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ESTIMATION OF HEPATIC BLOOD FLOW WITH INDOCYANINE GREEN *

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The Fick principle, plasma clearance, and indicator dilution techniques have been used for estimation of hepatic blood flow in man; however, each of these methods is limited by lack of an ideal test substance. Sulfobromophthalein (BSP) (1), urea (2), galactose (3), and radioiodinated rose bengal (4) have been largely employed in Fick principle studies. Radioactive chromic phosphate (5), colloidal gold (6), galactose (7), and I^{131}-denatured albumin (8) have been popularized for estimation of blood flow from plasma clearance; radioiodinated albumin (9) and chromated red cells (10) have been used in the indicator dilution technique.

Physical properties, physiological activities, and hepatic extraction of indocyanine green (ICG) suggest that it might also be of value in hepatic blood flow studies (11–13). Reports have appeared on its use in estimating hepatic blood flow by both the Fick principle (13–15) and by plasma clearance studies in man (15–18); however, its advantages, if any, in comparison with other test substances have not been established. The present study was undertaken further to assess its usefulness and limitations for blood flow studies. The investigation consisted of: *1*) a comparison of blood flow levels obtained with ICG and BSP, using the Fick principle in the same subject; *2*) evaluation of the reliability of estimating hepatic blood flow from initial or subsequent plasma clearance of ICG; and *3*) observations on the usefulness of oximetry for external monitoring and direct determination of ICG.

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MATERIALS AND METHODS

Studies were conducted in 28 healthy subjects and in 34 patients with liver disease. Each of the subjects had a percutaneous liver biopsy using either the Vim-Silverman or Menghini needle (19), and a series of liver function tests including serum bilirubin (20), plasma decay of BSP (21), serum alkaline phosphatase (22), cephalin-cholesterol flocculation (23), serum glutamic oxalacetic and pyruvic transaminase (24), and serum protein partitions. Red cell volume and plasma volume were determined by radioactive chromium-labeled red cells, and iodinated albumin or Evans blue, respectively (25). Fick blood flow studies were conducted in a basal state using hepatic vein catheterization. In this procedure the catheter was advanced to a wedged position utilizing the uppermost right hepatic vein and withdrawn just enough to permit free sampling (26). Cardiac output was determined by the indicator dilution technique using Evans blue (27). Plasma concentrations of ICG were determined in a Beckman DU spectrophotometer at 815 mμ; BSP concentrations were determined by alkalinization and reading in a Beckman DU spectrophotometer at 580 mμ or in a Leitz colorimeter at 560 mμ. In each instance readings were made at the wave length of maximal absorption as determined for the instrument used. The Beckman was more sensitive for BSP readings; however, a comparison of readings obtained on the same specimen using the Beckman and Leitz showed no significant difference.

Fick principle studies. A comparison was made of blood flow levels obtained with ICG and BSP by the Fick principle in the same subject. An attempt was made to use dosages employed by others and to secure comparable peripheral plasma concentrations in order to facilitate interpretation. In 14 normal subjects and in 5 patients with liver disease, one of these dyes was used initially for 45 to 60 minutes, and after a 30- to 60-minute rest period the other was employed; the order of use was reversed in alternate cases. Studies were repeated in the same individual on a subsequent day when results were inconstant for either dye. ICG was infused in 0.5 per cent albumin solution using a Bowman pump at a constant rate of 0.3 mg per m^2 body surface per minute after an initial loading dose of 10 mg. Dye concentrations were determined in blood samples taken simultaneously at 5-minute intervals from an indwelling arterial needle and the hepatic vein catheter. BSP was in-

fused in an identical manner, using 3.0 mg of dye per m^2 body surface per minute after an initial loading dose of 150 mg. Twenty patients were given both dyes simultaneously, in the conventional concentrations described above or in equimolar amounts consisting of 1.5 mg of ICG and 1.6 mg of BSP per m^2 body surface per minute after an initial loading dose of 10 mg of ICG and 150 mg of BSP. Blood flow studies were conducted for 1 to 2 hours in these patients. Neither ICG nor BSP interfered with the reading of the other when a mixture of conventional or equimolar concentrations of these dyes was read in the Beckman DU spectrophotometer. Administration of either of these dyes to a subject storing another of the dyes initially caused a slight increase in the hepatic vein concentration of the stored dye (11, 28). Estimated hepatic blood flow (EHBF) in liters per minute was calculated from the formula (1): $EHBF = R/(P - H)\ (1 - HCT)$, where R is the total dye removal rate and P is the concentration of dye in peripheral arterial blood, both in milligrams per liter. H is the concentration of ICG or BSP in hepatic venous blood in milligrams per liter, and HCT is the peripheral venous hematocrit (1). Total dye removal was determined during periods when peripheral dye concentration was constant for a minimum of 15 minutes; data requiring adjustment for increasing or decreasing concentrations was not accepted for analysis in this aspect of the study.

Clearance studies. Estimated blood flow was calculated from plasma clearance of ICG and radioactive colloidal gold (29). ICG clearance was studied during hepatic vein catheterization and was followed by an estimation of hepatic blood flow by the Fick principle, using a constant infusion of this dye; colloidal gold disappearance was studied prior to, during, or after the ICG studies.

In ICG studies arterial and hepatic venous bloods were obtained simultaneously at 2-minute intervals for 14 minutes after giving 0.25, 0.5, or 1.0 mg of ICG per kg intravenously. EHBF was calculated from clearance of ICG by dividing the initial clearance or the clearance after 20 minutes by its extraction ratio. Clearance was determined by the formula (22):

$$Cl = \frac{A}{C_1} \times \frac{\log_e C_1 - \log_e C_2}{t_2 \text{ (minutes)}},$$

where A equals amount of dye injected (milligrams), C_1 equals concentration at zero time, C_2 equals concentration at time t_2 (22). Extraction ratio was determined by the formula:

$$ER = \frac{a - HV}{a} \times 100,$$

where a equals arterial concentration and HV is the hepatic vein concentration. The disappearance rate constant (5) was also used to calculate blood flow for ICG and colloidal gold: $EHBF = \log_e(C_2/C_1) \times BV/t_{\frac{1}{2}}$, where $\log_e(C_2/C_1)$ is a constant K (0.693), BV represents the blood volume, and $t_{\frac{1}{2}}$ is that time in which the initial concentration of the test substance has undergone a 50 per cent decrease.

Colloidal gold, which had a particle size of 3 to 7 mμ and a maximum absorption peak of 266 mμ, was obtained from a commercial source.[1] Ten μc of this material dissolved in distilled water was given intravenously and its disappearance from the circulation determined by a scintillation counter placed over the left femoral artery at the inguinal area.

Oximetry. The Waters ear oximeter with a constant power supply (30) and the Norman-NEP ear oximeter (31) with an airflow attachment were compared for monitoring ICG levels. Both instruments were connected to a Shiner amplifier. The ear pieces were calibrated, attached to the patient, and a baseline established prior to

[1] Purchased from E. R. Squibb & Sons.

TABLE I

Comparison of EHBF determined with ICG (0.3 mg/m²/min) and BSP (3.0 mg/m²/min) in normal subjects *

Patient data					Indocyanine green				Sulfobromophthalein			
	No.	Sex	Age	BSA	Peripheral conc.	ER	Total removal rate	EHBF	Peripheral conc.	ER	Total removal rate	EHBF
			yrs	m^2	*mg%*	%	*mg/min*	*ml/m²/min*	*mg%*	%	*mg/min*	*ml/m²/min*
S.S.	1	M	34	1.95	0.28	71	0.96	470	0.87	70	5.05	730
B.W.	2	M	30	1.65	0.34	58	0.87	450	0.89	25	2.45	540
L.H.	3	M	42	1.87	0.28	66	1.35	410	1.28	65	4.95	660
T.S.	4	M	49	1.64	0.33	73	0.86	360	1.75	78	4.73	510
F.A.	5	M	47	1.65	0.23	79	1.08	590	2.40	33	3.28	670
B.M.	6	M	38	1.87	0.20	74	0.87	810	0.63	65	4.00	880
W.O'N.	7	M	34	1.95	0.34	83	0.91	300	1.95	54	6.18	480
W.McG.	8	M	53	1.89	0.42	70	2.08	590	2.59	52	5.91	680
T.S.	9	M	38	1.64	0.31	82	0.67	530	1.52	65	5.92	660
J.H.	10	M	36	1.69	0.39	60	1.14	490	1.32	64	5.25	580
J.R.	11	M	50	1.92	0.30	68	0.85	340	1.41	59	4.80	410
P.S.	12	M	30	1.88	0.36	64	1.04	450	0.95	63	3.50	500
E.G.	13	M	27	1.91	0.26	76	0.91	430	1.03	67	5.15	750
W.M.	14	M	36	1.46	0.15	53	0.35	520	0.77	92	5.40	780
				Mean	0.29	69.8	0.99	481.4	1.38	60.8	4.75	631
				±SD	±0.03	±19.9	±0.372	±123.0	±0.66	±16.1	±1.04	±128

* Only studies in which dye levels were constant are presented. EHBF = estimated hepatic blood flow; ICG = indocyanine green; BSP = sulfobromophthalein; ER = extraction ratio.

TABLE II

Comparison of EHBF determined with ICG (0.3 mg/m²/min) and BSP (3.0 mg/m²/min) in cirrhosis *

Patient data				Histological diagnosis	Clinical findings†	Biochemical tests						ICG		BSP	
	No.	Sex	Age			S. bil.	45-min BSP	Alk. phos.	Ceph. floc.	S. alb.	S. glob.	ER	EHBF	ER	EHBF
			yrs			*mg%*	%	BU‡		*g%*		%	*ml/m²/min*	%	*ml/m²/min*
J.S.	15	M	38	Cirrhosis	Heavy alcoholic ingestion; poor diet; H	0.5	16.5	6.1	4+	2.8	2.6	29	400	37	460
C.L.	16	M	57	Cirrhosis	Heavy alcoholic ingestion; poor diet; H; palmar erythema	0.3	31	4.1	Neg.	3.1	3.1	40	320	18	560
L.P.	17	M	41	Cirrhosis	Heavy alcoholic ingestion; poor diet; H; S	1.4	23	7.4	4+	3.1	2.8	43	430	51	510
A.A.	18	M	64	Cirrhosis	Poor diet; heavy alcoholic ingestion; J; A; H	6.3	48	6.8	4+	2.3	2.7	26	340	29	720
M.M.	19	F	44	Cirrhosis	Poor diet; alcoholic; J; A; H; S; fetor hepaticus	3.8	24	7.1	4+	2.6	2.1	15	400	16	570
										Mean		30.6	378.0	30.2	564
										±SD		±10.09	± 41.18	±12.9	± 88.8

* Only studies in which dye levels were constant are presented.
† H, hepatomegaly; S, splenomegaly; J, jaundice; A, ascites.
‡ Bodansky units.

TABLE III

EHBF determined with ICG (0.3 mg/m²/min) and BSP (3.0 mg/m²/min) given simultaneously

Patient data						ICG				BSP			
	No.	Sex	Age	BSA	Histological diagnosis	Peripheral conc.	ER	Total removal rate	EHBF	Peripheral conc.	ER	Total removal rate	EHBF
			yrs	*m²*		*mg%*	*%*	*mg/min*	*ml/m²/min*	*mg%*	*%*	*mg/min*	*ml/m²/min*
J.S.	20	M	48	1.93	Fatty liver	0.27	55	0.88	508	1.95	22	3.60	731
H.McG	21	M	55	1.95	Moderate cirrhosis	0.45	51	0.91	316	2.02	27	3.92	580
W.W.	22	M	42	1.86	Normal liver	0.33	81	0.825	336	1.20	50	5.17	761
J.Q.	23	M	54	1.98	Moderate cirrhosis	0.61	59	1.28	283	2.47	38	5.91	464
M.S.	24	F	49	1.85	Mild cirrhosis	0.69	31	1.48	543	2.30	24	4.95	714
					Mean	0.47	55	1.01	397	1.98	32.2	4.71	650
					±SD	±0.16	±16	±0.26	±107	±0.46	±10.4	±0.84	±112

TABLE IV

Comparison of EHBF with ICG (1.5 mg/m²/min) and BSP (1.6 mg/m²/min) given simultaneously

Patient data						ICG				BSP			
	No.	Sex	Age	BSA	Histological diagnosis	Peripheral conc.	ER	Total removal rate	EHBF	Peripheral conc.	ER	Total removal rate	EHBF
			yrs	*m²*		*mg%*	*%*	*mg/min*	*ml/m²/min*	*mg%*	*%*	*mg/min*	*ml/m²/min*
H.T.	25	M	46	1.76	Normal liver	1.44	84	5.36	450	0.650	50	2.20	473
R.J.	26	M	27	1.98	Normal liver	1.66	89	7.19	420	0.400	70	2.36	679
R.R.	27	M	32	1.85	Normal liver	1.16	76	5.82	537	0.530	60	3.52	868
E.K.	28	M	49	2.06	Normal liver	1.44	70	6.96	571	0.550	55	3.18	879
L.H.	3	M	44	1.90	Fatty liver	1.70	82	5.76	370	0.570	60	2.28	598
C.M.	29	M	41	1.85	Fatty liver	0.98	89	3.60	366	0.600	50	2.40	697
W.H.	30	M	51	1.65	Cirrhosis	1.90	75	4.95	335	1.400	35	2.52	491
S.S.	31	M	34	1.87	Cirrhosis	2.22	56	5.78	762	1.080	71	3.24	911
M.D.	32	F	44	1.74	Cirrhosis	1.57	77	5.56	718	0.840	44	2.82	761
					Mean	1.56	77.50	5.66	503	0.736	55	2.74	706
					±SD	±0.35	± 9.74	±1.00	±146	±0.375	±11	±0.45	±154

TABLE V

Effect of prolonged use of ICG on extraction ratio and EHBF in normal subjects and in patients with liver disease

Patient data				Histological diagnosis	Blood flow					
					at 20 min		at 1 hr		at 2 hrs	
	No.	Sex	Age		ER	EHBF	ER	EHBF	ER	EHBF
			yrs		%	*ml/m²/min*	%	*ml/m²/min*	%	*ml/m²/min*
J.McG	33	M	53	Cirrhosis	46	300	43	330	30	310
W.J.	34	M	29	Normal liver	69	770	74	790	65	750
L.C.	35	F	35	Fatty liver	47	560	43	510	39	620
T.K.	36	M	49	Normal liver	78	797	64	729	50	973
B.L.	37	M	48	Fatty liver	51	689	57	552	49	689
J.C.	38	M	42	Normal liver	67	511	47	681	53	600
E.F.	39	M	35	Fatty liver	60	408	58	477	60	478
				Mean	59	576	55	581	49	631
				±SD	±11	±172	±10	±148	±11	±193

administration of a loading dose of ICG. Trafuril,[2] a rubefacient cream, was applied 15 minutes before application of the earpiece and removed by brisk massage with a dry towel. After a stable arterial level was reached, the trace was brought back to the baseline and deviations from the baseline utilized to determine fluctuations of ICG arterial levels. Direct measurements of ICG blood levels were obtained by connecting the hepatic vein catheter and arterial needle through a Harvard dual syringe pump to two Colson densitometers (32). The densitometers were connected to a Shiner amplifier for recording.

RESULTS

Fick principle studies. Normal subjects who received conventional doses of the two dyes sequentially had a mean ICG blood flow of 481 ml per minute per m² and mean BSP blood flow of 630 ml per minute per m². An analysis of the difference of the means was statistically significant ($p < 0.001$; Table I). EHBF, using ICG, was also lower in each of five patients with moderate cirrhosis. In this group a mean ICG blood flow of 378 ml per minute per m² and a mean BSP blood flow of 564 ml per minute per m² were obtained, and analysis of the difference of the means was significant ($0.02 > p < 0.05$; Table II). An identical disparity occurred with simultaneous infusion of conventional dye dosage in five patients (Table III), and of equimolar concentrations in nine patients (Table IV). The same pattern was noted in Patient 3, whose blood flow was measured by both the sequential and simultaneous techniques of dye administration. The mean level of difference in calculated blood flow, using ICG and BSP, was of a similar magnitude with conventional and equimolar concentrations of these dyes.

It was possible to use 0.3 mg of ICG per m² per minute in estimating hepatic blood flow for periods of up to 2 hours in normal subjects and in patients with mild liver disease with only a slight decrease in extraction ratio in the latter phase of dye infusion (Table V). A mean extrac-

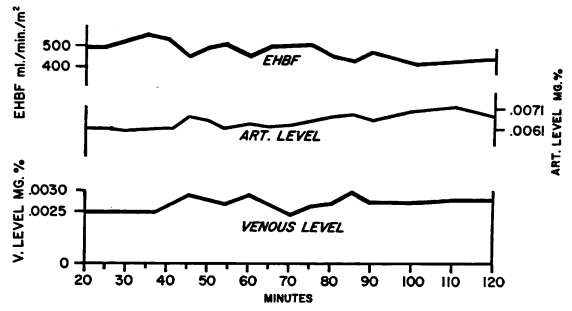

FIG. 1. ICG USED FOR ESTIMATION OF HEPATIC BLOOD FLOW (FICK) FOR 2 HOURS IN A NORMAL SUBJECT. Note the relative constancy of the hepatic venous and arterial dye concentrations.

[2] Supplied by Ciba Pharmaceutical Products, Inc.

TABLE VI

Effect of simultaneous administration of ICG (1.5 mg/m²/min) and BSP (1.6 mg/m²/min) on arterial concentration and extraction ratio in normal subjects

Patient	No.	Time after infusion started	ICG		BSP	
			Art. conc.	ER	Art. conc.	ER
		min	*mg%*	%	*mg%*	%
H.T.	25	20	1.30	87	0.70	61
		60	1.54	79	0.68	41
R.J.	26	20	1.63	91	0.65	72
		60	1.70	85	0.55	51
R.R.	27	20	1.02	80	0.53	60
		60	1.14	61	0.57	19
E.K.	28	25	1.14	81	0.63	71
		60	1.47	70	0.68	18

TABLE VII

Extraction ratios in patients in whom EHBF was possible with ICG but not with BSP

Patient data					Dye dosage (mg) and method of administration	ICG		BSP	
	No.	Sex	Age	Histological diagnosis		Art. conc.	ER	Art. conc.	ER
			yrs			*mg%*	%	*mg%*	%
J.O.	40	M	43	Viral hepatitis	0.3 ICG 3.0 BSP Sequentially	1.16	50	2.20	7
C.B.	41	M	36	Severe cirrhosis	0.3 ICG 3.0 BSP Sequentially	0.30	35	3.76	14
J.B.	42	M	39	Moderate cirrhosis	0.3 ICG 3.0 BSP Simultaneously	0.26	38	1.91	15
J.K.	43	M	57	Moderate cirrhosis	0.3 ICG 3.0 BSP Simultaneously	0.48	52	2.43	20
T.C.	44	M	29	Normal liver (after 120 mg norethandrolone daily for 10 days)	0.3 ICG 3.0 BSP Simultaneously	0.47	51	1.74	19
F.J.	45	M	69	Normal liver (after 120 mg norethandrolone daily for 10 days)	0.3 ICG 3.0 BSP Simultaneously	0.36	47	1.20	28

tion ratio was 59.9, 55.6, and 49.6 per cent at 20 minutes, 1 hour, and 2 hours, respectively, after beginning a constant infusion of ICG. There was remarkable constancy in both arterial and hepatic venous levels of ICG during the infusion (Figure 1). Similar results were obtained using 1.5 mg of ICG per m^2 per minute. When an equimolar concentration of BSP was given, its concentration was almost 50 per cent lower than that of ICG. With this dosage, there was little change in BSP concentration over a 60-minute period; however, the hepatic extraction ratio decreased significantly (Table VI).

ICG allowed estimation of hepatic blood flow, although BSP extraction was too low to permit blood flow studies in a patient with active viral hepatitis, in three patients with moderate cirrhosis, and in two normal subjects with abnormal BSP retention after receipt of 120 mg of norethandrolone [3] daily for 10 days (Table VII). Attempts to determine blood flow in six patients with severe cirrhosis, who exhibited clinical jaundice, ascites, and mental changes, were unsuccessful with both

TABLE VIII

Effect of prolonged simultaneous administration of ICG (0.3 mg/m²/min) and BSP (3.0 mg/m²/min) on arterial concentration and extraction ratio in cirrhosis

Patient	No.	Time after infusion started	ICG		BSP	
			Art. conc.	ER	Art. conc.	ER
		min	*mg%*	%	*mg%*	%
J.Q.	46	20	0.61	59	2.47	40
		60	0.63	61	3.45	13
		110	0.72	59	4.92	10
H.H.	47	25	0.32	46	1.08	30
		60	0.33	45	2.25	13
		100	0.36	39	3.85	9
J.K.	48	20	0.48	52	2.43	20
		60	0.44	57	3.39	12
		110	0.44	47	4.50	10
F.M.	49	20	0.52	76	2.62	51
		60	0.42	67	2.98	44
		120	0.56	70	5.0	21

TABLE IX

Comparison of extraction ratio and per cent disappearance rate using various doses of ICG in normal subjects

Subject	No.	ICG dose	ER	Disappearance rate
		mg/kg body wt	%	%
J.F.	50	0.15	69	17.8
		0.25	68	19.1
		0.50	67	18.4
J.K.	51	0.15	74.8	19.6
		0.25	77.1	20.2
		0.50	81.2	19.1
		Mean	72.85	19.03
		±SD	± 5.23	± 0.78

[3] 17α-Ethyl-17-hydroxynorandrostenone.

dyes. In four patients with cirrhosis in whom blood flow studies were performed for 100 to 120 minutes using conventional doses of ICG and BSP, there was little change in either the peripheral concentration or extraction ratio of ICG, whereas BSP blood levels increased and extraction ratios decreased and prohibited calculation of blood flow during the latter period of study (Table VIII).

Clearance studies. No significant difference between extraction ratio and percentage disappearance rate was noted when 0.15, 0.25, and 0.5 mg of ICG per kg body weight was given to two normal subjects (Table IX). For clearance studies, 0.5 mg of ICG per kg was selected. At no time after its injection was there 100 per cent hepatic extraction of ICG; during the decelerated phase of removal of dye from plasma, the extraction ratio exhibited a further decrease (Figure 2). There was a good correlation ($r = 0.8$) (33) between blood flow as calculated from the disappearance rate constant using ICG and colloidal gold both in normal subjects (Table X) and in patients with liver disease (Table XI). In contrast, neither of these substances provided a good correlation with blood flow results obtained by the Fick principle using ICG. A similar lack of agreement was noted between Fick principle results and blood flow calculated by dividing plasma clearance by extraction ratio ($r = 0.4$) or by using the disappearance rate constant ($r = 0.3$) (Table XI).

Oximetry. The Norman-NEP ear oximeter was found to provide a stable record of arterial

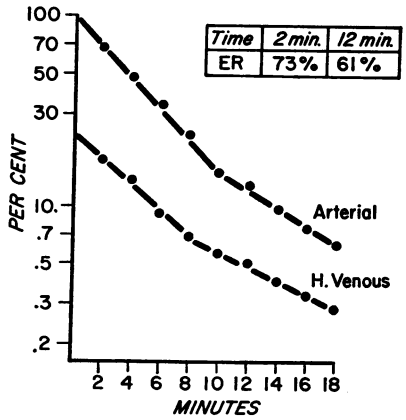

FIG. 2. PLASMA CLEARANCE OF ICG. Note the decrease in dye extraction during the decelerated phase of decay.

ICG blood levels. Unlike the Waters ear oximeter, its air-cooling system permitted continuous oximetry for 2 hours without discomfort. An arterial level of 0.001 mg of ICG produced a deflection of 0.5 cm on the recorder. Rapidly increasing levels of serum concentration of this dye, characteristic of saturation, were readily demonstrated. Fluctuations incident to changes in blood volume and flow through the ear occurred but were minimized by head fixation; these alterations did not interfere with the over-all usefulness of the monitoring system. Two Colson densitometers or cuvets were used to determine ICG concentration simultaneously in hepatic vein and arterial blood for calculation of blood flow. This permitted instantaneous determination of extraction ratio and blood flow after standardization of the system, and was

TABLE X

Comparison of EHBF using ICG and colloidal gold in normal subjects

Patient data				ICG blood flow calculated from disappearance constant	Colloidal gold blood flow calculated from disappearance constant	ICG blood flow calculated by Fick principle	ICG blood flow calculated from plasma clearance/extraction ratio
	No.	Age	Sex				
		yrs		*ml/m²/min*		*ml/m²/min*	
J.R.	52	32	M	1,000	960	670	
S.S.	1	34	M	510	520	470	439.6
B.W.	2	30	M	560	510	450	
L.H.	3	42	M	460	400	410	
T.S.	4	49	M	650	740	360	
F.A.	5	47	M	820	580	590	322.6
W.O'N	7	34	M	470	440	300	304.3
T.S.	9	38	M	690	890	530	597
J.H.	53	36	M	580	580	380	391.5
			Mean	637.7	624.4	462.0	411.0
			±SD	± 167.58	±185.24	±111.7	±104.3

TABLE XI

Comparison of EHBF using ICG and colloidal gold in patients with liver disease

Patient data				Histological diagnosis	Clinical findings*	Biochemical tests						ICG blood flow calculated by Fick principle	ICG blood flow calculated from disappearance constant	ICG blood flow calculated from plasma clearance/extraction ratio	Colloidal gold blood flow calculated from disappearance constant
	No.	Sex	Age			S. bil.	Alk. phos.	S. alb.	S. glob.	45-min BSP	Ceph. floc.				
			yrs			*mg%*	*BU*†	*g%*		%		*ml/m²/min*		*ml/m²/min*	
C.L.	16	M	57	Cirrhosis	Alcoholic; poor diet; H; palmar erythema	0.3	4.1	3.1	3.1	31	Neg.	320	330	227.0	440
J.S.	15	M	38	Cirrhosis	Alcoholic; poor diet; H	0.5	6.1	2.8	2.6	16.5	4+	400	450	429.9	
R.D.	54	M	52	Cirrhosis	Alcoholic; poor diet; H	0.1	3.4	3.3	2.3	6	2+	340	280	145.6	360
L.P.	17	M	41	Cirrhosis	Alcoholic; poor diet; H; S	1.4	7.4	3.1	2.8	23	4+	430	380	250.7	450
A.A.	18	M	64	Cirrhosis	Alcoholic; poor diet; J; A; H	6.3	6.8	2.3	2.7	48	4+	340	140		590
W.S.	55	M	46	Cirrhosis	Alcoholic; poor diet; H	0.1	2.5	2.9	3.5	2	1+	300	240		
H.S.	56	M	45	Fatty liver	Alcoholic; poor diet; obese; H	0.1	4.8	3.7	2.9	0.5	Neg.	400	390		
C.P.	57	M	28	Cirrhosis	Alcoholic; poor diet; H	1.8	7.0	3.8	2.6	16.5	1+	180	390		
L.B.	58	M	51	Cirrhosis	Alcoholic; poor diet; A; S	1.6	4.4	2.8	2.7	32	4+	350	160		330
G.O.	59	M	49	Cirrhosis	Alcoholic; poor diet	0.8	0.0	3.4	3.1	16.5	2+	570	340		580

* H, hepatomegaly; S, splenomegaly; J, jaundice; A, ascites.
† Bodansky units.

valuable in confirming the location of the catheter in a hepatic vein.

DISCUSSION

Results of these investigations provided a lower level than that previously established for EHBF utilizing BSP and the Fick principle in normal man. Winkler and Tygstrup (14) and Caesar and co-workers (15) have also obtained lower blood flow levels with ICG than with BSP when these dyes were given simultaneously to patients with hepatic disease. Methods of comparison constitute a problem, since the use of either conventional or equimolar concentrations (34) of these dyes given sequentially or simultaneously may theoretically alter results. This consideration was eliminated by utilizing each of these approaches. Also, variations in the level of dye in plasma produce divergent results, since peripheral BSP levels of less than 1.0 mg per 100 ml may give a falsely high hepatic blood flow (35). This was not responsible for observed differences, since representative peripheral levels of BSP were of the same, higher, and lower magnitude than those usually employed.

Technical considerations or differences in physical properties and physiological activities of ICG and BSP presumably account for the observed discrepancy in calculated blood flow. Factors which may influence results of blood flow, such as hemolysis (36), use of different hepatic veins, and variation in cardiac output, were excluded. This, therefore, focuses attention on the fact that ICG, unlike BSP, is not excreted as an amino acid conjugate, does not undergo an enterohepatic circulation, and exhibits negligible extrahepatic removal (11–13). Neither BSP conjugation, which should not influence results in the Fick formulation, nor enterohepatic circulation of this dye, which could produce a late effect, appears to account for the higher EHBF when BSP is used. Consequently, it seems likely that extrahepatic removal of BSP is principally responsible for the difference. It has been repeatedly demonstrated that BSP is taken up by skeletal muscle or bone marrow, sequestered in the splanchnic bed, and excreted in the urine (27, 37–39). It has been assumed that the loading dose of BSP saturates extrahepatic sites and that calculated removal represents hepatic uptake of this dye (1). Actually, there is continuous extrahepatic removal of BSP, as demonstrated by femoral arteriovenous differences (40) and maintenance of a constant BSP arterial level in the face of decreasing hepatic extraction when only 1.6 mg per m^2 per minute of this dye is used. In contrast, the concentration of ICG is 100 per cent higher than that of BSP when equimolar concentrations of the dyes are used, because the extrahepatic removal of ICG is negligible.

ICG permits estimation of hepatic blood flow in many patients with liver disease in whom it is impossible to use BSP. This appears to be true regardless of the dosage of BSP, although it is not possible to obtain an acceptable plasma concentration of BSP when the conventional ICG dose (0.3 mg per m^2 per minute) is used. The two patients treated with norethandrolone illustrate this point; the subcellular lesion produced by this drug alters extraction and calculated blood flow when BSP is used, but has no effect on extraction or blood flow with ICG. The difference in extraction occurs with both conventional and equimolar concentrations of these dyes (40). No significant alteration in hepatic extraction of ICG occurred during its continuous infusion over a period of 2 hours or more. This has permitted longer periods of reliable blood flow study during metabolic investigations. In contrast, when either 3.0 or 1.5 mg of BSP is used, there is a rapid decrease in extraction ratio so that in many instances blood flow levels are inaccurate after 60 minutes.

Other features of ICG which are valuable for measurement of hepatic blood flow by the Fick method include: its physical properties, which permit recognition of accumulation of the dye by ear oximetry; its suitability for serial blood flow studies using the Colson densitometer; its failure to cause local irritation or untoward reactions on repeated administration. Also, of particular importance is the recent demonstration in our laboratory that hepatic hypoxia induced by glucagon or by 10 per cent oxygen does not influence hepatic extraction or blood flow when ICG is used, whereas it regularly decreases extraction of BSP and increases calculated blood flow when the latter dye is used (41).

Considerable interest has been manifest in the possibility of evaluating hepatic blood flow from

clearance of substances which are removed to the extent of 90 to 100 per cent by a single passage through the liver. This approach is of limited value since it is generally agreed that liver disease or dysfunction which alters extraction, independent of blood flow, invalidates the use of clearance studies for this purpose (42–44). Unfortunately, neither blood flow determined by multiplying initial or subsequent clearance of ICG by the disappearance rate constant and blood volume, nor results derived by dividing initial clearance of ICG by its extraction ratio, provided a reliable and reproducible value for hepatic blood flow. The lack of correlation of blood flow calculated from the disappearance constant and from the Fick principle is probably due to the fact that the mean initial extraction of ICG was only 77 per cent and decreased during latter phases of its removal. The poor correlation of Fick data and of results obtained by dividing initial clearance of ICG by its extraction is more difficult to rationalize and must be attributed to inaccuracy of calculated extraction ratios after a single injection of ICG.

SUMMARY AND CONCLUSIONS

1. Studies were undertaken to determine the usefulness and limitations of indocyanine green in estimating hepatic blood flow. Indocyanine green consistently provided a lower estimate of blood flow by the Fick principle than did sulfobromophthalein when comparisons were made using conventional or equimolar concentrations of these dyes given sequentially or simultaneously. With indocyanine green, a mean blood flow for normal adults was found to be 480 ml per m^2 body surface area as compared with 630 ml by the sulfobromophthalein method in the same individuals. The lower level for blood flow is attributed to the fact that indocyanine green, unlike sulfobromophthalein, is removed to a negligible extent by extrahepatic tissues.

2. Advantages of indocyanine green for estimation of hepatic blood flow include the fact that it provides reliable blood flow data in patients with liver disease or hepatic dysfunction in whom sulfobromophthalein extraction is too low for calculation of blood flow; it regularly allows accurate blood flow measurements for 2 hours or more in normal subjects; its peripheral concentration may be monitored continuously by ear oximetry; and its administration has not evoked any local or systemic reactions.

3. Estimated hepatic blood flow calculated from indocyanine green clearance showed a good correlation with that obtained from plasma disappearance of radioactive colloidal gold. Neither provided an accurate index to hepatic blood flow when results obtained by the Fick principle with indocyanine green were used as a reference. Multiplying indocyanine green clearance by the disappearance rate constant, as well as dividing its clearance by its extraction ratio, provided incorrect estimates of hepatic blood flow.

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