



contained ICG was carried out for 18 hours in 3 systems: 1) butanol: acetic acid: water (10:4:8, vol/vol); 2) methylethyl ketone: propionic acid: water (75:25:30, vol/vol); and 3) pyridine: water: butanol (3:4:2, vol/vol). S and S no. 598 filter paper was used. Four spots were chromatographed with each buffer system: dye in aqueous solution, human bile to which dye had been added, human bile into which dye had been secreted, and a sample of bile containing no dye.

*Site of removal from plasma.* Five patients without evidence of kidney or liver disease were studied to determine whether ICG is ordinarily excreted in the urine. They were given 0.5 mg per kg body weight of ICG intravenously, and total urine collections were made during a 6 hour period following dye administration. Urine was collected in bottles containing 5 ml of a 5 per cent solution of human serum albumin in order to prevent deterioration of dye.

Appearance and disappearance of ICG was studied in bile taken from T-tube drainage of two patients who recently had undergone cholecystectomy for gallstones. Each received 2 mg per kg of dye as a single intravenous dose. Samples of bile were collected at intervals during periods of 6 and 19 hours, respectively. Two drops of a 5 per cent solution of human serum albumin were added to each 5 ml bile sample which was then diluted as necessary with distilled water to produce a dye concentration suitable for spectrophotometric analysis.

Peripheral arteriovenous differences of ICG were studied in one normal subject. He was given a priming dose of 10 mg of ICG intravenously, and a constant infusion of the dye at a rate of about 0.5 mg per minute was administered through a polyethylene catheter inserted into an antecubital vein of the left arm. During the infusion, simultaneous sampling was done at 5-minute intervals from a Cournand needle placed in the left brachial artery and from a polyethylene catheter inserted into a right antecubital vein.

*Plasma decay.* Plasma decay of ICG and sulfobromophthalein<sup>3</sup> (BSP) was studied in a group of 9 patients without clinical or laboratory evidence of liver

<sup>3</sup> Bromsulphalein; Hynson, Westcott, and Dunning, Baltimore, Md.

disease (Table I) and in 16 patients with confirmed liver disease (Table II). Fourteen of the latter had cirrhosis, in 8 of whom it was associated with chronic alcoholism and in 6 of unknown etiology. Two patients with cirrhosis had normal liver function tests, but in both, hepatic histology was consistent with the diagnosis. Two patients had infectious hepatitis.

All subjects were given ICG, 0.5 mg per kg body weight, and BSP, 5 mg per kg body weight as single, rapid, intravenous injections at least 2 hours apart. Venous samples were taken at 3 and 5 minutes from the time of dye injection and at 5-minute intervals thereafter for at least 1 hour and in some cases for 2 or 3 hours. One patient with infectious hepatitis had 3 determinations of ICG and BSP decay during the acute illness and convalescent period. Indocyanine green was also administered to 7 of the 9 control subjects (Table I) in amounts of 1.0 mg per kg body weight and to 3 additionally in doses of 2.0 mg per kg body weight, sampling being made as stated above. BSP concentration in serum was determined by alkalization and reading in an Evelyn colorimeter at 580 m $\mu$ .

Plasma dye decay rate was calculated from the following formulae (6):

$$R = 1 - d$$

and

$$\log d = \frac{\log C_2 - \log C_1}{t_2 - t_1}$$

where R is the rate of decay per minute, d is the fraction retained per minute, C<sub>2</sub> and C<sub>1</sub> are concentrations in plasma at two points during the exponential decay period, and t<sub>2</sub> and t<sub>1</sub> are the times in minutes corresponding to C<sub>2</sub> and C<sub>1</sub>.

*Hepatic extraction.* Hepatic extraction rates of ICG and estimated splanchnic blood flow were determined in 7 subjects without liver disease, using right hepatic vein catheterization. The catheter was advanced to wedge position, then withdrawn just far enough to permit free sampling, in order to minimize reflux from the inferior vena cava. ICG was infused in 0.5 per cent albumin solution with the use of a Bowman pump at a constant rate of from 0.3 to 0.7 mg per minute after an initial intravenous loading dose of 10 to 15 mg had been given.

TABLE I  
*Clinical and laboratory data on patients without evidence of liver disease*

Subject	Sex	Age	Diagnosis	Cephalin flocculation	Serum bilirubin mg/100 ml
AW	M	27	Pneumococcal pneumonia	0	0.8
JW	M	54	Peptic ulcer	1+	0.3
BO	M	49	Alcoholism	2+	0.7
FW	M	58	Pneumococcal pneumonia	1+	1.9
EE	M	51	Alcoholism	0	0.2
JT	M	43	Alcoholism	1+	0.5
JM	M	42	Peptic ulcer	2+	0.7
CF	M	45	Alcoholism	1+	0.4
CW	M	48	Post-traumatic epilepsy	0	0.6

TABLE II  
*Clinical and laboratory data on patients with liver disease*

Subject	Sex	Age	Clinical features*	Clinical diagnosis	Liver histology	Cephalin flocculation	Serum bilirubin mg/100 ml
JL	M	49	H, S	Cirrhosis Of the alcoholic	Fibrosis, fat, inflammation	4+	16.8
JS	M	49	H, S	Of the alcoholic		1+	2.1
BB	F	53	H	Of the alcoholic		1+	1.6
AB	F	59	H, A, S	Of the alcoholic	Fibrosis, alc. hyaline	3+	12.8
ZM	F	39	None	Of the alcoholic	Fibrosis, fat	0	0.5
EC	F	45	H	Of the alcoholic	Fibrosis, fat	2+	0.4
JM	M	34	H	Of the alcoholic	Fibrosis, mild bile stasis	2+	1.8
MS	M	36	H, A, S	Of the alcoholic		1+	1.7
DG	M	58	H, S, P-C	Etiology unknown	Portal cirrhosis	2+	1.4
JC	M	70	H, S, P-C	Etiology unknown	Postnecrotic cirrhosis	2+	3.9
BM	M	47	None	Etiology unknown		2+	0.6
MM	F	65	H, S	Etiology unknown	Fibrosis, fat	3+	0.5
AV	M	57	H, S	Etiology unknown		2+	0.2
RR	M	48	None	Etiology unknown	Fibrosis	1+	0.4
RB	M	32	H, S	Viral hepatitis		4+	5.8
EG	M	29		Viral hepatitis			
	5-1-59		H, S			3+	1.0
	5-8-59		None			2+	0.6
	5-18-59		None			2+	0.4

\* H = hepatomegaly; S = splenomegaly; A = ascites; P-C = portacaval shunt.

The addition of this amount of albumin had been shown to stabilize dye solutions during the infusion periods. Dye concentration was determined in blood samples taken simultaneously at 10-minute intervals from an indwelling brachial arterial needle and the hepatic vein catheter.

Extraction rate (E.R.) was calculated from the formula:

$$E.R. = \frac{A - HV}{A}$$

where A is the arterial dye concentration and HV is the dye concentration in hepatic venous blood.

Estimated hepatic blood flow (EHBF) in liters per minute was calculated from the formula:

$$EHBF = \frac{R}{(P - H)(1 - HCT)}$$

where R is the total ICG removal rate in milligrams per minute, P is the concentration of ICG in peripheral arterial blood in milligrams per liter, H is the concentration of ICG in hepatic venous blood in milligrams per liter, and HCT is the peripheral venous hematocrit.

Total ICG removal rate (R) was taken as equal to the infusion rate in milligrams per minute during periods when peripheral dye concentration was constant (7).

#### RESULTS

*Physical properties.* Initial volume of distribution of indocyanine green, calculated from plasma

dye decay curves, and the volume of distribution of radioiodinated albumin at 5 minutes showed close agreement in the four patients studied (Table III). There was close correspondence between the absorption spectrum of ICG in a 5 per cent solution of human serum albumin and in normal human plasma (Figure 2). Absorption maximum was at 815  $m\mu$  in both cases. The absorption spectrum of the dye in aqueous solution was distinctly different from the spectra found for the two albumin-containing dye solutions, the absorption maximum being at 790  $m\mu$ . On starch block electrophoresis, ICG was found to migrate

TABLE III  
*Comparison of volume of distribution of radioiodinated albumin and initial volume of distribution of indocyanine green*

Subject	$I^{131}$ -albumin volume of distribution at 5 min	Initial volume of distribution of ICG
	ml	ml
JR	4,202	4,351
BO	3,100	2,970
CE	2,908	2,810
JT	3,636	3,801

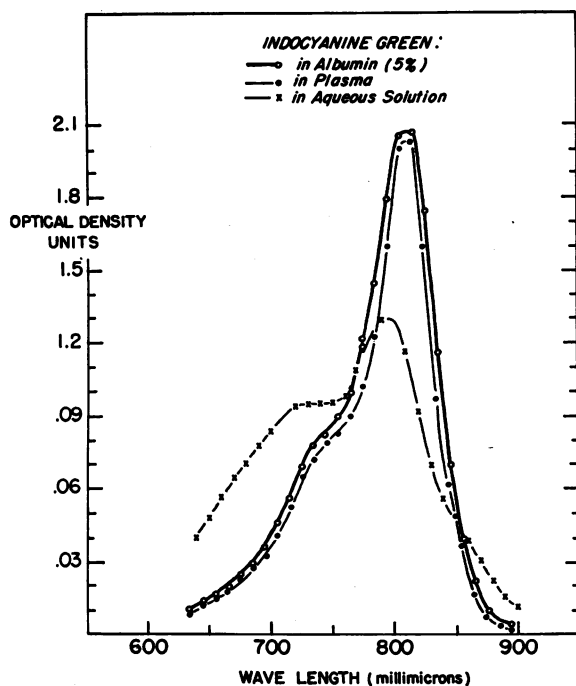


FIG. 2. ABSORPTION SPECTRA OF INDOCYANINE GREEN.

with albumin and to be bound to the other plasma proteins in only small amounts (Table IV). Chromatographic studies carried out in three different systems showed no differences in the rate or pattern of movement of dye in aqueous solution as compared with dye which had been secreted into bile and dye which had been added to bile (Figure 3).

*Site of removal from plasma.* Urine collected from five patients during a 6 hour period following the intravenous administration of ICG contained no dye. Following its intravenous injection, ICG appeared at about 8 minutes in the T-tube bile from one patient and at about 15 min-

TABLE IV

*Plasma protein-binding of indocyanine green as determined by starch block electrophoresis—average of four determinations*

Plasma protein	Recovered ICG associated with plasma protein
	%
Albumin	95.0
$\alpha_1$ -globulin	2.4
$\alpha_2$ -globulin	2.0
$\beta$ -globulin	0.6
$\gamma$ -globulin	0.0

TABLE V

*Appearance and disappearance of indocyanine green in bile of patient following rapid intravenous administration of indocyanine green \**

Following dye injection	Concentration of ICG in bile
<i>min</i>	<i>mg/100 ml</i>
15	0.010
30	0.022
60	0.283
120	0.750
240	0.354
540	0.163
600	0.134
900	0.067
1,020	0.024
1,080	0.005
1,140	0.002

\* Two mg per kg body weight.

utes in the other. When sampling of bile was discontinued at 6 hours after dye injection in the former case, ICG continued to be present in high concentration. Although removal from plasma was very rapid, there was distinct delay in biliary excretion, the peak concentration in bile occurring at 120 minutes (Table V). Sampling of bile was

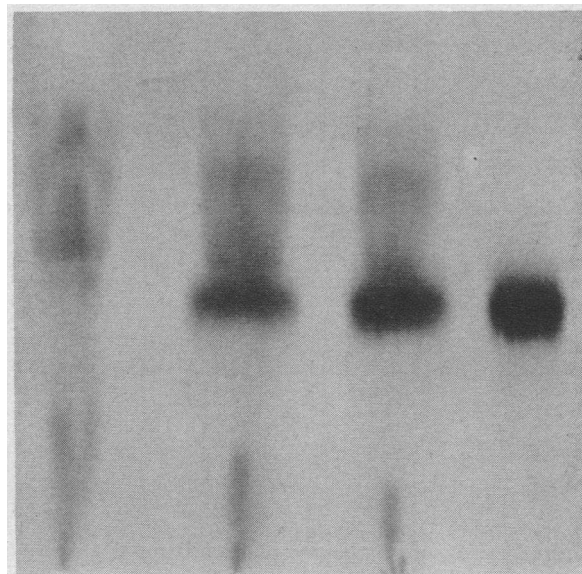


FIG. 3. ASCENDING CHROMATOGRAMS DEVELOPED IN BUFFER SYSTEM OF METHYLETHYL KETONE: PROPIONIC ACID: WATER (75:25:30, VOL/VOL). Left to right: human bile; human bile containing endogenously-secreted ICG; human bile containing exogenously-added ICG; ICG in aqueous solution. Green color was restricted to same regions (very dense areas) in three chromatograms containing ICG.

TABLE VI  
Peripheral arterial and venous concentrations of indocyanine green following start of intravenous dye infusion in contralateral arm

Following start of dye infusion	Artery concentration of ICG	Vein concentration of ICG
min	mg/100 ml	mg/100 ml
5	0.168	0.166
10	0.164	0.164
15	0.154	0.156
20	0.147	0.143
25	0.138	0.142
30	0.128	0.126
35	0.133	0.137
40	0.136	0.134
45	0.140	0.139
50	0.152	0.154

continued in the second case until 19 hours following dye administration, at which time only a trace of ICG was present. No attempt was made at quantitative recovery of dye in the bile since T-tubes do not collect all of the biliary drainage externally. Peripheral arterial and venous samples simultaneously collected during a 50 minute period from a patient receiving an intravenous infusion of ICG revealed no consistent differences in dye concentration (Table VI). Therefore, detectable peripheral tissue uptake of ICG did not occur.

*Plasma decay.* Decay of ICG in plasma of normal subjects occurred in nearly exponential fash-

TABLE VII  
Comparison of plasma removal of indocyanine green with plasma removal of sulfobromophthalein

	Indocyanine green 0.5 mg/kg body weight			Sulfobromophthalein 5.0 mg/kg body weight		
	Initial rate of decay	Half-life	Retained after 20 min	Initial rate of decay	Half-life	Retained after 45 min
	%/min	min	%	%/min	min	%
Normal						
AW	20.9	3.0	2.5	11.0	6.0	1.4
JW	16.2	3.9	4.5	15.6	4.1	1.5
BO	15.4	4.1	4.9	13.1	5.0	2.8
FW	15.6	4.2	4.5	11.1	6.1	4.4
EE	18.7	2.5	4.2	14.4	4.4	0.4
JT	20.1	3.1	2.2	12.7	5.1	1.2
JM	15.1	4.2	4.7	13.9	4.7	0.9
CF	20.8	3.0	3.4	16.0	4.0	1.3
CW	24.0	2.7	3.7	16.9	3.8	1.3
Mean ( $\pm$ SD)	18.5 ( $\pm$ 3.1)	3.4 ( $\pm$ 0.7)	3.8 ( $\pm$ 1.0)	13.8 ( $\pm$ 2.1)	4.8 ( $\pm$ 0.8)	1.7 ( $\pm$ 1.2)
Cirrhosis—diagnosis by clinical and laboratory findings; biopsy confirmation in 7 cases						
JL	1.2	110.0	77.4	1.6	47.6	51.1
JS	4.6	14.4	38.2	2.6	26.4	33.4
BB	5.1	13.3	34.9	4.7	14.2	16.1
AB	1.7	46.8	70.9	2.8	29.7	43.1
ZM	18.8	3.3	4.2	9.8	6.8	5.3
EC	15.9	4.1	5.7	9.3	7.1	5.9
JM	6.8	10.0	24.6	3.8	19.1	31.6
MS	4.0	44.8	72.5	2.5	27.5	33.0
DG	3.3	20.4	50.6	2.1	43.0	49.0
JC	2.9	23.6	55.4	1.8	47.0	50.7
MM	14.6	4.4	7.7	5.1	13.0	17.9
AV	15.0	4.3	5.7	5.7	11.9	18.0
Mean	7.8	25.0	37.3	4.3	24.4	29.6
Cirrhosis—diagnosis by biopsy; all clinical and laboratory findings normal						
BM	17.0	3.7	5.0	12.2	5.6	6.2
RR	16.4	3.9	4.4	11.2	5.8	1.2
Viral hepatitis						
RB	16.0	3.9	5.1	6.3	21.4	23.4
EG						
5/1/59	16.5	3.9	5.3	4.0	20.2	34.7
5/8/59	20.0	3.1	2.5	9.9	6.8	7.3
5/18/59	19.3	3.1	2.8	10.3	6.2	3.7

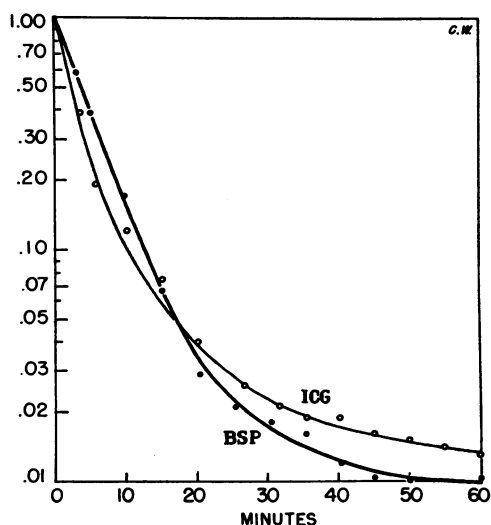


FIG. 4. DECAY OF ICG AND BSP IN PLASMA OF CONTROL SUBJECT (CW), PLOTTED WITH ZERO-TIME CONCENTRATION TAKEN AS UNITY.

ion for 10 to 20 minutes, exhibiting falling concentrations on a straight line on semilogarithmic paper. Following that initial period, deceleration of decay occurred in all cases (Figure 4). The approximate rate of decay, calculated during the exponential decay segment, was 18.5 per cent per minute (SD 3.1) for ICG as compared with 13.8 per cent per minute (SD 2.1) for BSP (Table VII).

Among the control subjects of this series, the mean BSP level 45 minutes after injection was 1.7 per cent (SD 1.2) of the initial concentration. ICG removal was so rapid that the percentage retained after 20 minutes was selected for comparison. The mean value in the normal group was 3.85 per cent (SD 1.0). When the amount of ICG injected was raised to 1 mg per kg body weight, the percentage retained at 20 minutes remained unchanged (mean, 3.9 per cent). When the dosage was further increased to 2.0 mg per kg body weight, the mean value for 20 minute retention was 4.0 per cent.

Plasma decay of ICG characteristically was suppressed in patients with cirrhosis. An example of marked defect in dye clearance is illustrated in Figure 5. The BSP decay curve is shown on the same plot for comparison. The group of subjects with cirrhosis contained patients with widely differing degrees of liver damage (Table II).

Rates of ICG decay in plasma of those patients, as expected, varied markedly as did decay rates for BSP (Table VII). Average values for removal of both dyes, therefore, have little meaning in that group. The correlation "R" between initial rates of decay for the two test substances was 0.92 ( $p < 0.01$ ). Two patients with cirrhosis had no clinical stigmata of that disease and showed no evidence of impaired liver function. They nevertheless had histological liver changes which unequivocally established the diagnosis of cirrhosis. If those two are excluded, the correlation is 0.91 ( $p < 0.01$ ). Although the mean value for initial decay rate of ICG has a high degree of correlation with the mean initial decay rate of BSP within the group of patients with cirrhosis, there are individual cases that belie the average values (Table VII). In most cases ICG was removed somewhat more rapidly than BSP. Two patients (MM and AV) with distinctly abnormal BSP decay curves cleared ICG in normal fashion. Two patients (AB and JL) with extreme impairment of hepatic function removed BSP more rapidly than ICG.

Both patients with infectious hepatitis had distinctly abnormal BSP decay curves but probably normal rates of removal and 20 minute levels of ICG (Table VII). One of those patients (EG) had two repeat studies during convalescence. BSP removal showed a return to normal, while ICG removal, already within the normal range, nevertheless showed definite increase in rate of plasma disappearance.

During the course of plasma decay studies, ICG

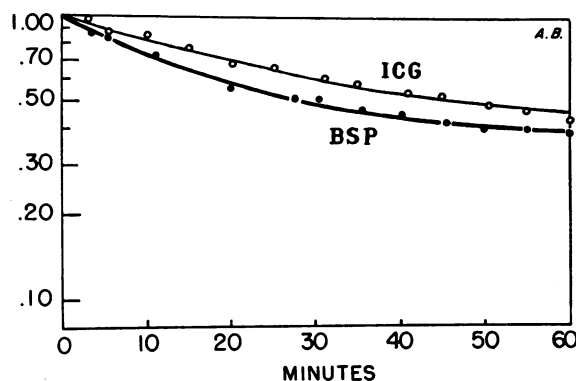


FIG. 5. DECAY OF ICG AND BSP IN PLASMA OF PATIENT WITH DECOMPENSATED CIRRHOSIS (AB), PLOTTED WITH ZERO-TIME CONCENTRATION TAKEN AS UNITY.

was inadvertently extravasated subcutaneously in three subjects. No ill effects resulted. Neither single nor repeated injections or infusions of ICG were associated with untoward systemic reactions.

*Hepatic extraction.* Stabilization of both arterial and hepatic venous levels of ICG usually occurred within 20 minutes and could be maintained for periods as long as 2 hours. Extraction rates of ICG determined at the earliest point of plasma dye stabilization varied between 0.59 and 0.83 in seven normal subjects. Estimated hepatic blood flow calculated from these data ranged from 0.70 to 1.80 L per minute per m<sup>2</sup> of body surface area. The results of studies of hepatic flow will be reported in detail in a later communication (8).

#### DISCUSSION

Indocyanine green appears to be rapidly and completely bound to plasma protein following its intravenous administration. This can be inferred from the data which show that at zero time the space occupied by the dye is about the same as that of plasma volume (Table I). If it were not bound to a nondiffusible molecule, an appreciable amount of the dye would quickly leave the intravascular compartment. The shift which occurs in the absorption spectrum of ICG when albumin is present in solution (Figure 2) is evidence that the dye is bound to albumin. The similarity of the absorption spectrum of the dye in human plasma and in albumin solution, and the identity of the absorption maxima suggest that plasma proteins other than albumin are not important in the binding of ICG. These data are supported by the results of starch block electrophoresis (Table II).

Chromatography of ICG in three different buffer systems fails to show evidence that the dye is secreted into bile in conjugated form. ICG differs in that respect from BSP which has been reported to be secreted, in part, in conjugation with one or more amino acids (9-12). The present studies further indicate that, unlike BSP, ICG is not cleared by the kidney and that peripheral tissue uptake of the dye is negligible. Renal clearance (13) and tissue uptake (6, 14, 15) of BSP are known to occur.

In normals and in most patients with cirrhosis ICG is removed more rapidly than is BSP. It

appears likely, on the basis of decay characteristics of ICG in patients with cirrhosis, that the dye has good discriminative value in selecting those subjects who have a significant degree of liver disease. In the entire group of patients with cirrhosis, its sensitivity compared favorably with BSP, the correlation "R" between the initial decay rate of the two test substances being 0.92 ( $p < 0.01$ ). ICG appears in a less favorable light when individual cases are examined, however, for it revealed no abnormality in two patients (AV and MM) in whom BSP decay was significantly impaired. At the same time, ICG was more strikingly abnormal in two jaundiced cirrhotics (JL and AB) than was BSP. In the two cases of viral hepatitis studied, ICG failed entirely to reflect hepatic dysfunction. At the time the dye studies were made the clinical disease was mild, and both patients had normal serum bilirubin concentrations and cephalin flocculation tests.

There are several possible explanations for the failure, in certain subjects, of the two dyes to behave similarly. First, it is known that the mechanisms whereby the two substances are handled by the liver are not identical; BSP is partially conjugated during its biliary secretion while ICG is not. There may be other differences, not recognized at present, between clearance mechanisms of the two dyes. On the other hand it may be that physiologically equivalent amounts of BSP and ICG had not been given. The dosage of 0.5 mg per kg body weight was most extensively used in these studies. On the basis of seven ICG decay curves when 1.0 mg per kg body weight had been injected, there is no indication that dye decay is different at these two dosage levels. The percentage of initial plasma dye concentration remaining at 20 minutes was the same in both cases. A dose of 2.0 mg per kg was studied in three subjects. The percentage of dye remaining at 20 minutes and the shape of the decay curve did not differ significantly from those found when ICG had been given in the two smaller amounts. Nothing is known of the toxicity of ICG in amounts exceeding 2.0 mg per kg body weight. Investigations using such large amounts of dye were therefore not undertaken.

Another explanation for the occasional disparate behavior of the two dyes lies in the fact that BSP plasma decay is partially accounted for by extra-

hepatic removal, while no such extraction has been demonstrated for ICG. In hepatectomized, eviscerated dogs, 50 per cent of the initially-injected dose of BSP has been shown to be removed in 50 minutes (13). It is likely, therefore, that in subjects with very severe liver disease extrahepatic modes of BSP removal might become very significant. In these patients ICG may be a better relative index of liver function because there is apparently no alternate means for removal.

For clinical purposes, the retained percentage of ICG, 20 minutes after a single injection of 0.5 mg per kg body weight, seems to give information equivalent to the rate of decay during the exponential portion of the plasma decay curve. In this small series, inadequate to establish norms, it appeared that 20 minute dye retention of less than 5 to 6 per cent and initial decay of greater than 15 per cent per minute were within normal limits.

If its insensitivity to minor degrees of liver dysfunction limits its practical value in the evaluation of liver disease, ICG seems to be an excellent substance for estimating hepatic blood flow by the Fick principle (8). It is apparently extracted from the plasma exclusively by the liver. The faster decay of ICG than of BSP in some subjects with mild liver disease may mean that blood flow studies can be carried out in such persons with ICG when those studies would not be feasible with BSP because of poor hepatic extraction and excessive plasma retention of the dye. ICG extraction rates of a magnitude suitable for calculating hepatic blood flow were found in these studies.

#### SUMMARY AND CONCLUSIONS

Following its intravenous injection, indocyanine green (ICG) appears to be rapidly and completely bound to plasma protein, of which albumin is the principal carrier. The dye is excreted in bile in unconjugated form. It is not cleared by extrahepatic mechanisms in detectable amounts. ICG was nonirritating when inadvertently introduced subcutaneously and produced no untoward reactions upon single or repeated intravenous injections or infusions.

In normal subjects and in selected patients with liver disease, plasma decay of ICG was similar to

that of sulfobromophthalein (BSP). In subjects without liver disease mean initial ICG decay rate was 18.5 per cent per minute as compared with 13.8 per cent for BSP. In patients with cirrhosis, the correlation "R" between initial decay rates of the two substances was 0.92. While patients with mild liver disease seem to remove ICG more rapidly than BSP, persons with marked hepatic dysfunction may remove BSP more rapidly than ICG.

Indocyanine green should provide a reliable means of estimating hepatic blood flow by use of the Fick principle. Its physical properties, degree of hepatic removal, tolerance by human subjects, and rapid decay even in the presence of mild liver disease make it an ideal dye for that purpose.

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