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THE EFFECT OF ACUTE RENAL FAILURE ON THE PHARMACOKINETICS OF INDOCYANINE GREEN IN THE RAT

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Abstract—The pharmacokinetics of indocyanine green (ICG) have been studied in control rats and rats with acute renal failure (ARF). Three models of ARF were investigated, and these were produced in the following ways: by bilateral ureteral ligation (surgical model), or by parenteral injection of either uranyl nitrate or glycerol. In both control and uraemic rats, from all three models of ARF, the plasma disappearance of ICG was biexponential and from the plasma disappearance curves the rate constants for transfer from plasma to liver (k_{12}), liver to plasma (k_{21}); and liver to bile (k_{23}) were calculated. The initial removal of ICG from plasma was significantly slowed and k_{12} significantly decreased in male rats with surgically or uranyl nitrate-induced ARF. The plasma clearance of ICG was not affected by surgically-induced ARF but clearance was reduced in the uranyl nitrate model. More complex changes occurred in male rats with glycerol-induced ARF as k_{12}, k_{21}, k_{23} and plasma clearance of ICG were all significantly decreased. Female rats with glycerol-induced ARF showed significant decreases in k_{12}, and plasma clearance, but these changes were much smaller than in uraemic males.

Of the three models of ARF studied the glycerol model was preferred because surgery alone had a pronounced effect upon the kinetics of ICG and wet liver weight was significantly decreased in the uranyl nitrate model. These changes complicated interpretation of the results from the surgical and uranyl nitrate models. The results from the glycerol model suggest that the hepatic uptake of ICG, and possibly its biliary excretion, were reduced in rats with ARF. In addition, liver function in female rats appears to be more resistant than that in males to the effects of glycerol-induced ARF.

Acute and chronic renal failure in the rat [1] and chronic renal failure in man [2] are known to affect the rate of biotransformation of many drugs by the liver. Recent studies in rats with acute renal failure [3] and in patients with chronic renal failure [4] have shown that the presystemic clearance of drugs may also be decreased. Furthermore, the hepatic metabolism of carbohydrates, lipid and protein are also thought to be altered in the uraemic state [5]. Thus there is evidence to suggest that many of the processes that contribute to the normal function of the liver are perturbed in renal failure. However, few investigations have been carried out to discover whether the uptake, storage and biliary excretion of substances by the liver are altered in uraemia. Evidence from an animal model [6] and man [7] suggests that renal failure can result in decreased uptake, storage and biliary excretion of substances like rose bengal and bromosulphophthalein. The cause of these changes and their effects on the disposition and elimination of drugs have received little attention.

The present study was designed to investigate the effect of acute renal failure (ARF)† upon liver function in the rat by determination of the pharmacokinetics of indocyanine green (ICG). ICG is a convenient compound to use because there is sufficient evidence to believe that it has little extravascular distribution [8–10] and that it is excreted exclusively into the bile without biotransformation [9, 11–13]. A further aim of the study was to investigate various models of ARF in the rat in order to identify the most suitable model for pharmacokinetic studies of liver function in ARF. ARF in the rat was produced in three different ways: by bilateral ligation of the ureters and by parenteral administration of either uranyl nitrate or glycerol. These models were chosen because they represent three different categories of ARF [14] namely; ARF caused by tubular obstruction (ureter ligation), nephrotoxic ARF (uranyl nitrate) and haemodynamically-mediated ARF (glycerol). The glycerol model of ARF resembles the 'crush syndrome' in man since intramuscular injection of glycerol results in myohaemoglobinuria which leads to renal ischaemia [14].

Whilst each model produced characteristic changes in the pharmacokinetics of ICG, the common finding was a decrease in the rate of primary uptake of ICG into the liver, which suggests that the biliary excretion of ICG may be delayed. A preliminary report of this work has appeared [15].

MATERIALS AND METHODS

Chemicals. ICG was purchased from Hynson, Wescott and Dunning Ltd. (Baltimore, MD). Reagents for the determination of total protein, urea and bilirubin were obtained from Sigma Chemical Co. (Poole, U.K.). All other reagents were available commercially and of analytical grade.

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† Abbreviations used: ICG, indocyanine green; ARF, acute renal failure; BSP, bromosulphophthalein; bovine serum albumin, BSA.

Renal failure induced by bilateral ligation of ureters. ARF was produced in male Wistar albino rats by bilateral ligation of the ureters under halothane anaesthesia (1.5% v/v halothane in 95% O_2:5% CO_2). Control rats (sham operated) received the same surgical treatment during which both ureters were manipulated. Both groups of rats were studied 24 hr after surgery.

Uranyl nitrate-induced ARF. Male Wistar albino rats were injected, via the tail vein, with uranyl nitrate (5 mg/kg) dissolved in sterile saline [16]. Control rats received an equal volume of saline. Both groups of animals were studied five days after the injection.

Glycerol-induced ARF. Acute renal failure was also produced by intramuscular injection of glycerol [17]. Male and female Wistar albino rats were deprived of drinking water for 24 hr but allowed food *ad lib.* An intramuscular injection of 50% v/v glycerol in sterile saline (0.9% NaCl w/v), 10 ml/kg body wt, was then administered, under ether anaesthesia, in divided doses to two sites in each of the hind limbs. The drinking water was immediately restored. Control rats (sham injected) were similarly dehydrated but injected with sterile saline only, (10 mg/kg body wt). Both groups of rats were studied 48 hr after the i.m. injection of either saline or glycerol.

Experimental protocol. Rats were anaesthetised with pentobarbitone (60 mg/kg, body wt, i.p.): a tracheal cannula was inserted and artificial respiration was maintained with a Miniature Ideal Pump (BioScience) (ventilation rate 80 strokes/min and stroke volume 10 ml/kg body wt). Cannulae were also inserted into the left jugular vein and right carotid artery. Body temperature was maintained at 37° by means of a heating lamp.

ICG (7.5 mg/kg) was administered i.v. (jugular vein) as an aqueous solution (10 mg/ml). Heparinised blood samples (0.1 ml) from the carotid artery were taken for 60 min. After each sample was collected, blood was replaced by an equal volume of saline infused through the venous cannula. Packed cell volume (PCV) was determined for blood samples taken prior to the injection of ICG (0 min) and after the last blood sample (60 min). The PCV was about 6% lower in all groups of rats at the end of the sampling period.

Measurement of plasma ICG concentration. Standard solutions of ICG were prepared by dissolving the dye in distilled water containing 4% w/v bovine serum albumin (BSA). The dye–BSA solutions were diluted ten-fold with rat plasma and calibration curves were done by addition of 50 μl, of the rat plasma–BSA standards, to 1.5 ml of distilled water. The absorbance of these solutions was measured promptly, after the addition of distilled water, at 800 nm [18]. The final concentration range of the dye standards was between 0.25 and 4.0 μg/ml. It was found necessary to prepare a new calibration curve for each batch of ICG used.

For the assay of ICG in plasma, 25 or 50 μl of plasma were diluted with 1.5 ml of distilled water and absorbances were measured immediately at 800 nm with a Cecil 272 linear readout u.v. spectrophotometer [18].

Total plasma protein, urea and bilirubin. Standard spectrophotometric assays were used: total plasma protein was measured by the biuret method (BSA as standard), urea by reaction with diacetyl monoxime and total bilirubin (unconjugated and conjugated derivatives) by coupling with diazotized sulphanilic acid to produce azobilirubin.

Histological methods. Livers were removed from male rats with glycerol-induced ARF and samples of tissue were taken from the two major lobes, fixed in 10% buffered formal-saline and embedded in paraffin wax. Sections 5 μm thick were cut and stained using standard staining methods, namely haematoxylin and eosin and periodic acid Schiff both before and after diastase treatment, reticulin stain and methyl green-pyronin. Stained sections were examined by light microscopy.

Pharmacokinetic analysis. The concentration–time curves were fitted to a biexponential equation ($Ae^{-\alpha t} + Be^{-\beta t}$) by non-linear least squares regression analysis [19]. Experimental values were weighted inversely proportional to the slope of the fitted curve.

Rate constants for transport of ICG from plasma into liver (k_{12}): for efflux from liver to plasma (k_{21}) and for transport from liver to bile (k_{23}) were calculated using the equations given by Gibaldi and Perrier [20]. These rate constants are in accordance with a two-compartment model with elimination of ICG from the peripheral compartment (Fig. 1). It is reasonable to assume that the central compartment corresponds to plasma and the peripheral compartment to the liver, because ICG has little extra-vascular distribution [8–10]; it is highly protein bound [18, 21] and it is eliminated exclusively by the liver into bile [9, 10]. Other pharmacokinetic parameters were calculated from the following relationships.

(a) *Apparent volume of the central compartment (Vc).* The apparent volume of the central compartment was calculated as:

$$Vc = \frac{\text{Dose}}{A + B}$$

where A and B are the intercept values at zero time for the individual α- and β-phases [20].

(b) *Apparent volume of distribution at steady-state (Vdss).* The apparent volume of distribution at

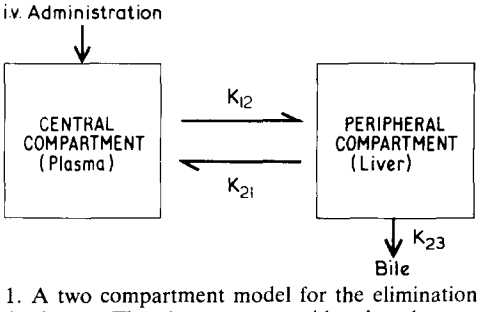

Fig. 1. A two compartment model for the elimination of ICG in the rat. The plasma was considered as the central compartment and the liver as the peripheral compartment from which elimination occurs into bile. The rate constants for primary uptake into the liver (k_{12}), reflux from the liver (k_{21}) and transport from liver to bile (k_{23}) were calculated using the equations given by Gibaldi and Perrier [20].

steady-state was calculated from the equation

$$Vdss = Vc\frac{k_{12} + k_{21} + k_{23}}{k_{21} + k_{23}}$$

The equation for calculation of Vdss was derived by the method given by Riggs [22].

(c) *Apparent volume of the peripheral compartment (Vp)*. The apparent volume of the peripheral compartment was calculated from:

$$Vp = Vdss - Vc$$

(d) *Plasma clearance (Clp)*. Plasma clearance was calculated rom the equation

$$Clp = \frac{Dose}{AUC_{i.v.}}$$

where $AUC_{i.v.}$ is the area under the concentration–time curve from zero to infinity following intravenous administration of ICG. $AUC_{i.v.}$ is given by the equation

$$AUC_{i.v.} = \frac{A}{\alpha} + \frac{B}{\beta}$$

where α- and β- are the slopes of the individual α- and β-phases.

Statistical analysis. Results are expressed as mean ±S.D. and statistical comparison was made by the non-paired Student's *t*-test.

RESULTS

Bilateral ligation of ureters

Table 1 shows the results for the analysis of blood and plasma obtained from both sham operated and ligated male rats 24 hr after surgery. In the ligated rats, there was a large increase in plasma urea concentration and a significant decrease in PCV when compared to the corresponding value for the sham-operated animals. Total plasma protein concentration and wet liver weight were not significantly affected by ureter ligation.

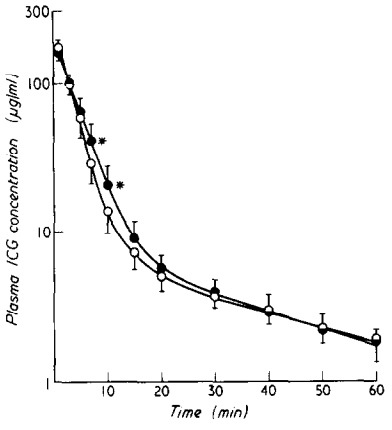

Fig. 2. Plasma concentrations of ICG (7.5 mg/kg i.v.) in male sham operated rats (○) and male rats with ureter ligation (●). Each point is the mean ±S.D. of eight sham operated rats and six rats with ligated ureters. Key: * significantly different from control values ($P < 0.05$).

Mean plasma concentrations of ICG are shown in Fig. 2. The two groups differed only in a greater mean plasma concentration at 7 and 10 min in the uraemic rats. However, the rats with ARF had a significantly greater α-phase half-life and a smaller value of k_{12} than the sham-operated animals (Table 2), suggesting a reduction in the rate of primary hepatic uptake of ICG. There was no significant change in the plasma clearance of ICG possibly because the decrease in k_{12} was accompanied by a significant increase in Vc (Table 2). The latter change may be the result of fluid retention in the uraemic rats.

Comparison of the pharmacokinetic data from normal and sham-operated male rats revealed a number of differences (Table 2). In the sham-operated rats, k_{12} was significantly decreased but, by contrast, a significant increase in k_{23} was observed. In addition, Vp and Vdss were significantly smaller after the

Table 1. Body weight, liver weight, packed cell volume (PCV), total plasma protein and urea concentrations in the rat for the surgical, uranyl nitrate and glycerol models of acute renal failure*

Group	Body weight (g)	Liver weight (g/100 g body wt)	PCV (%)	Plasma protein (g/100 ml)	Plasma urea (mg/100 ml)
Surgical model					
Normal male	336 ± 34(12)	3.7 ± 0.4 (12)	48 ± 2(9)	N.D.	22 ± 6(12)
Sham-operated male	294 ± 20(8)	3.8 ± 0.2(8)	47 ± 2(6)	6.5 ± 1.1(7)	25 ± 5(8)
Ligated male	308 ± 28(6)	3.8 ± 0.2(6)	43 ± 3(6)†	6.1 ± 0.4(5)	164 ± 38(6)‡
Uranyl nitrate model					
Control male	326 ± 42(6)	3.9 ± 0.2(4)	47 ± 3(6)	6.3 ± 0.5(6)	52 ± 6(6)
Uraemic male	319 ± 25(6)	3.1 ± 0.3(6)†	47 ± 4(6)	6.0 ± 0.8(6)	313 ± 82(6)‡
Glycerol model					
Control male	332 ± 28(9)	3.8 ± 0.4(9)	47 ± 2(9)	6.1 ± 0.5(9)	23 ± 3(9)
Uraemic male	345 ± 25(9)	3.5 ± 0.2(9)	43 ± 4(8)†	6.4 ± 0.6(9)	104 ± 53(9)‡
Control female	211 ± 34(9)	3.9 ± 0.4(9)	45 ± 3(7)	6.9 ± 0.3(5)	17 ± 4(9)
Uraemic female	221 ± 18(9)	4.0 ± 0.2(9)	38 ± 5(7)†	6.8 ± 0.5(5)	152 ± 85(9)‡

* Results are given as mean ±S.D. and number of rats in parentheses.
N.D. = not determined.
† $P < 0.01$; ‡ $P < 0.001$ relative to respective control group.
Measurements were done 24 hr after bilateral ureteral ligation (surgical model); 5 days after i.v. uranyl nitrate and 48 hr after i.m. glycerol.

sham operation and these changes could not be accounted for by a difference in mean body weight between the two groups of rats (Table 1). Although the sham operation did not alter the plasma clearance of ICG it is clear from these data that surgery alone had a significant effect upon the pharmacokinetics of ICG. For this reason the surgical model of renal failure was not investigated further.

Uranyl nitrate-induced ARF

The control rats used in these experiments had higher plasma urea levels than the control counterparts of the other models of ARF (Table 1); but the reason for this is not clear. However, plasma urea levels were significantly elevated five days after the intravenous administration of uranyl nitrate (Table 1). In these uraemic rats there was no significant change in either the total plasma protein concentration or PCV; but wet liver weight was significantly decreased.

The plasma concentration–time data on ICG in this series of experiments are shown in Fig. 3. Mean plasma concentrations of ICG, in the period 5–20 min after administration, were higher in the rats with ARF than in the controls. Consequently the half-life of the α-phase was significantly increased and there was a significant decrease in both k_{12} and the plasma clearance of ICG (Table 3). These changes are similar to those observed in the surgical model of ARF and clearly indicate that the primary rate of hepatic uptake of ICG was decreased in the uraemic rats. However, wet liver weight was significantly lower in the uraemic rats, and so the decrease in k_{12} may be the result of a decrease in the functional mass of the liver, rather than an effect of ARF upon liver function.

Glycerol-induced ARF in the male rat

Forty-eight hours after the injection of glycerol, plasma urea levels were significantly increased and similar to those obtained for the surgical model of ARF (Table 1). Glycerol-induced ARF resulted in a significant decrease in PCV but no change was observed in wet liver weight or plasma protein concentration. Also, the mean total plasma bilirubin concentration in control rats (0.16 ± 0.06 mg/100 ml; $N = 8$) was not significantly different from that in rats with ARF (0.18 ± 0.10 mg/100 ml; $N = 7$).

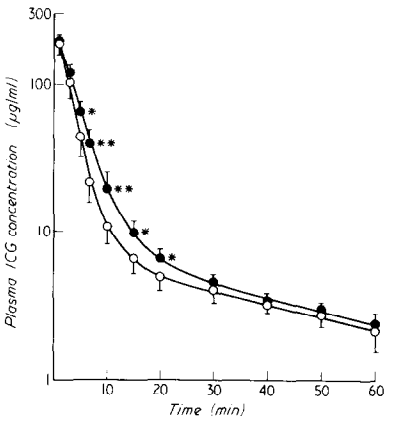

Fig. 3. Plasma concentrations of ICG (7.5 mg/kg i.v.) in male control (saline injected) rats (○) and male rats with uranyl nitrate-induced ARF (●). Each point is the mean ±S.D. of six rats. Key: * $P < 0.05$; ** $P < 0.01$ relative to respective control value.

The effect of glycerol-induced ARF on the plasma concentration–time data for ICG is shown in Fig. 4 and the pharmacokinetic parameters derived from these data are given in Table 4. The half-lives of both α- and β-phases were significantly prolonged in the uraemic rats and there were significant decreases in k_{12}, k_{21} and k_{23}. The percentage decrease in k_{12} (46%) was larger than that for k_{21} (35%) or k_{23} (27%). The decrease in these rate constants was accompanied by a significant reduction in the plasma clearance of ICG (Table 4). No significant changes were observed for Vdss or Vp; but Vc was increased in the rats with ARF. The percentage increase in Vc (32%) was similar to that observed in the surgical model of ARF (23%).

Glycerol-induced ARF in the female rat

Table 1 shows that a similar degree of renal failure developed in the female rats because there was no significant difference between the mean plasma urea concentrations for male and female rats with ARF.

Table 2. Effect of sham operation and bilateral ligation of the ureters on the pharmacokinetics of ICG (7.5 mg/kg, i.v.) in male rats*

Pharmacokinetic parameters	Normal rats ($N = 12$)	Sham-operated rats ($N = 8$)	Ligated rats ($N = 6$)
$t_{0.5}$ α (min)	1.7 ± 0.2	2.1 ± 0.3‡	2.7 ± 0.4‖
$t_{0.5}$ β (min)	35.6 ± 3.7	31.6 ± 3.5†	32.0 ± 8.3
k_{12}(min^{-1})	0 4009 ± 0.0532	0.3240 ± 0.0474‡	0.2528 ± 0.0362‖
k_{21}(min^{-1})	0.0079 ± 0.0012	0.0084 ± 0.0005	0.0073 ± 0.0017
k_{23}(min^{-1})	0.0201 ± 0.0021	0.0230 ± 0.0028†	0.0237 ± 0.0063
Vc (ml)	8.8 ± 1.2	9.0 ± 1.0	11.1 ± 1.4‖
Vp (ml)	126.8 ± 21.4	92.1 ± 12.3§	95.6 ± 27.9
Vdss (ml)	135.6 ± 22.0	101.0 ± 12.6§	106.7 ± 29.3
Clp (ml/min/100 g body wt)	0.75 ± 0.09	0.72 ± 0.10	0.69 ± 0.10

* Results are given as mean ± S.D.
† $P < 0.05$; ‡ $P < 0.01$; § $P < 0.001$ relative to normal rats.
‖ $P < 0.01$; relative to sham-operated rats.

Table 3. Effect of uranyl nitrate-induced acute renal failure on the pharmacokinetics of ICG (7.5 mg/kg i.v.) in male rats*

Pharmacokinetic parameters	Control rats ($N = 6$)	Uraemic rats ($N = 6$)	% Change
$t_{0.5}$ α (min)	1.7 ± 0.2	2.4 ± 0.4‡	+41.2
$t_{0.5}$ β (min)	37.6 ± 10.5	35.7 ± 4.9	−5.1
k_{12} (min^{-1})	0.3939 ± 0.0392	0.2838 ± 0.0491‡	−28.0
k_{21} (min^{-1})	0.0090 ± 0.0014	0.0077 ± 0.0007	−14.4
k_{23} (min^{-1})	0.0201 ± 0.0055	0.0203 ± 0.0026	+1.0
Vc (ml)	8.6 ± 1.1	9.3 ± 1.2	+8.1
Vp (ml)	119.7 ± 26.2	93.4 ± 12.5	−22.0
Vdss (ml)	128.3 ± 26.3	102.7 ± 13.1	−20.0
Clp (ml/min/100 g body wt)	0.72 ± 0.12	0.59 ± 0.06†	−18.1

* Results are given as mean ±S.D.
† $P < 0.05$; ‡ $P < 0.01$ relative to respective control group.

Table 4. Effect of glycerol-induced acute renal failure on the pharmacokinetics of ICG (7.5 mg/kg, i.v.) in male rats*

Pharmacokinetic parameters	Control rats ($N = 9$)	Uraemic rats ($N = 9$)	% Change
$t_{0.5}$ α (min)	1.6 ± 0.2	3.0 ± 0.6‡	+87.5
$t_{0.5}$ β (min)	29.5 ± 4.5	42.8 ± 12.2†	+45.1
k_{12} (min^{-1})	0.4393 ± 0.0649	0.2361 ± 0.0492‡	−46.3
k_{21} (min^{-1})	0.0083 ± 0.0018	0.0054 ± 0.0012‡	−34.9
k_{23} (min^{-1})	0.0245 ± 0.0038	0.0178 ± 0.0050†	−27.3
Vc (ml)	8.5 ± 1.6	11.2 ± 1.4†	+31.8
Vp (ml)	116.7 ± 33.8	117.9 ± 29.3	+1.0
Vdss (ml)	125.3 ± 35.1	129.2 ± 30.5	+3.1
Clp (ml/min/100 g body wt)	0.84 ± 0.18	0.59 ± 0.14†	−29.8

* Results are given as mean ±S.D.
† $P < 0.01$; ‡ $P < 0.001$ relative to respective control group.

Table 5. Effect of glycerol-induced acute renal failure on the pharmacokinetics of ICG (7.5 mg/kg, i.v.) in female rats*

Pharmacokinetic parameters	Control rats ($N = 9$)	Uraemic rats ($N = 9$)	% Change
$t_{0.5}$ α (min)	1.4 ± 0.2	2.0 ± 0.4‡	+42.9
$t_{0.5}$ β (min)	29.4 ± 2.3	35.3 ± 8.4	+20.1
k_{12} (min^{-1})	0.4781 ± 0.0714	0.3567 ± 0.0690‡	−25.4
k_{21} (min^{-1})	0.0099 ± 0.0021	0.0091 ± 0.0019	−8.1
k_{23} (min^{-1})	0.0242 ± 0.0018	0.0211 ± 0.0045	−12.8
Vc (ml)	6.2 ± 1.0	7.2 ± 1.0†	+16.1
Vp (ml)	87.5 ± 20.5	86.1 ± 18.8	−1.6
Vdss (ml)	93.7 ± 21.4	93.4 ± 19.2	−0.3
Clp (ml/min/100 g body wt)	1.00 ± 0.16	0.80 ± 0.18†	−20.0

* Results are given as mean ±S.D.
† $P < 0.05$; ‡ $P < 0.01$ relative to respective control group.

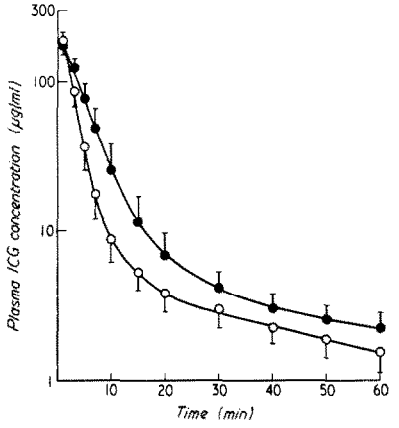

Fig. 4. Plasma concentrations of ICG (7.5 mg/kg i.v.) in male control (saline injected) rats (○) and male rats with glycerol-induced ARF (●). Each point is the mean ±S.D. of nine rats. With the exception of the 1 min sample, all concentrations in plasma from rats with ARF were significantly different from control values ($P < 0.05$).

Renal failure was accompanied by a significant decrease in PCV but no changes in liver weight or plasma protein concentration were observed (Table 1).

In contrast to the male rats, glycerol-induced ARF in the females produced a significant decrease in k_{12} only and a significant decrease in the plasma clearance of ICG (Table 5). No significant changes in Vdss or Vp were found although Vc was increased in the rats with ARF. The changes in the rate constants and plasma clearance of ICG in the uraemic female rats (Table 5) were considerably smaller than those for their male counterparts (Table 4); although the severity of renal failure in both groups of animals was similar (Table 1). These results suggest that the processes involved with the removal of ICG from blood are less sensitive in female rats than in males to the effects of glycerol-induced ARF.

Histological examination of liver slices from male rats with glycerol-induced ARF

A total of thirteen livers were examined by light microscopy. Three livers appeared normal; eight had minor changes in the centrilobular cells consisting of pallor, due to loss of ribosomes, and in some cases hydropic vacuolation was evident. Two livers showed more pronounced changes in that small areas of coagulative necrosis were found; but this lesion appeared to be of 3–5 days duration and may have been due to coincidental liver infection. However, in all livers examined the vast majority of liver cells appeared normal.

DISCUSSION

It is clear from the results that the pharmacokinetic behaviour of ICG was modified in all three models of ARF. The principal change was a decrease in the rate constant for the entry of ICG into the peripheral compartment (k_{12}) and this suggests that the primary rate of hepatic uptake was decreased in rats with ARF. The changes that occurred in the individual models of ARF are discussed in the following paragraphs.

Surgical model of ARF

Surgical models have been used to study the effects of chronic renal failure in the rat upon liver function and showed that the hepatic uptake of rose bengal [6] or *p*-aminobenzoic acid [23] were diminished. Our findings, using rats with ARF, were similar in that the primary rate of hepatic uptake (k_{12}) of ICG was decreased. No change in the plasma clearance of ICG was observed possibly because the change in k_{12} was accompanied by a significant increase in Vc (Table 2).

Giacomini *et al.* [24] have pointed out that surgical models of ARF have limitations such as a high rate of mortality and poor reproducibility of the degree of uraemia. We did not experience any of these difficulties but it was clear from the results (Table 2) that the sham-operation alone had a pronounced effect upon the kinetics of ICG. In view of these problems and those encountered by Giacomini *et al.* [24], it would appear that this is not a good model with which to study the effect of renal failure upon liver function.

Uranyl nitrate model of ARF

The induction of ARF with uranyl nitrate is a simple procedure and the mortality rate associated with it is low (no deaths occurred). However, wet liver weight was significantly decreased and this could have been responsible for the changes both in k_{12} and in the plasma clearance of ICG (Table 3). The severe degree of uraemia that developed in the rats with ARF, and/or a hepato-toxic effect of uranyl nitrate may be the cause of the decrease in wet liver weight. The latter seems unlikely because Giacomini *et al.* [24] found no evidence of gross hepatic toxicity in the rat, after a single injection of uranyl nitrate, in that glutamic–pyruvic transaminase levels in serum were normal. Because of this decrease in liver weight the uranyl nitrate model was not investigated any further.

Glycerol model of ARF

The changes in the kinetics of ICG were more complex in this model of ARF than in the surgical or uranyl nitrate models (Table 4). In addition to a significant decrease in k_{12}, both k_{21} and k_{23} were also decreased, but to a lesser extent. The decreases in both k_{12} and k_{23} suggest that the biliary excretion of ICG may be slowed in this model. The plasma clearance of ICG was decreased despite a significant increase in the volume of the central compartment (Vc). The latter change was probably the result of plasma expansion, due to fluid retention in the uraemic rats, as this stage of ARF is characterised by severe oliguria [17].

At the dose of ICG used (7.5 mg/kg), the removal of dye from plasma is partially dependent upon liver blood flow [25]. Decreased blood flow to the liver cannot explain the decrease in ICG clearance because at the time when these experiments were done (48 hr after glycerol injection) liver blood flow is known to be increased [26]. ICG is highly bound to plasma proteins [18, 21] and ARF in the rat can

decrease the binding of many substances to plasma proteins [27]. An increase in the unbound fraction of ICG is more likely to result in increased plasma clearance of the dye because a proportional relationship exists between the unbound fraction of dyes with high hepatic clearances and their removal from plasma [28, 29]. In view of this evidence it is unlikely that changes in afferent liver blood flow or plasma protein binding were responsible for the altered kinetics of ICG in rats with glycerol-induced ARF.

It is possible that the pharmacokinetic changes observed in this model were brought about by a toxic effect of glycerol upon the liver. However, there is some evidence, albeit indirect, to suggest that the changes found in the kinetics of ICG were due to ARF alone. Firstly, the major difference between control rats and those with ARF was a significant decrease in k_{12}. Since this also occurred in the surgical model, then the change in k_{12} must in part, if not wholly, be due to the effect of ARF on hepatic function. Secondly, changes in liver function similar to those found in the glycerol model have been observed in rats with surgically-induced chronic renal failure [6, 23]. Thirdly, there was no evidence of serious hepato-cellular damage in the rats with ARF, although a minor toxic lesion was observed, but hepatic lesions are often found in association with renal damage produced by a variety of surgical, chemical and dietary procedures [30]. Furthermore, recent studies in which we have ameliorated the degree of uraemia, by administration of 0.9% w/v saline as drinking water for 14 days and omission of dehydration before injection of glycerol, showed that there was no significant difference in the kinetics of ICG between control rats and rats with a minor degree of ARF (Bowmer and Yates, unpublished observations).

Renal failure and liver function

Overall the present results suggest that in the rat, acute renal failure can induce changes in liver function manifested as a decreased hepatic uptake of ICG. A hypothesis that renal failure can affect the liver's ability to remove highly cleared substances from plasma is supported by Tse *et al.* [6] who found that the clearance and biliary excretion of rose bengal were both decreased in rats with chronic renal failure. Furthermore, Howie and Bourke [23] showed that the uptake of *p*-aminobenzoic acid by isolated perfused livers from rats with chronic renal failure was decreased but, by contrast, the biliary excretion of this compound was increased. Similar changes in liver function may also occur in man. The capacity of the liver to store bromosulphophthalein (BSP), and to a lesser extent the hepatic transport maximum of BSP, were significantly decreased in patients with chronic renal failure [7]. Wernze and Spech [7] concluded that the uptake and binding of BSP to cytoplasmic proteins of the liver were affected more than the transport of BSP into bile.

There appears to be no clear explanation for the pharmacokinetic changes observed in this study and for the alterations in hepatic function found by others [6, 7, 23]. It is possible that some endogenous substance(s) which accumulates in plasma during renal failure may inhibit the transport of these dyes across the hepatocyte membrane. Some common uraemic metabolites have been tested, but without success, for their ability to inhibit the uptake of BSP by rat isolated perfused livers [31]. Alternatively, it has been suggested that altered hepatic protein metabolism could be responsible for the decreased uptake and storage of BSP by the liver in patients with chronic renal failure [7].

Sex difference in the effect of renal failure upon liver function

The changes in the kinetics of ICG in glycerol-induced ARF were much more pronounced in male than in female rats even though there was no difference in plasma urea concentration between these two groups of animals (Table 1). This suggests that liver function in female rats is much less sensitive than in males to the effects of ARF. Results similar to these were obtained by Wernze and Spech [7] who found that in male patients with chronic renal failure, the hepatic storage capacity and transport maximum of BSP were reduced by 59 and 22% of their respective values in normal subjects. By contrast, there was only a 34% decrease in hepatic storage capacity and no significant change in transport maximum in female patients. The reasons why a sex difference should exist in the response of the liver to renal failure are not clear but it may be related to the difference in hormonal status between male and female animals [32, 33].

In conclusion, it is clear that the hepatic uptake of ICG was decreased in all three models of ARF. Our findings also indicate that the glycerol model is the most satisfactory with which to study the effects of ARF upon liver function. In view of the results from this and other studies [6, 7, 23] it seems reasonable to suggest that the ability of the liver to remove compounds from plasma, to store them and excrete them into bile is diminished in renal failure. The cause of these changes remains to be determined but more detailed studies are in progress using the glycerol model of ARF.

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