepatic function with indocyanine green. *Gastroenterology*, **39**, 713–724.

KEIDING, S. (1976). Hepatic elimination kinetics: the influence of hepatic blood flow on clearance determinations. *Scand. J. clin. Lab. Invest.*, **36**, 113–118.

KEIDING, S. & ANDREASEN, P.B. (1979). Hepatic clearance measurements and pharmacokinetics. *Pharmacology*, **19**, 105–110.

KLAASSEN, C.D. & PLAA, G.L. (1969). Plasma disappearance and biliary excretion of indocyanine green in rats, rabbits and dogs. *Tox. appl. Pharmac.*, **15**, 373–384.

KRARUP, N. & LARSEN, J.A. (1976). The influence of dye infusion rate and hepatic plasma flow on indocyanine green clearance. *Scand. J. clin. Lab. Invest.*, **36**, 183–188.

LEAMAN, D.M., LEVENSON, L., ZELIS, R. & SHIROFF, R. (1978). Effect of morphine on splanchnic blood flow. *Br. Heart J.*, **40**, 569–571.

LEEVY, C.M., MENDENHALL, C.L., LESKO, W. & HOWARD, M.M. (1962). Estimation of hepatic blood flow with indocyanine green. *J. clin Invest.*, **41**, 1169–1179.

NOWAK, J. & WENNMALM, A. (1978). Influence of indomethacin and of prostaglandin E_1 on total and regional blood flow in man. *Acta physiol. scand.*, **102**, 484–491.

PAUMGARTNER, G., PROBST, P., KRAINES, R. & LEEVY, C.M. (1970). Kinetics of indocyanine green removal from the blood. *A. New York Acad. Sci.*, **170**, 134–147.

ROWELL, L.B., BLACKMON, J.R., KENNY, M.A. & ESCOURROU, P. (1984). Splanchnic vasomotor and metabolic adjustments to hypoxia and exercise in

humans. *Am. J. Physiol.*, **247,** H251–H258.

SONNENBERG, G.E., KELLER, U., PERRUCHOUD, A., BURCKHARDT, D. & GYR, K. (1981). Effect of somatostatin on splanchnic hemodynamics in patients with cirrhosis of the liver and in normal subjects. *Gastroenterology*, **80,** 526–532.

STEEL, R.G.D. & TORRIE, J.H. (1960). *Principles and Procedures of Statistics*. New York: McGraw-Hill Book Company, Inc.

STEKIEL, W.J., KAMPINE, J.P., BANASZAK, E.F. & SMITH, J.J. (1960). Hepatic clearance of indocyanine in the dog. *Am. J. Physiol.*, **198,** 881–885.

STOECKEL, K., MCNAMARA, P.J., MCLEAN, A.J., DUSOUICH, P., LALKA, D. & GIBALDI, M. (1980). Non linear pharmacokinetics of indocyanine green in the rabbit and rat. *J. Pharmacokin. Biopharm.*, **8,** 483–496.

WHEELER, H.O., CRANSTON, W.I. & MELTZER, J.I. (1958). Hepatic uptake and biliary excretion of indocyanine green in the dog. *Proc. Soc. exp. Biol. Med.*, **99,** 11–14.

WHEELER, H.O., EPSTEIN, R.M., ROBINSON, R.R. & SNELL, E.S. (1960). Hepatic storage and excretion of sulfobromophthalein sodium in the dog. *J. clin. Invest.*, **39,** 236–247.

WIEGAND, B.D., KETTERER, S.G. & RAPAPORT, E. (1960). The use of indocyanine green for the evaluation of hepatic function and blood flow in man. *Am. J. Dig. Dis.*, **5,** 427–436.

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