PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL AND BIOLOGY MEDICINE 150, 612–617 (1975)

Differences between the Plasma Indocyanine Green Disappearance Rates of Normal Men and Women (39090)

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Indocyanine green (ICG), a water-soluble tricarbocyanine dye, is rapidly removed from the circulation by the liver and secreted into the bile without conjugation (1–3). Currently, its major medical use is for indicator dilution studies during cardiac catheterization. However, its rapid excretion via the liver without significant extrahepatic removal (1–4) or enterohepatic circulation (1, 3) and freedom from the problems of local tissue necrosis or anaphyllaxis, which may occur with sulfobromophthalein (5), have prompted the increasing use of plasma ICG disappearance curves as a test of liver function. The most commonly used format for this purpose involves determination of the plasma fractional disappearance rate (k) or half-life ($T_{1/2}$) of ICG following the rapid intravenous injection of dye at a dose of 0.5 mg/kg body weight.

We present the results of plasma ICG disappearance studies in a large group of young, healthy normal volunteer subjects. The data unexpectedly indicate a significant difference in the plasma fractional ICG disappearance rates between normal men and women, which must be taken into account in interpreting similar studies in patients with either suspected or established liver disease.

Methods. Subjects studied. Plasma ICG disappearance studies were performed in 51 healthy normal individuals, ages 18–29, who had been admitted to the Clinical Center of the National Institutes of Health for the specific purpose of serving as control subjects for a variety of studies. Eighteen were men and 33 were women. All met strict, previously defined criteria for "normality" (6), and none had taken medications of any type within 4 weeks of the studies. Each of the 51 volunteers underwent plasma ICG disappearance studies employing a dose of 0.5 mg/kg body weight. In addition, 19 of the volunteers (9 men and 10 women) underwent three additional studies, employing indocyanine green doses of 2.0, 3.5, and 5.0 mg/kg. Informed consent was obtained from all subjects, and the study was approved by the Clinical Research Committee of the Medical Board of the National Institutes of Health.

Plasma ICG disappearance studies. Crystalline indocyanine green (Hynson, Wescott and Dunning, Baltimore, Md.) was dissolved immediately before injection in a distilled water diluent provided by the manufacturer. For the standard 0.5 mg/kg dose, the solution was made up to a concentration of 5 mg/ml in accordance with the manufacturer's direction. At zero time, 0.1 ml of this solution/kg body weight was rapidly injected into an antecubital vein through a running intravenous solution of normal saline. For studies at higher doses, concentrations of the initial solutions were varied slightly according to body weight to maintain an injection volume of approximately 10–15 ml. For doses of 2.0, 3.5, and 5.0 mg/kg, concentrations of the injected solutions averaged 12.5, 20.0, and 33.3 mg/ml, respectively. In all cases, zero time was taken to be the end point of the injection, and blood samples were withdrawn from the opposite arm via an indwelling scalp vein needle. In addition to a preinjection sample, heparinized blood samples were drawn (a) either at $3\frac{1}{2}$, 5, $6\frac{1}{2}$, 10, 12 and 15 min after injection, or (b) at 1-min intervals from 5 to 26 min. The protocol employed for blood sampling was found not to influence the results (*vide infra*), and the data obtained by both protocols were pooled for subsequent analysis.

The plasma indocyanine green concentration was determined spectrophotometrically, using a Beckman DU-2 spectrophotometer. Sample optical densities were read at 805 nm, and the ICG concentration calculated by means of a standard curve constructed for each study from serial dilutions in the subject's own plasma of the intial solution injected.

Data analysis. Plasma ICG concentrations were plotted semilogarithmically as a function of time. The fractional ICG disappearance rate [$k(\text{min}^{-1})$] was determined from a least-squares regression performed by means of a Hewlett Packard 9830A digital computer over the region of the curve extending from 5–12 min. Data obtained during this time period were almost invariably log-linear. Although the exponential portion of the ICG disappearance curve usually extended well beyond 12 min, the 5–12 min period was selected in all cases so that there was no possible effect of varying time periods on the calculated values of k.

In virtually all studies, the appearance of a somewhat slower second component to the plasma ICG disappearance curve became apparent at some time after 12 min. In most instances, including all of the studies employing the 0.5 mg/kg dose, the plasma ICG concentrations at this point were too low to permit reliable determination of the value of a second exponential to the plasma disappearance curve.

As previously described (3, 7, 8, 9, 10), the initial volume of distribution of ICG (VD_{ICG}) was estimated for each 0.5 mg/kg study from the injected dose of ICG and the computer-extrapolated value of the curve at zero time, according to the relationship,

$$VD_{ICG}\ (\text{ml/kg}) = \frac{\text{injected dose (mg/kg)}}{\text{plasma ICG concentration at zero time (mg/ml)}}. \quad [1]$$

Indocyanine green clearance (C_{ICG}), the volume of plasma from which ICG is irreversibly removed per min by the liver was then estimated as

$$C_{ICG}\ (\text{ml/min/kg}) = k(\text{min}^{-1}) \cdot VD_{ICG}\ (\text{ml/kg}). \quad [2]$$

As noted above, this calculation does not take into account the slower second component of the plasma ICG disappearance curves. Although there is no simple mathematical relationship between this second component and any single biological process (11), by analogy with studies with both BSP (12) and bilirubin (6, 13), one factor contributing to its appearance may be the reflux of small amounts of ICG from liver back to plasma. As a result, C_{ICG}, as calculated from Eq. [2], may overestimate slightly net hepatic ICG extraction (*vide infra*).

Results. Over the time range 5–12 min, semilogarithmic plots of plasma ICG concentration versus time were linear, as indicated by a range of 0.9666 to 0.9998 for the calculated correlation coefficients.

The experimental plasma fractional ICG disappearance rate (k's) are presented in Table I. The k's in normal women were significantly faster than those in normal men for the 0.5 mg/kg ($P < 0.01$) and 2.0 mg/kg ($P < 0.05$) doses, but were essentially the same for both sexes at 3.5 and 5.0 mg/kg. The ranges observed at the standard 0.5 mg/kg dose are presented in Fig. 1.

The volume of distribution of ICG during the 0.5 mg/kg studies averaged 39.2 ± 1.2 ml/kg (mean ± SE), and was not signifi-

TABLE I. PLASMA FRACTIONAL ICG DISAPPEARANCE RATES [k (min^{-1})]

	Dose (mg/kg)			
	0.5	2.0	3.5	5.0
Normal men[a]	0.205 ± 0.005[b]	0.179 ± 0.007	0.181 ± 0.011	0.179 ± 0.008
Normal women[a]	0.243 ± 0.006	0.204 ± 0.009	0.172 ± 0.007	0.178 ± 0.005
Student's t test	$P < 0.01$	$P < 0.05$	$P > 0.5$	$P > 0.9$

[a] Studies at 0.5 mg/kg were performed in 18 men and 33 women. Studies at all other doses were performed in nine men and 10 women.

[b] All values = mean ± SE.

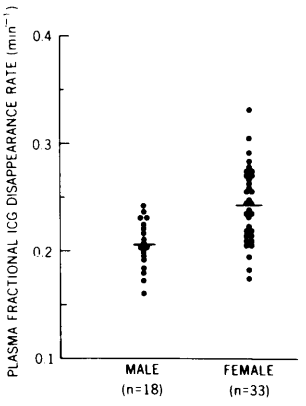

FIG. 1. Plasma fractional disappearance rates for indocyanine green in normal men and women following rapid intravenous injection of a dose of 0.5 mg/kg.

cantly different in men than in women (men: 40.5 ± 1.8 ml/kg; women: 38.4 ± 1.6 ml/kg; $P > 0.4$). As a result of these values for VD_{ICG}, the increase in ICG clearance observed in women was not statistically significant (women: 9.3 ± 0.4 ml/kg vs men: 8.3 ± 0.4 ml/kg; $0.05 < P < 0.1$), and C_{ICG} averaged 8.9 ± 0.3 ml/min/kg in the total population of 51 normal individuals.

Discussion. The principal finding of the present study is the observation of a significant difference between the plasma fractional disappearance rates for ICG in normal men and women, following administration of the usually employed 0.5 mg/kg dose. This difference is both unexpected and not readily explainable. Previous studies of plasma ICG disappearance rates following administration of this dose to normal subjects have been performed in predominantly male populations (2, 3, 14). Sex differences in the plasma disappearance rates of other organic anions such as BSP and bilirubin have not previously been reported. A review of data accumulated in this laboratory over approximately 8 yr indicates no sex differences in the initial disappearance rates of either a 5 mg/kg dose of BSP (male: 0.116 ± 0.003 min^{-1}; female: 0.123 ± 0.008 min^{-1}; $P > 0.4$) or of a tracer dose of radiolabeled bilirubin (male: 0.028 ± 0.002 min^{-1}; female: 0.030 ± 0.002 min^{-1}; $P > 0.6$) in normal subjects.

Although the plasma fractional ICG disappearance rate (k) in women was significantly greater than in men for an 0.5 mg/kg dose, there was no significant sex difference in the calculated values for clearance (C_{ICG}, ml/min/kg). While this may, at first, appear inconsistent, we believe that this discrepancy largely reflects the fact that methodological factors in the calculation of C_{ICG} subject the values for this parameter to considerable statistical uncertainty, whereas the values for k can usually be determined with a statistical error of less than ±5%. Although Eqs. [1] and [2] have been used previously by several authors to estimate both VD_{ICG} and C_{ICG} (3, 7, 8, 9, 10), it has not been adequately appreciated that estimation of these variables from single injection studies is subject to methodologic limitations not applicable to substances cleared from plasma more slowly. Because the $T_{1/2}$ of the injected ICG is short relative to the usually assumed intravascular mixing time of 4–5 min, calculation of VD_{ICG} by the usual dilution equation may be subject to errors resulting from nonuniform mixing. A further potential error arises from the extrapolation of the steep log-linear plot of plasma ICG concentration vs time to $T = 0$. Although the statistical uncertainty in the logarithmic intercept in technically adequate studies is usually small, conversion to the antilogarithm frequently results in a statistical uncertainty of ±15% or more in the estimated plasma ICG concentration at zero time, and a corresponding uncertainty in VD_{ICG} and C_{ICG}. Furthermore, as noted above, calculation of C_{ICG} from Eqs. [1] and [2] ignores the presence of a second exponential component to the plasma ICG disappearance curve. This introduces yet another source of error in the calculation of C_{ICG} which is small in normal subjects, but which may be appreciable in individuals with liver disease. For these reasons, analysis of plasma ICG disappearance curves in terms of initial fractional disappearance rates is more reliable than calculation of clearance, although the latter may be intellectually more appealing.

The plasma fractional disappearance rate for ICG depends on the following recognized factors: (a) the rate of delivery of dye to the liver as determined by hepatic plasma

flow rate, (b) innate efficiency of the liver cell for extracting ICG from plasma, and (c) plasma levels of endogenous substances competing for or otherwise influencing hepatic ICG uptake. In addition, when a wide range of doses is employed, some of which partially saturate the hepatic uptake mechanism for ICG, the injected dose, plasma volume, and initial dye concentration in the plasma may also influence the value of k observed at doses approaching saturation.

In an extensive review of the literature on hepatic blood flow in man, only two studies were found which contained data on a significant number of normal women (16, 17). When the data from these two studies were used to calculate hepatic plasma flow (HPF) (ml/min/kg), virtually identical results were obtained in men and women, irrespective of whether the BSP infusion technique (16) or colloidal gold disappearance method (17) was used to determine hepatic flow. These calculations are summarized in Table II. Thus, although the relatively high efficiency of indocyanine green extraction (calculated as C_{ICG}/HPF), compared with that of either BSP or bilirubin (Fig. 2), suggests that hepatic ICG uptake might be particularly sensitive to differences in plasma flow, the published data do not support the hypothesis that a sex difference in HPF is the basis for the observed sex differences in the plasma ICG disappearance rate.

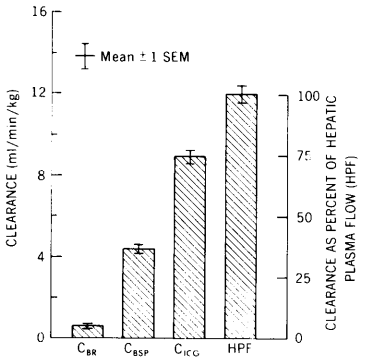

FIG. 2. Clearance values for bilirubin (C_{BR}), sulfobromophthalein (C_{BSP}), and indocyanine green (C_{ICG}) in healthy young volunteers of both sexes. These values are compared with measurements of hepatic plasma flow (HPF) in normal subjects, as calculated from published data (16, 17).

The available data on hepatic ICG extraction, as measured during hepatic vein catheterization studies, were obtained almost exclusively in men and provide no information about a possible sex difference in this parameter (9, 10, 15, 18). Interestingly, if hepatic ICG extraction is calculated as above from C_{ICG}, the resulting value of 74 ± 4% for 51 normal subjects of both sexes is similar to the figure of 70 ± 6% determined by Leevy *et al.* (9) during hepatic vein catheterization studies in normal men. This agreement suggests that the failure to take into account the small second exponential component of the plasma ICG disappearance curve introduces only a very small error when C_{ICG} is calculated from Eq. [2] in normal volunteers.

In the present study, the sex difference in k was observed only at the lowest doses of ICG employed, at which possible saturation of the hepatic uptake mechanism would

TABLE II. CALCULATED HEPATIC PLASMA FLOW (HPF) IN NORMAL HUMAN SUBJECTS

Method	Hepatic plasma flow (ml/min/kg)				Difference[a] between sexes
	Male	n	Female	n	
BSP infusion (16)[b]	11.9 ± 0.3[c]	73	12.6 ± 0.6	18	$P > 0.3$
Colloidal gold clearance (17)	12.1 ± 0.7	18	11.2 ± 0.8	7	$P > 0.3$
Combined	11.9 ± 0.3	91	12.2 ± 0.5	25	$P > 0.6$
Difference between methods[a]	$P > 0.7$		$P > 0.1$		

Calculated normal HPF for 116 subjects of both sexes: 12.0 ± 0.3 ml/min/kg

[a] Student's t test.

[b] Original data were expressed per m² body surface area. These values were converted to per kg body weight by assuming that 1.73 m² is equivalent to 70 kg.

[c] All values mean ± SE.

appear to be unlikely. In any case, at the 0.5 mg/kg dose, neither the initial plasma ICG concentration nor the initial volume of distribution of ICG were significantly different in men from the corresponding values in women.

The possibility that there is a sex difference in the plasma levels of some endogenous compound which influences ICG uptake is intriguing but difficult to evaluate. One can speculate that a compound in the circulation of males slows fractional hepatic ICG uptake by competing for binding to a hepatic receptor site, or conversely, that a compound in the circulation of women accelerates fractional hepatic ICG uptake by competitive displacement of ICG from albumin. While this hypothesis is entirely speculative, the competitive nature of the proposed mechanism would explain why a sex difference in hepatic ICG uptake is observed with small (0.5–2.0 mg/kg) but not with larger (3.5–5.0 mg/kg) doses of dye. In further support of this hypothesis, rates of hepatic indocyanine green uptake (V, mg/min/kg), calculated as $V = k \times$ Dose, were fitted to the Michaelis–Menten equation on a Univac 1108 digital computer, using the Simulation, Analysis and Modeling (SAAM) program of Berman and Weiss (19). The applicability of the Michaelis–Menten equation to the saturable hepatic uptake process for indocyanine green has previously been demonstrated by Paumgartner *et al.* (20). The data for both sexes were fitted to the Michaelis–Menten equation simultaneously under the single constraint that V_{max} (male/V_{max} (female) = 1.0 ± 0.2, i.e., that the values for V_{max} in both sexes did not differ by more than 20%. Under this single constraint, calculated values for V_{max} and k_m were 4.7 mg/min/kg and 23 mg/kg, respectively, in men, and 4.3 mg/min/kg and 19 mg/kg, respectively, in women. The residual variance for the constrained computer solution was not significantly different from that obtained without the above constraint (Fisher's F test, $P > 0.25$). Values for V_{max} obtained in both men and women are similar to the value of 3.6 ± 0.6 mg/min/kg reported by Paumgartner *et al.* in a study employing ICG doses up to 10 mg/kg in men without liver disease (20) The finding of a higher K_m in males is consistent with, but certainly does not prove, the hypothesis that the sex difference in k observed at low doses of ICG result from the presence of sex specific levels for a competitor of ICG binding to either the liver cell membrane or plasma albumin.

We cannot provide more than a speculative explanation for the observed sex difference in the plasma fractional ICG disappearance rate at this time. However, the consistent presence of a statistically significant sex difference as this relatively large series was accumulated suggests that it is a real biological phenomenon, and not merely statistical artifact. Although the measurement of plasma fractional ICG disappearance rate after the injection of the recommended dose of 0.5 mg/kg body weight has been shown to be a somewhat less sensitive indicator of hepatic dysfunction than conventional BSP tests employing a dose of 5.0 mg/kg, the sensitivity of the ICG technique can be made comparable to that of BSP by increasing the injected dose of ICG to 5.0 mg/kg (14). Nevertheless, at present an ICG dose of 0.5 mg/kg is most widely used for clinical liver function studies, and the higher dose can only be employed on an investigational basis with special FDA approval. Therefore, recognition that the ICG plasma disappearance rate in a patient must be compared with a sex-specific normal range may increase the sensitivity of this technique as an indicator of hepatic dysfunction.

The authors are indebted to Mrs. Sharon McFadden and Mrs. Phyllis Melton for rapid and accurate preparation of the manuscript.

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Received July 11, 1975. P.S.E.B.M. 1975, Vol. 150.