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Frequency Distribution of Bilirubin Intrinsic Clearance in Adult Male Sprague–Dawley Rats

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Abstract □ The intrinsic body clearance of bilirubin was determined in 48 adult male Sprague–Dawley rats that received an intravenous infusion of bilirubin. The intrinsic body clearance was calculated from the infusion rate, the steady-state plasma concentration of total (free and protein-bound) bilirubin, and the free fraction of bilirubin in plasma. The intrinsic body clearance of bilirubin ranged from 5.05 to 13.20 liters/kg/min and was bimodally distributed, with half of the animals in each group. The plasma free fraction of bilirubin ranged from 0.00025 to 0.00077 (mean 0.00053) in the 24 intrinsically rapid metabolizers of bilirubin and from 0.00048 to 0.00103 (mean 0.00075) in the intrinsically slow metabolizers of the pigment. Thus, interindividual differences in the *total* clearance of bilirubin in the rats are due to differences in both intrinsic body clearance and plasma protein binding.

Keyphrases □ Bilirubin—intrinsic body clearance, frequency distribution, rats □ Clearance, intrinsic body—bilirubin, frequency distribution, rats □ Pigments—bilirubin, intrinsic body clearance, frequency distribution, rats

The heme pigment bilirubin is eliminated in humans and animals almost entirely by conjugative pathways (1). The concentration of bilirubin in plasma is a frequently used diagnostic index because it reflects changes in the formation and elimination rates of the pigment that may be caused by hemolysis, liver disease, and other pathologic conditions.

Preliminary studies on a small group of rats revealed pronounced interindividual differences in the total body clearance of bilirubin which could be ascribed largely to corresponding differences in the plasma protein binding of the pigment (2). This study has been extended to a total of 48 animals to determine the magnitude of interindividual differences in total body clearance, intrinsic clearance, and plasma free fraction of bilirubin.

EXPERIMENTAL

Forty-eight male Sprague–Dawley rats[1], 300–400 g, were maintained on a standard diet[2] and received an infusion of bilirubin into the right jugular vein. Forty animals received bilirubin at a rate of 0.8 mg/kg/min for 15 min and then 0.32 mg/kg/min for up to 4 hr; the other eight rats were infused at a rate of 0.8 mg/kg/min for the entire period (except for three of these animals that received 2 mg/kg/min for the first 15 min). Blood samples were obtained periodically, and plasma was assayed for free (3) and total (4) unconjugated bilirubin.

Steady-state conditions were ascertained as previously described (2). The total body clearance of bilirubin was calculated by dividing the maintenance infusion rate by the steady-state concentration of total bilirubin in plasma. The intrinsic body clearance was determined (5) by dividing the total body clearance by the free fraction of bilirubin in plasma (free fraction = free ÷ total concentration of bilirubin). These experiments were carried out over 2 years, 1–4 weeks after receipt of the animals.

Histograms to describe the frequency distribution of intrinsic clearance and log intrinsic clearance values were constructed by iteratively changing the class interval to maximize the number of bars in the respective graph while minimizing the number of regional reversals (modes and antimodes), as suggested by Martin *et al.* (6). For the bimodal characterization, the mean value, standard deviation, and fraction of the total population in each Gaussian component were estimated (7) by dividing the appropriate histogram at the antimode and considering each segment of the population as a separate Gaussian or log-normally distributed component.

[1] Blue Spruce Farms, Altamont, N.Y.
[2] Charles River Formula 4RF.

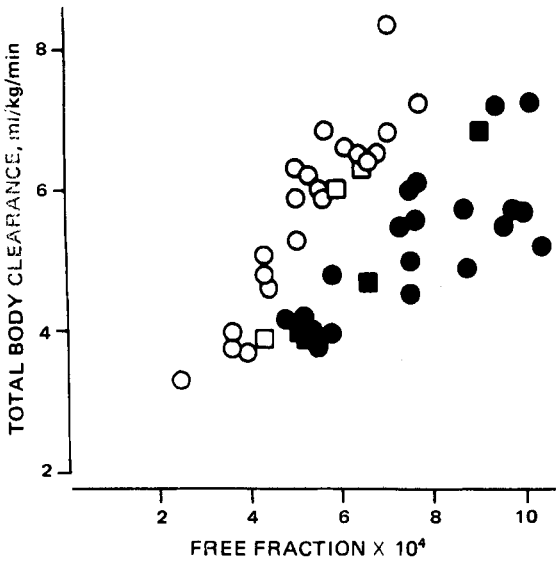

Figure 1—*Relationship between total body clearance of bilirubin and the free fraction of bilirubin in the plasma of individual rats. The 48 animals yielded data indicative of two separate populations, designated by open and closed symbols. Key: ○, ●, animals that received a bilirubin "loading" infusion of 0.8 mg/kg/min for 15 min and a maintenance infusion of 0.32 mg/kg/min thereafter; and □, ■, animals that received a maintenance infusion of 0.8 mg/kg/min (three animals first received a "loading" infusion of 2 mg/kg/min for 15 min). Correlation coefficients are 0.91 (p < 0.001) for data represented by open symbols and 0.79 (p < 0.001) for data represented by closed symbols.*

The various frequency distribution curves were fitted to the respective frequency–clearance value or frequency–log clearance value histograms by a standard method (8). The expected frequencies for overlapping components were summed prior to evaluating the agreement of the distribution model with the histogram by a χ-square test. For this test, classes were pooled when the expected frequency was three or less.

RESULTS

The average bilirubin concentration in the plasma of the rats before infusion of exogenous bilirubin was 0.06 mg/100 ml, which is less than 2% of the steady-state concentration during the infusion of the pigment. Thus, no correction had to be made for endogenous bilirubin.

The total body clearance of bilirubin in the 48 rats ranged from 3.3 to 8.3 ml/kg/min, and the plasma free fraction of bilirubin ranged from 0.00025 to 0.00103, indicating 99.897–99.975% protein binding. There is a statistically significant correlation ($r = 0.53$, $p < 0.001$) between total body clearance and free fraction values. This correlation becomes stronger if the data are treated as being representative of two different populations (Fig. 1). The existence of two different populations becomes even more apparent in the histogram of the intrinsic body clearance values (Fig. 2).

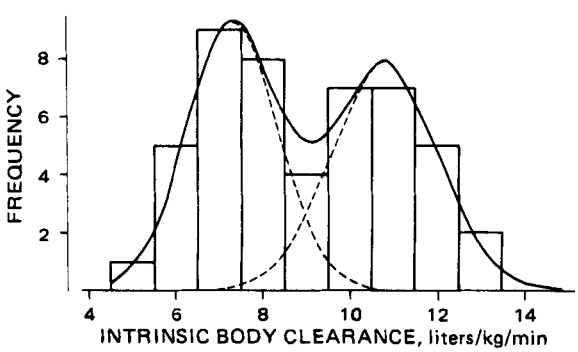

Figure 2—*Histogram showing the frequency distribution of bilirubin intrinsic body clearance by 48 male Sprague-Dawley rats. Also shown is the bimodal theoretical distribution curve, with the dotted line indicating the overlapping portions of the individual Gaussian components.*

Table I—Evaluation of Different Types of Frequency Distribution Curves for Bilirubin Intrinsic Clearance by Sprague–Dawley Rats

Type of Distribution	Mean(s), liters/kg/min	*SD*	χ^2	Degrees of Freedom	*p*
Normal	8.91	2.12	5.23	4	>0.26
Log-normal	8.65	+2.42, −1.89	4.59	3	>0.20
Bimodal	7.30	1.00	0.50	2	>0.77
	10.80	1.20			
Log-bimodal	7.24	+1.08, −0.93	2.18	1	>0.13
	10.72	+1.52, −1.39			

The data are best described by a bimodal frequency distribution curve, with one-half the animals in each Gaussian component (Table I). The intrinsic clearance data were examined for a possible seasonal or time-related effect (9), and none of the runs above or below the median for any one of the populations or for the combined group of 48 animals exceeded the length predictable entirely by chance ($0.10 < p < 0.50$).

There is a statistically significant negative correlation between the intrinsic body clearance and the plasma free fraction of bilirubin for the entire 48 animals ($r = -0.711$, $p < 0.001$) and for the intrinsically rapid and slow metabolizers of bilirubin considered separately (Fig. 3). Taken as a whole, the 48 plasma free fraction values are log-normally distributed.

DISCUSSION

The total body clearance of bilirubin in male Sprague–Dawley rats, as determined in this investigation, is significantly lower than their liver perfusion rate (about 93 ml of blood/kg/min) (10). Therefore, an approximately linear relationship between total body clearance and plasma free fraction may be expected on theoretical grounds if the intrinsic body clearance is relatively constant (5). Such a linear relationship is apparent in Fig. 1, but the data in this figure suggest that there may be two populations, one consisting of intrinsically rapid metabolizers of bilirubin (high intrinsic body clearance) and the other consisting of intrinsically slow metabolizers (low intrinsic body clearance).

The existence of such bimodality was verified by statistical analysis of the frequency distribution of the intrinsic body clearance values for bilirubin in the 48 animals (Fig. 2). However, there is also an unanticipated, although relatively weak, negative correlation between the intrinsic body clearance and the plasma free fraction of bilirubin (Fig. 3).

A change in plasma free fraction has a relatively modest effect on the intrinsic body clearance when the rapid and slow metabolizers are considered separately. This result suggested the possibility that the negative correlation apparent in Fig. 3 may be a consequence of a small influence of the hepatic perfusion rate on the body clearance of bilirubin. Such an

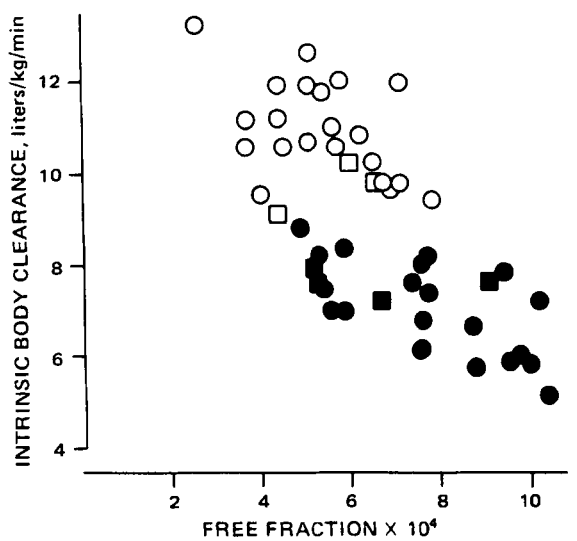

Figure 3—*Relationship between intrinsic body clearance of bilirubin and free fraction of bilirubin in the plasma of individual rats. Key: same as Fig. 1. Correlation coefficients are −0.426 (p < 0.05) for data represented by open symbols and −0.651 (p < 0.001) for data represented by closed symbols.*

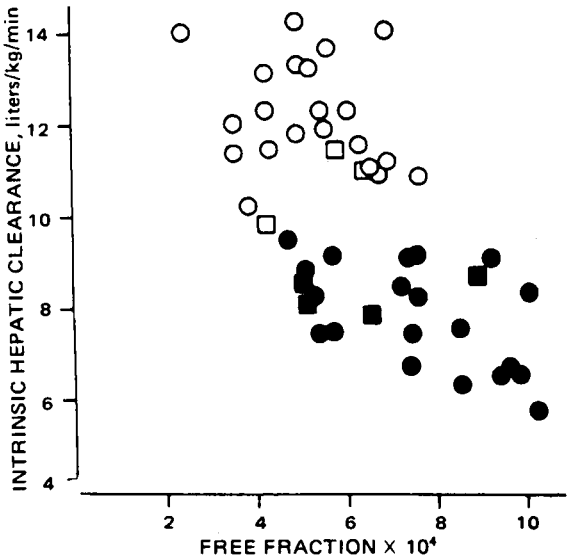

Figure 4—*Relationship between intrinsic hepatic plasma clearance of bilirubin and the free fraction of bilirubin in plasma of individual rats. Key: same as Fig. 1. Correlation coefficients are −0.194 (p > 0.30) for open symbols and −0.527 (p < 0.01) for closed symbols.*

influence, although small in magnitude, cannot be neglected in the interpretation of the data. The total body clearance values were, therefore, converted to total *hepatic* plasma clearances on the basis of the relationship (11):

$$\text{total body clearance} = \frac{\text{THPC} \times \text{PPR}}{\text{THPC} + \text{PPR}} \qquad \text{(Eq. 1)}$$

where THPC is the total hepatic plasma clearance and PPR is the plasma perfusion rate of the liver, which was assumed to be 55.8 ml/kg/min (60% of blood flow). Determination of plasma rather than blood clearance presents no interpretative difficulty since less than 10% of the bilirubin in rat blood is in or on erythrocytes. The total hepatic plasma clearance was divided by the plasma free fraction value to obtain the intrinsic hepatic plasma clearance (IHPC).

The relationship between the intrinsic hepatic plasma clearance and the plasma free fraction of bilirubin is shown in Fig. 4. The correlation between these variables is statistically not significant ($r = -0.194$, $p > 0.30$) for the intrinsically rapid metabolizers but significant ($r = -0.527$, $p < 0.01$) for the slow metabolizers. Even if the plasma perfusion rate is decreased by one-third to 37.2 ml/kg/min, the negative correlation between the intrinsic hepatic plasma clearance and the free fraction is marginally significant ($r = -0.448$, $p < 0.05$).

The reason for this negative correlation is not readily apparent. One possibility, entirely speculative, is that certain endogenous inhibitor(s) of bilirubin binding to plasma proteins may be eliminated by the same conjugative pathways as bilirubin itself. Rapid intrinsic metabolizers of bilirubin may then also be rapid eliminators of the endogenous inhibitor(s), resulting in lower inhibitor concentrations and more extensive plasma protein binding of bilirubin.

The results of this investigation show that there are appreciable interindividual differences in the total body clearance of bilirubin by adult male Sprague–Dawley rats and that these differences are due to interindividual differences in both determinants of the total body clearance, plasma protein binding and intrinsic clearance. The intrinsic body clearance values were bimodally distributed. No similar study has been carried out in human subjects, but it has been established that there are significant interindividual differences in plasma protein binding of bilirubin in normal adult volunteers and in newborn infants (12).

Indirect evidence, namely an inverse correlation between the conjugation of salicylamide with glucuronic acid and the plasma concentration of bilirubin in newborn infants together with pronounced interindividual differences in the formation of salicylamide glucuronide (13), suggests that the intrinsic metabolic clearance of bilirubin differs appreciably among human neonates. Similar differences are likely in adults, particularly in relation to changes in liver function. For example, Pirotte (14) found that the total body clearance of exogenous bilirubin ranged from 22.7 to 66.8 ml/min in normal adult humans and from 2.25 to 78.6 ml/min in patients with cirrhosis of the liver. Therefore, the results of this study probably apply in principle also to humans.

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