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Hepatic Uptake and Biliary Excretion of Indocyanine Green in the Dog.* (24229)

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Indocyanine green is a water-soluble tricarbocyanine dye developed by Brooker and introduced by Fox *et al.*(1,2) for measurement of cardiac output by the indicator dilution technic. For this purpose its absorption spectrum and protein-binding characteristics (1) are ideally suited. The results of the present investigation indicate that the dye may also be of great value in study of hepatic function.

Methods. All studies were conducted on 2 trained unanesthetized female dogs weighing 18.9 and 22.0 kg. Several months previously each had undergone splenectomy, cholecystectomy, and preparation of a permanent duodenal fistula using the device described by Thomas(3), which permitted access to the common bile duct. The dogs remained in good health, and hepatic function, as judged by repeated measurements of sulfobromophthalein (BSP) transfer maximum(4), continued unimpaired. At the time of each study an olive-tipped ureteral catheter (5 or 6 Fr.) was inserted through the ampulla of Vater and advanced about 5 to 6 cm into the common bile duct. The dogs were then placed upright in a sling and bile was collected by gravity or, when it was particularly viscous, by gentle aspiration with a tuberculin syringe. Intravenous injections were given through a polyethylene catheter introduced through a large bore needle in a vein of the foreleg and advanced to the region of the right atrium.

Indocyanine green§ was made up to 250 mg% in distilled water for injections. In one study a continuous infusion of the dye was given. This was prepared by mixing 24 ml of 500 mg% indocyanine green with 51 ml of normal saline and 5 ml of dog plasma. (Indocyanine green is unstable on standing in aqueous solution, and the presence of plasma was found empirically to retard its breakdown.) Concentration of indocyanine green in plasma was measured in a Beckman DU spectrophotometer at 810 mμ after 11-fold dilution with normal saline. Concentration standards for this determination were also prepared in dog plasma. (Dilute aqueous standards are unsatisfactory because of marked instability.) Bile samples were diluted 25 to 100 times with water. One ml of diluted bile was then added to 1 ml of dog plasma and 9 ml of normal saline and the absorption measured at 810 mμ. Although the dye is quite stable in bile even when diluted, the addition of plasma, for reasons not apparent, was necessary for full color development. BSP concentrations

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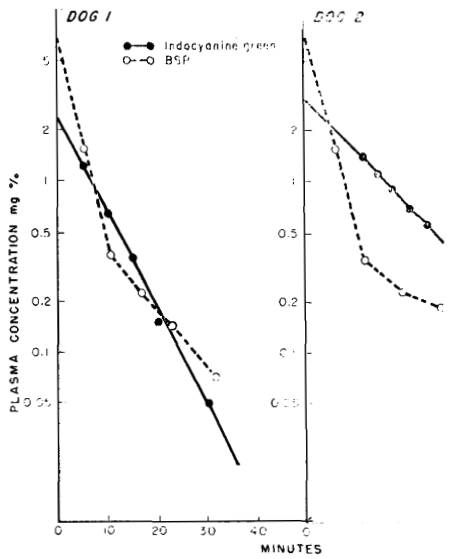

FIG. 1. Comparison of plasma concentrations of indocyanine green following administration of 20 mg intrav. with plasma concentrations of BSP following administration of 50 mg. Values are plotted semi-logarithmically against time after inj.

in plasma and bile were measured at 580 mμ after suitable dilution and addition of alkali. Measurement of the concentration of either dye was unaffected by the presence of the other.

Results. A. Disappearance from plasma: A single intravenous injection of 20 mg of indocyanine green was administered to each dog. During the succeeding 30 minutes plasma concentration fell exponentially with respect to time (Fig. 1). The half-times of the disappearance curves were 5.2 minutes and 10.5 minutes in dogs 1 and 2, respectively. Percentage disappearance rates(5) were 13.3% per min. and 6.6% per min. The curves were extrapolated back to zero time and this concentration value was divided into the administered dose to provide a rough estimate of the initial volume of distribution of injected material. This calculation yielded values of 830 and 706 ml compared to the measured plasma volumes (I^{131} labelled albumin) of 871 and 868 ml in these 2 dogs. The approximate agreement between volume of distribution and plasma volume strongly suggests that the initial distribution is limited to the vascular compartment. Following its introduction the dye must therefore be very rapidly and completely bound to plasma protein.

For comparison, an intravenous injection of 50 mg of BSP was given on another occasion to each of these dogs. Its initial disappearance rate was more rapid than that of indocyanine green (Fig. 1), but in contrast to the behavior of indocyanine green the disappearance of BSP did not follow an exponential course.

B. Biliary excretion. The recovery from bile of an intravenously administered dose of indocyanine green was close to 100% (Table I). No dye was detectable in the urine. Appreciable concentrations of dye were detectable in the bile for over 5 hours after injection in dog 1 and 4 hours in dog 2, long after its disappearance from the plasma (Fig. 2). These findings support the view that hepatic removal accounts for rapid disappearance of the dye from the plasma. Apparently indocyanine green, like some of the phthalein and flavine dyes(4,6,7,8,9), is rapidly stored in the liver and then gradually secreted into the biliary tree. The completeness with which it can be recovered in the bile by a colorimetric method suggests that it does not undergo major chemical alteration during this process. Moreover, in ascending paper chromatography with water saturated n-butanol-acetic acid the migration of indocyanine green recovered from several bile specimens was uniform and identical with that of the administered material. In contrast, BSP is excreted in several chromatographically distinct forms (10). The quantity of the latter dye identifiable in bile by colorimetric methods is rarely over 70 to 80% of an administered dose(6,7), and the measurement of S^{35} labelled BSP in the bile indicates that color is partially lost as a result of chemical alteration of this dye by the liver(11). In view of the behavior of in-

TABLE I. Recovery of Indocyanine Green in Bile.

Dog	Intrav. dose, mg	% recovered in bile
1	20	101.5
2	20	94.2
2	45	95.9
1	142	97.7
	Avg	97.3

locyanine green such modifications of the molecule do not appear to be essential either to storage or to transport.

C. Interference by indocyanine green with BSP uptake. A priming dose of BSP of approximately 10 mg/kg was administered intravenously and followed by a constant infusion of about 3.8 mg per min—a rate close to the BSP "Tm"(4) measured previously in these dogs and therefore designed to maintain a constant plasma level. After 30 minutes of infusion, control blood and bile specimens were obtained during three 10-minute intervals and then 45 mg of indocyanine green was administered to dog 2. During the first 10

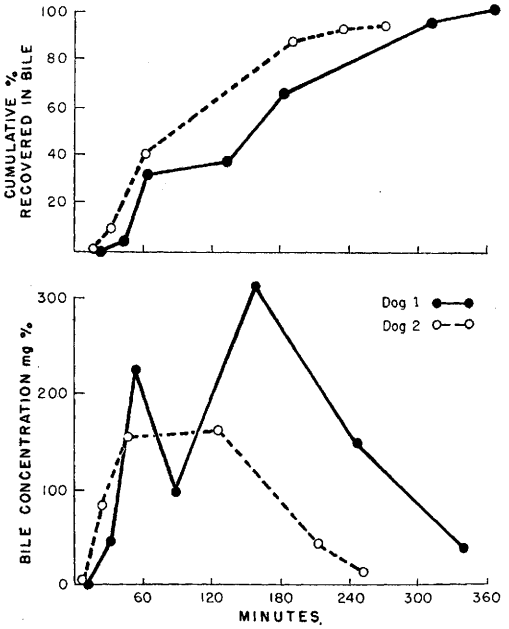

FIG. 2. Recovery and concentration of indocyanine green in bile following intrav. administration of 20 mg of the dye. Bile concentrations are plotted at mid-point of each collection period. Fluctuations in bile concentration in dog 1 were related to changes in bile flow.

minutes after this injection plasma concentration of BSP rose sharply from 3.6 to 5.1 mg % and then returned gradually to the control level over the next 100 minutes as the indocyanine green disappeared (Fig. 3). The biliary excretion rate of BSP showed a slight downward trend throughout the experiment and it was difficult to be sure whether this was modified during the period of maximum indocyanine green excretion. The ultimate

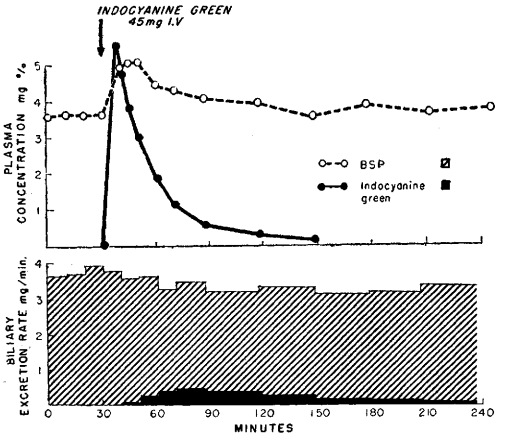

FIG. 3. Effect of a single intrav. inj. of indocyanine green given during a continuous infusion of BSP. Thirty-one min. before time zero 200 mg of BSP was administered intrav. as a priming dose. At the same time an infusion of 3.81 mg/min. was started and maintained throughout the study.

recovery of indocyanine green in the bile was 95.9% (Table I).

In dog 1, after a similar control period, a 45 mg dose of indocyanine green was followed by an infusion of 2.02 mg/min over the next 48 minutes (total quantity administered, 142 mg). Again the BSP plasma concentration rose sharply (from 2.9 to 4 mg %), then continued to rise very slowly throughout the indocyanine green infusion, after which it remained elevated and constant (Fig. 4). The

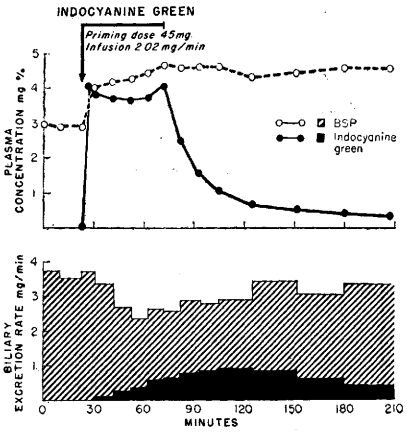

FIG. 4. Effect of combined infusions of indocyanine green and BSP. Forty-five min. before time zero 250 mg of BSP was administered intrav. as a priming dose. At the same time an infusion of 3.91 mg/min. was started and maintained throughout the study. Bile was collected for 3 hr beyond the time shown above in order to measure total recovery of indocyanine green.

biliary excretion rate of BSP appeared to be reduced during the period of maximum indocyanine green excretion. It rose thereafter, but since it never quite returned to the control level it is difficult to interpret this observation with certainty. Eventual recovery of indocyanine green from the bile in this study was 97.7% of total administered dose (Table I).

It is clear that indocyanine green interfered with hepatic uptake of BSP from the plasma in both dogs. However, this interference apparently was overcome by an elevation of the BSP concentration so that, in spite of the continued presence of indocyanine green, BSP removal rate from plasma again became practically equal to the infusion rate. It is understandable that this should occur since the removal of BSP from plasma does not appear to be a rate limited process (in contrast to biliary secretion), and rate of removal is normally accelerated whenever there is an increment in plasma concentration(4). Whether indocyanine green partially inhibits an hepatic BSP uptake mechanism, whether the 2 dyes compete for a common mechanism, or whether indocyanine green displaces BSP previously stored in the liver cannot be deduced from the present data. Although the last study (Fig. 4) suggests that indocyanine green may also interfere or compete with BSP in the biliary secretory process, further clarification of this and other possibilities must await the availability of much larger quantities of the new dye.

D. Intestinal absorption. Through the Thomas fistula 20 ml of aqueous solution containing 25 mg of indocyanine green was introduced into the duodenum of dog 2. Bile was collected over the next 4 hours. Low concentrations of indocyanine green were detectable in the bile after one hour, reaching a maximum during the third hour and diminishing thereafter. The total quantity recovered in 4 hours was 0.47 mg. or 1.9% of the administered dose.

To the same dog on another occasion 31 ml of bile (from the study on dog 1 described in the preceding section) containing 46 mg of indocyanine green, was administered into the duodenum. There was no detectable indocyanine green in the bile collected during the next 3 hours.

It is concluded that the intestinal absorption of indocyanine green is minimal and that reabsorption and recirculation of material excreted in the bile should introduce no significant error into studies undertaken without bile collection.

Summary. 1. Indocyanine green given intravenously to dogs is distributed in the plasma compartment and rapidly removed by the liver. 2. Biliary excretion of the dye is delayed, but an average of 97.3% of the administered dose is eventually recovered from the bile in apparently unaltered form. Indocyanine green does not appear in the urine. 3. Indocyanine green interferes with hepatic uptake of BSP from the plasma. 4. Absorption of indocyanine green from the bowel is minimal.

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