THE PLASMA REMOVAL OF INDOCYANINE GREEN AND SULFOBROMOPHTHALEIN: EFFECT OF DOSAGE AND BLOCKING AGENTS

By DONALD B. HUNTON, JESSE L. BOLLMAN AND HARRY N. HOFFMAN, II

(From the Mayo Clinic and Mayo Foundation, Rochester, Minn.)

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In 1950 Brauer and Pessotti (1) observed that the concentration of sulfobromophthalein in bile reached maximal values which were not influenced by further increments in infusion rate. In 1956 Combes, Wheeler, Childs and Bradley (2) introduced the concept of maximal biliary transport capacity for sulfobromophthalein. It is now generally held that when sulfobromophthalein is infused at a rate that exceeds the maximal biliary transport capacity, the biliary excretion of the dye remains relatively constant.

The effect of the initial dosage or infusion rate of sulfobromophthalein on the various parameters measuring hepatic uptake is not so uniformly agreed upon, due in part to confusion about the meaning of the parameters themselves. These include: *1*) hepatic extraction—the percentage of dye removed in one passage through the liver; *2*) clearance rate—the milliliters of plasma completely cleared of dye in 1 minute; and *3*) disappearance rate or removal rate—herein defined as the percentage of remaining dye removed each minute. Unless specified, removal rate will not be used to express the number of milligrams removed per minute.

Casselman and Rappaport (3), Andrews, Maegraith and Richards (4) and Combes and associates (2) have suggested that hepatic extraction is constant or nearly constant, regardless of dosage or infusion rate. Myers (5) and Cook, Lawler, Calvin and Green (6) observed the exact converse; that is, that extraction became less efficient with increasing dose. Lavers and colleagues (7) found that variation in the initial single dose did not affect the rate of removal of sulfobromophthalein from the plasma. Again, the converse has been reported: Fauvert (8) stated that increasing the initial dose decreased the plasma disappearance rate of the dye.

Wheeler and associates (9, 10) recently described the hepatic uptake of sulfobromophthalein under special circumstances in which a constant infusion of dye is in excess of the maximal biliary transport capacity. Under these circumstances a portion of plasma removal of the dye is accounted for by bile excretion; this is a constant. The remainder of the dye taken up by the liver from the plasma is stored in the hepatic cells. These authors have shown that hepatic storage is directly proportional to plasma concentration. Consequently, the higher the plasma concentration, the more milligrams of dye can be stored. Limitations were not observed for any potential maximal capacity for this removal mechanism, but it should be noted that the phenomenon is not rate limited. This description does not apply to the mechanics of removal of a single injection of dye, however, because the storage compartment is empty at the outset and, obviously, no constant proportional relationship between storage and plasma concentration would be possible.

Several authors (1, 11–13) reported that sodium dehydrocholate will slow the removal of sulfobromophthalein from the plasma and will delay its excretion in the bile. Cantarow, Wirts, Snape and Miller (14) and Fauvert (8) suggested that competition for hepatic uptake exists between bilirubin and sulfobromophthalein, the latter being selectively removed. Brauer and Pessotti (1) observed that rose bengal slowed the excretion of sulfobromophthalein in bile, but Popper and Schaffner (15) and Fauvert (8) pointed out that sulfobromophthalein is selectively taken up by the liver when administered simultaneously with rose bengal. Wheeler, Cranston and Meltzer (16) reported that the hepatic uptake of sulfobromophthalein was faster than that of indocyanine green; Cherrick, Stein, Leevy and Davidson (17) reported the converse. However, the last two reports were done on different species with different dosage relationships, and the dyes were not administered simultaneously.

In recent studies of hepatic function, indocyanine green has demonstrated the following advantages over other protein-binding dyes: essentially complete recovery in bile (16, 18–20), single exponential plasma removal pattern (16, 17, 19–23), distribution limited to the intravascular volume (16, 24), no demonstrable uptake by extrahepatic tissues (18), no urinary excretion (18–20, 23), no enterohepatic circulation (16), and no reabsorption in the lymphatic circulation (19, 20). Because of these advantages, indocyanine green was employed in these studies in an attempt to clarify the various phenomena and to resolve some of the apparent conflicts.

METHODS

The technic used for the determination of the concentration of indocyanine green in plasma has been described elsewhere (19). Briefly, it consists of measurement of transmittance of light in a spectrophotometer at 805 mμ after appropriate dilution of the specimen.

The concentration of sulfobromophthalein in the plasma was determined by measurement of light transmittance in a spectrophotometer at 580 mμ after dilution and alkalinization. Turbid or hemolyzed specimens were not used.

Determination of the concentration of indocyanine green and sulfobromophthalein in the same specimens of plasma with simultaneous administration of both dyes was performed as follows. Transmittance of light at 805 mμ was measured at neutral pH which thus measured only the indocyanine green, the sulfobromophthalein being decolorized. Then transmittance was measured in the same specimens at 580 mμ after alkalinization. This measured the concentration of sulfobromophthalein, since at this wave length and in this concentration indocyanine green, while not decolorized, transmits 98 to 100 per cent of light (25). In these studies the dosage of sulfobromophthalein was five times greater than that of indocyanine green in order that the indocyanine green be sufficiently dilute not to interfere with transmittance at 580 mμ, and that no intervening dilutions be required between measurements, since indocyanine green absorbs light more intensively than does sulfobromophthalein.

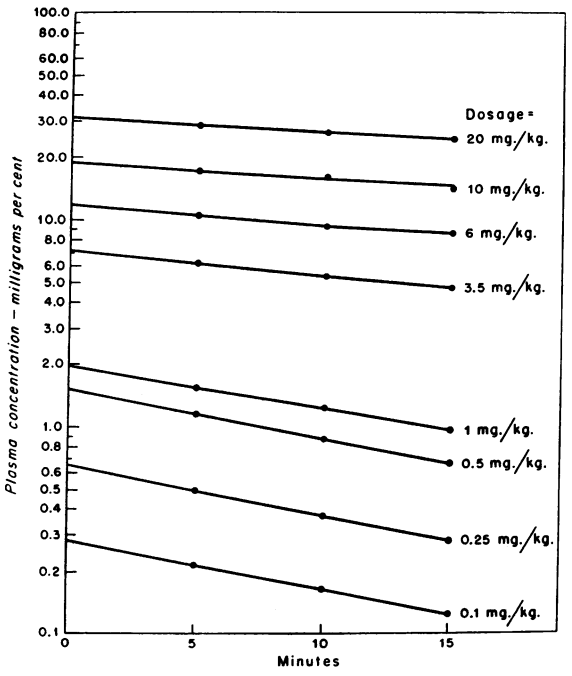

FIG. 1. PLASMA DISAPPEARANCE RATE OF INDOCYANINE GREEN IN DOG A.

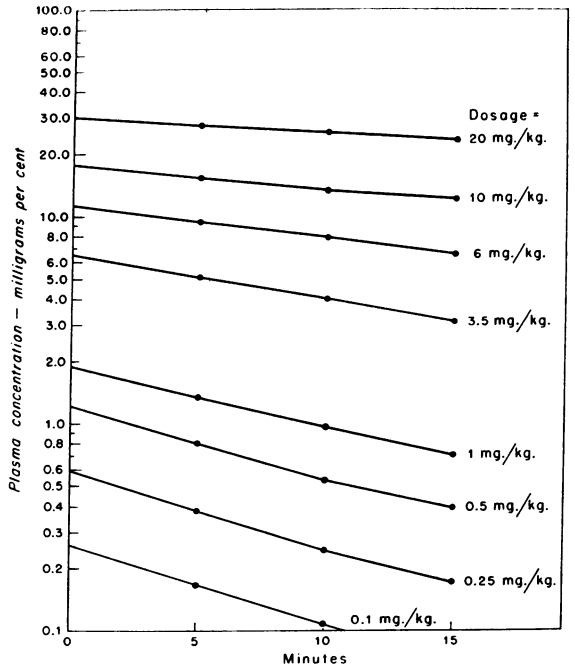

FIG. 2. PLASMA DISAPPEARANCE RATE OF INDOCYANINE GREEN IN DOG B.

The method of calculation of the plasma disappearance rate has been described previously (8, 19). We have found (19, 20), as have others (16, 18), that the plasma disappearance rate of indocyanine green in dogs is exponential for at least 15 minutes, and usually for 30 to 60 minutes, in essentially all cases. Since the present studies of dose effect and blocking are concerned with phenomena occurring in the early phase of removal from the plasma, blood samples were taken at 5, 10 and 15 minutes after injection of dye.

The plasma removal of sulfobromophthalein in dogs and rats does not follow a single exponential pattern, but for description of the phenomena in the early phase of removal, a useful approximation can be reached by taking two points on the early portion of the disappearance curve and substituting these values in the equation as if the

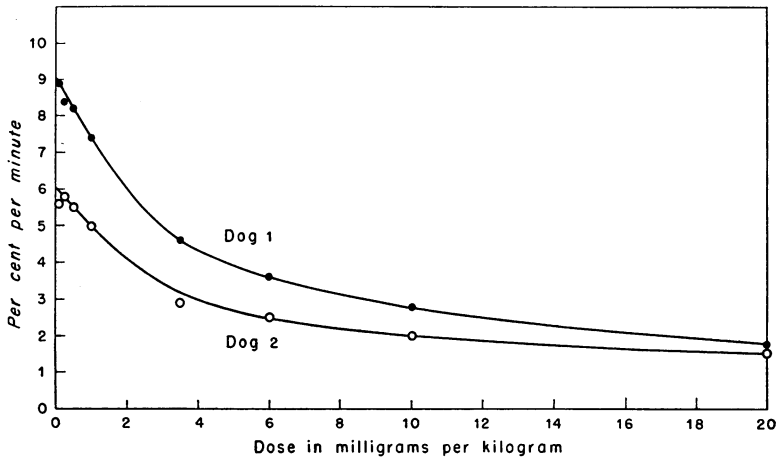

FIG. 3. THE DOSE AND PLASMA DISAPPEARANCE RATE ARE RELATED INVERSELY; LARGER DOSES HAVE PROPORTIONATELY SMALLER EFFECTS.

pattern were exponential. The removal rates of sulfobromophthalein in this study were so obtained and apply only to this time.

RESULTS

The plasma disappearance rate of indocyanine green [1] was determined in each of two unanesthetized dogs on eight separate occasions with doses of dye which varied from 0.1 to 20.0 mg per kg body weight. Blood was sampled at 5, 10 and 15 minutes. The removal curves were consistently exponential for the 15-minute observation (Figures 1 and 2). As the initial dose increased, the plasma disappearance rate decreased but not in linear fashion. The apparent similarity of the slopes of the lines in Figures 1 and 2 is the result of the scale including 3 log cycles. The larger dosage of dye exerted a proportionately smaller slowing effect (Figure 3).

When the plasma disappearance rate was multiplied by the initial dose (per cent per minute times milligrams per kilogram), an expression of the actual number of milligrams per minute removed in the initial moments was obtained (milligrams per minute per kilogram). This figure applies only to the initial moments because, with a constant percentage being removed (exponential rate), the milligrams per minute will decrease steadily with time.

When this initial removal rate in milligrams per minute per kilogram was plotted logarithmically against the initial dose (Figure 4), it was seen that with larger doses a larger amount of dye was removed, even though the percentage was smaller. Logarithmic plots were made of the difference between the observed removal rate and various arbitrary possible asymptotes to the curve in Figure 4. With 0.4 mg per kg weight per minute

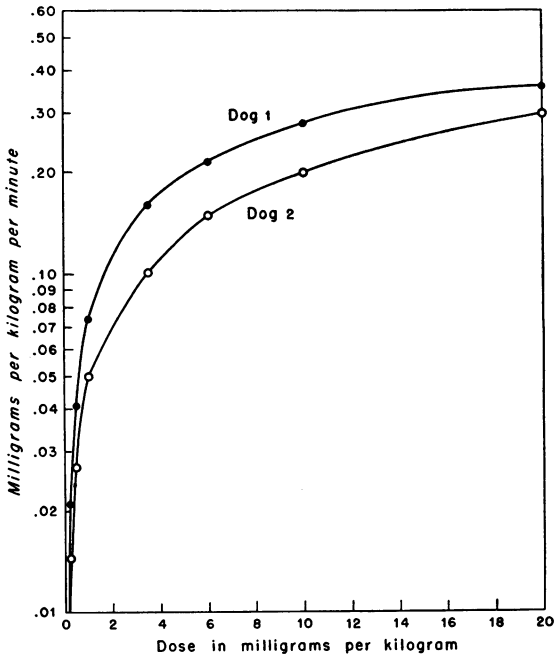

FIG. 4. THE REMOVAL RATE IN MILLIGRAMS PER MINUTE PER KILOGRAM INCREASES WITH INCREASING DOSAGE BUT AN ASYMPTOTE IS APPROACHED AT 0.4 MG PER MINUTE PER KG, WHICH REPRESENTS THE MAXIMAL CAPACITY FOR REMOVAL OF DYE.

[1] Supplied as cardio-green through the courtesy of Hynson, Westcott & Dunning Pharmaceutical Laboratories, Baltimore, Md.

as an asymptote, the log plot of the difference was a straight line. This suggests that the asymptotic value is the maximal initial (rate limited) removal capacity.

The initial plasma disappearance rate of sulfobromophthalein was estimated in two unanesthetized dogs. Blood was sampled at 5, 10 and 15 minutes, and the 5-minute and 10-minute values were substituted in the equation as described. Initial doses of 3.3 and 33.0 mg of sulfobromophthalein per kg body weight were used in separate tests. The initial plasma disappearance rate (Figure 5) of Dog C decreased from 18.6 to 13.6 per cent per minute when dosage of dye was increased tenfold; the plasma disappearance rate of Dog D decreased from 20.4 to 12.6 per cent per minute. Thus, sulfobromophthalein removal rates in dogs were decreased by increasing the dosage.

Two unanesthetized dogs were subjected to a series of tests in which various agents were injected simultaneously with a standard dose of indocyanine green to test the effect of each agent on the plasma disappearance rate of the indocyanine green. One mg of indocyanine green equals 0.0013 mmole. In each case the dose of the blocking agent, that is, sulfobromophthalein, rose bengal, bilirubin, sodium dehydrocholate, or indocyanine green itself, was molecularly 10 times the standard dose of indocyanine green or 0.013 mmole (Table I). Bilirubin also was given in twice the molecular dose, or 0.0026 mmole. Blood was sampled at 5, 10 and 15 minutes and revealed exponential removal patterns (Dog B, Figure 6).

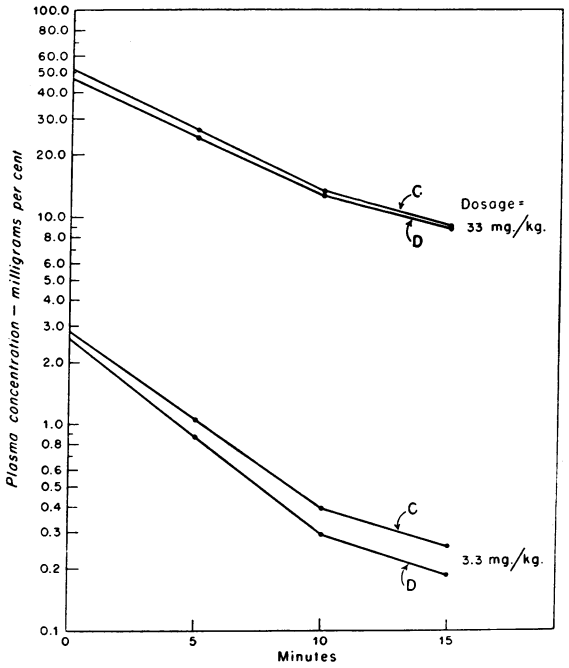

FIG. 5. THE PLASMA DISAPPEARANCE RATE OF SULFOBROMOPHTHALEIN IN DOGS IS NOT EXPONENTIAL. The initial rate is estimated from the early portion of the curve. Rates are shown for Dogs C and D.

The extent to which sulfobromophthalein, rose bengal, bilirubin, and indocyanine green itself, slowed the removal rate of the indocyanine green was the same when equivalent doses of these blocking agents were used (Figure 6, Table I). The effect of bilirubin in a dose of 0.0026 mmole was significantly less than the effect of the larger amount. Bilirubin was dissolved in 0.1 M sodium

TABLE I

Effect of blocking drugs on removal of indocyanine green

Drug	Formula		Dose	Rate	
				Dog A	Dog B
		mol wt	*mg/kg*	*%/min*	
Indocyanine green	$C_{43}H_{47}O_6N_2S_2Na$	775	1.0*	4.5–5.0	6.1–7.4
			11.0†	3.3	4.0
Sulfobromophthalein	$C_{20}H_8Br_4Na_2O_{10}S_2$	838	10.9	2.8	4.1
Rose bengal	$C_{20}H_2O_5I_4Cl_4Na_2$	1,018	13.2	3.3	4.0
Bilirubin	$C_{32}H_{36}N_4O_6$	573	7.4	2.2	3.6
			1.5	3.1	5.8
Sodium dehydrocholate	$C_{24}H_{33}NaO_5$	415	5.4	4.5	7.1

* Standard dose of indocyanine green.
† Standard dose of indocyanine green plus the tenfold blocking dose.

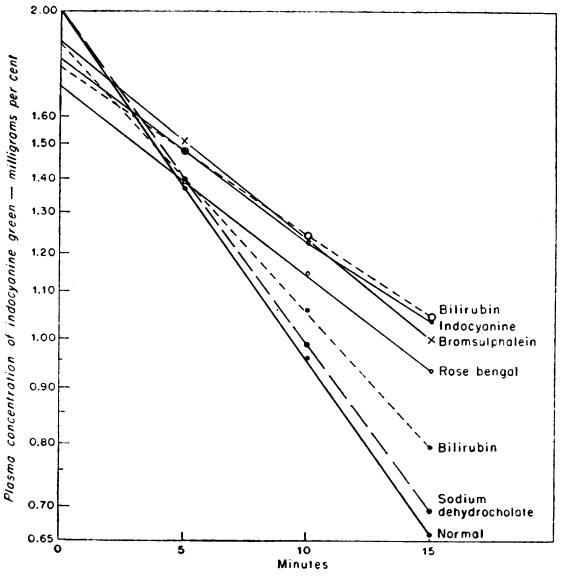

FIG. 6. THE REMOVAL RATES OF INDOCYANINE GREEN WITH THE VARIOUS BLOCKING DRUGS ARE EXPONENTIAL. Similarity of removal rate is denoted by similarity of slope. The slower slope applies to the larger dose of bilirubin. Data for Dog A are not illustrated but are similar to those shown here for Dog B.

carbonate for intravenous administration. The same amount of 0.1 M sodium carbonate given alone had no effect on the plasma disappearance rate of indocyanine green. Sodium dehydrocholate, when given in the same molecular dose of 0.013 mmole, did not produce a measurable decrease in the plasma disappearance rate of indocyanine green (Table I); but, when given in a much larger dose of approximately 260 mg per kg body weight (10 ml of a 20 per cent solution), it produced definite slowing of the plasma disappearance rate (1.2 and 2.1 per cent per minute in the two dogs).

The initial plasma disappearance rate of sulfobromophthalein was measured in two unanesthetized dogs by giving an initial dose of 5 mg of sulfobromophthalein per kg body weight and by sampling blood at 5, 10 and 15 minutes. The removal rates were not exponential, but the initial estimated plasma disappearance rates were 23.9 and 21.0 per cent per minute, respectively. Then 1.0 mg of indocyanine green per kg body weight was administered simultaneously with the standard dose of 5 mg sulfobromophthalein per kg body weight. Under these circumstances the estimated initial plasma disappearance of sulfobromophthalein in the two dogs was 14.8 and 16.9 per cent per minute, respectively (average of three tests in each dog). Thus it was seen that indocyanine green could slow or block the removal rate of sulfobromophthalein.

The initial plasma disappearance rate of sulfobromophthalein was determined in four Sprague-Dawley rats weighing approximately 250 g. The rats were under ether anesthesia, and cardiac blood was sampled 3, 6 and 9 minutes after injection of 5 mg sulfobromophthalein (approximately 20 mg per kg body weight; Table II). The plasma removal pattern was not exponential, but the initial plasma disappearance rates taken from specimens withdrawn at 3 and 6 minutes after injection averaged 35.6 per cent per minute.

Next, the plasma disappearance rate of indocyanine green was determined in six Sprague-Dawley rats weighing approximately 250 g under the above mentioned conditions after injection of 1.0 mg indocyanine green (approximately 4 mg per kg body weight). The results (Table II) revealed nonexponential removal rates (19) averaging 34.0 per cent per minute in the initial 6 minutes. Then 1.0 mg indocyanine green and 5 mg sulfobromophthalein were given simultaneously to four Sprague-Dawley rats weighing ap-

TABLE II

Plasma disappearance rates of indocyanine green and sulfobromophthalein in Sprague-Dawley rats

Dog	Indocyanine green		Sulfobromophthalein	
	Dose	Rate	Dose	Rate
	mg	*%/min*	*mg*	*%/min*
1	1.0	26.7		
2	1.0	30.1		
3	1.0	31.5		
4	1.0	31.5		
5	1.0	40.8		
6	1.0	43.3		
Average (1–6)		34.0		
7			5.0	30.1
8			5.0	33.0
9			5.0	38.5
10			5.0	40.8
Average (7–10)				35.6
11	1.0	11.2	5.0	21.0
12	1.0	14.1	5.0	25.7
13	1.0	16.1	5.0	36.5
14	1.0	19.2	5.0	30.1
Average (11–14)		15.1		28.3

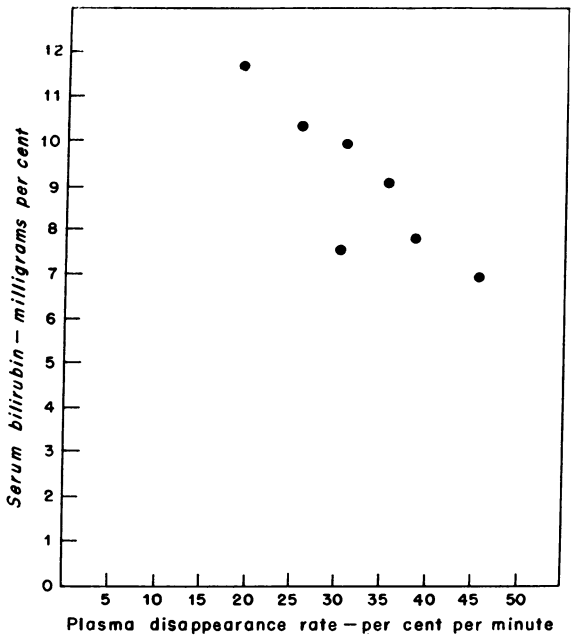

FIG. 7. PLASMA REMOVAL OF INDOCYANINE GREEN IN GUNN RATS. With one exception the points form a nearly straight line, indicating an inverse linear relationship between plasma disappearance rate of indocyanine green and the total serum bilirubin.

proximately 250 g each, and the removal rate of each dye was determined as before. The results (Table II) indicate that the removal rate of each dye was decreased. The amount of slowing was more prominent with indocyanine green, because in this instance the dose of the blocking dye, sulfobromophthalein, was five times greater than the dose of the test dye. Conversely, the slowing with sulfobromophthalein was less pronounced, but here the blocking dye, indocyanine green, was given in only one-fifth of the dose of the test dye.

It had been reported previously by the authors (20) that the plasma disappearance rate of indocyanine green in a group of congenitally jaundiced Gunn rats was not statistically different from the plasma disappearance rate in normal Sprague-Dawley rats. However, it had been noted that the range of removal rates was wide and so an attempt was made to compare the plasma disappearance rate with the degree of jaundice. The plasma disappearance rate of indocyanine green, therefore, was determined in seven Gunn rats weighing approximately 250 g, with the rat under ether anesthesia during sampling of cardiac blood after administration of 1.0 mg of indocyanine green. Total serum bilirubin was measured by the Malloy-Evelyn method (Figure 7). An approximately inverse linear relationship exists between the plasma disappearance rate and the serum bilirubin. The group was too small for the reliable application of statistical methods permitting more precise description of the relationship.

CONCLUSIONS

These studies confirm the previous observation that the plasma disappearance rate of sulfobromophthalein is slower with large initial doses than with small doses of the dye. A similar phenomenon has been demonstrated for the plasma disappearance of indocyanine green. It has been shown further that the relationship between dye dosage of indocyanine green and the plasma disappearance rate is a nonlinear inverse one. It would therefore appear that, when removal rates of one dye are compared with those of another (16, 17), the comparison must be qualified for the dose used. However, these studies did indicate that, milligram for milligram or millimole for millimole, sulfobromophthalein was more rapidly removed from dog and rat plasma in the initial phase than was indocyanine green. If it is kept in mind that the present study is limited to the removal of a single dose and that the storage compartment is unsaturated at the outset, it appears that the capacity of the liver to take up indocyanine green from the plasma of dogs has a maximal rate limit (Figure 4).

Since smaller initial doses are more quickly removed from the plasma, these studies also seem to indicate that dye already absorbed by the liver inhibits subsequent absorption of dye. Were this not the case, as the plasma concentration of dye decreases with time, the removal rate of the smaller remaining amount should increase rather than continue at the same rate. Previous studies (19, 20) indicate that indocyanine green is not reabsorbed into the hepatic lymph, and hence it appears unlikely that reabsorption would explain this observation. These additional observations on uptake of dye by the liver in no way disagree with the phenomena described by Wheeler and his colleagues (9, 10).

These studies do not appear to confirm the con-

cept of *a priori* selective uptake of some dyes during simultaneous administration. All of the dyes studied exert the same blocking effect when given in equimolecular doses, and it has been shown that sulfobromophthalein and indocyanine green show mutual blocking. The blocking effect seems to be molecular competitive inhibition, since a single agent, indocyanine green, blocks itself similarly to the other blocking dyes. The blocking produced by sodium dehydrocholate is less pronounced, and its nature is not clarified by these studies. The demonstration that bilirubin blocks the removal of indocyanine green in dogs, and that the plasma disappearance rate is related inversely to the degree of jaundice in Gunn rats, is in accord with an observation made earlier (19), that obstructive jaundice in human beings produces an inverse linear slowing effect on the plasma disappearance rate of indocyanine green.

SUMMARY

It has been shown that the removal of indocyanine green from dog plasma is an inverse function of the initial single dose. A maximal initial transfer capacity for hepatic uptake after a single dose of dye has been found.

The mechanism whereby several dyes, taken up by the liver, compete with each other or block each other appears to be molecular competitive inhibition. The data do not support the concept that some of these dyes are, *a priori,* taken up selectively.

It has been shown that bilirubin blocks the removal of indocyanine green as do the dyes, and that the rate of removal of indocyanine green in Gunn rats is dependent on the degree of bilirubinemia.

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