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Indocyanine Green-Plasma Half Time Clearance (T ½) in Normal Beagles.* (30140)

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Indocyanine green (Cardio-Green®)† when given in a single injection has been reported to be an excellent and relatively simple test for detecting liver damage in humans and dogs (1-4). This tricarbocyanine dye when injected intravenously is rapidly bound to the plasma albumin (95%) and cleared exponentially during the first 20 minutes(1,2). This clearance is accomplished almost exclusively by the liver as shown by Wheeler(5), who recovered 96% of the total injected dye in the bile of dogs. There is also evidence that indocyanine green (ICG) is equal, if not superior to, Bromsulphalein (BSP) when used to assess liver function(3,4,6).

The following study was undertaken to establish normal values for ICG clearance when the dye was given in a single injection to normal adult beagles.

Animals. Eight purebred beagles, 4 males and 4 females, were used in this study. Weights ranged from 8.9 to 13.4 kg with a mean of 11.2 kg. Ages ranged from 4 to 6 years with a mean of 5 years and 2 months.

The animals were housed in comfortable kennels, were fed a commercial ration once a day and water was available at all times. Food was withheld for 24 hours prior to injection with ICG.

The animals were divided into 2 groups of 4 animals each. Five clearance T ½ values were determined on each animal with a 2- to 3-day interval between consecutive determinations.

Methods. The method used for determining the plasma half time clearance of ICG was essentially the same as that reported by Bonasch *et al*(4). The dye was prepared to a concentration of 2.5 mg/ml and adminis-

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† Cardio-Green® was provided through the courtesy of Hynson, Westcott & Dunning, Baltimore, Md.

tered on the basis of 0.5 mg/kg body weight. Injections were given *via* the jugular vein. Using a heparinized syringe and with the aid of a stop watch blood samples were drawn from the opposite jugular at 3, 6, 9, and 12 minutes after injection. The samples were centrifuged at 2000 rpm for 10 minutes and the plasma removed. Pre-injection blood samples were taken for the preparation of a standard and a blank. The plasma was prepared according to the method of Bonasch *et al*(4) and optical density (O.D.) of each sample was read on a Beckman DU spectrophotometer at a wavelength of 805 mμ, the absorption maximum for ICG.

The concentration of the dye in each sample was calculated using the following expression(4):

$$\text{mg of dye/100 ml plasma} = \frac{\text{O.D. sample}}{\text{O.D. standard}} \times 0.0025 \times \frac{100}{0.5}$$

These concentrations were plotted against time after injection on 2-cycle semilogarithmic graph paper. The 4 points were then fitted by a straight line. This line was extrapolated to zero time and the value at zero time was reduced by half and found on the fitted straight line. The time corresponding to this one half value was then read as the ICG plasma clearance half time.

Plasma volumes were calculated using the formula(4):

$$\text{Volume of distribution (plasma volume in ml)} = \frac{\text{mg injected}}{\text{extrapolated concentration at 0 time (mg/ml)}}$$

Results. Individual half time clearance values (T ½) are presented in Table I. Analysis of variance of these observations produced a significant F value at the 5% significance level. Further analyses using Student's "t" test showed that this difference was due to sex. It was found that the female dogs cleared ICG significantly more slowly than the males ($P = 0.05$). The mean clearance curves of males and females are presented in Fig. 1. The mean T ½ of the males was 5.6 ± 0.8‡ minutes with a range of 4.2 to 8.1 minutes, and that of the females was 7.0 ± 1.5 minutes with a range of 4.7 to 10.0 minutes. The mean slopes of these curves were .127 ± .014 for the males, and .102 ± .021 for the females. This difference is significant at the 5% level.

Variances within and among males and within and among females were also tested and not significant.

TABLE I. Individual Half-Times (Min).

	Males					Females			
Dog No.	D-25	D-27	D-58	D-85	D-72	D-88	D-65	5267	
	5.7	5.5	5.8	5.5	8.1	5.4	7.1	5.7	
	6.1	5.3	6.3	5.1	7.6	5.6	5.6	6.1	
	8.1	4.2	6.1	5.8	9.5	5.7	7.0	10.0	
	5.9	5.5	5.9	4.9	8.4	4.7	7.2	7.1	
	6.0	5.7	5.0	4.4	9.5	7.0	7.2	6.1	
Mean ± 1 S.D.	6.36 (.98)	5.24 (.59)	5.82 (.49)	5.14 (.54)	8.62 (.85)	5.68 (.83)	6.82 (.68)	7.00 (1.75)	
Mean (males) = 5.64 ± .80					Mean (females) = 7.03 ± 1.48				

‡ Mean ± 1 standard deviation.

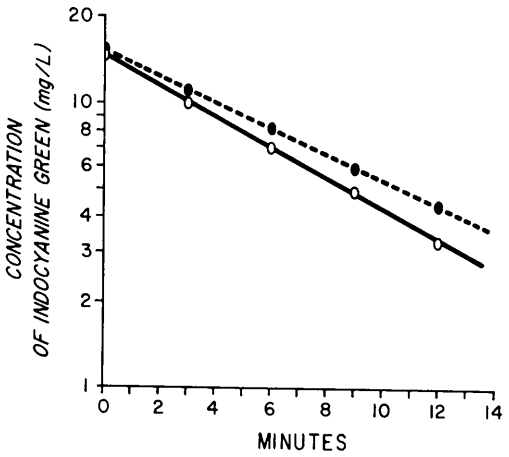

FIG. 1. Indocyanine green plasma clearance in normal male and female beagles. Each point is a mean of 20 observations. ○——○ represents males with T ½ = 5.6 ± 0.8 (S.D.). ●– – –● represents females with T ½ = 7.0 ± 1.5 (S.D.). Each point at zero time is a mean of 20 extrapolated values.

Individual plasma volumes ranged from 21.8 to 52.6 ml/kg with a mean of 35.1 ± 8.7 ml/kg. This mean plasma volume is somewhat lower than that reported by other investigators using the same method. To check our results, 11 additional dogs, mongrels and beagles, ranging in weight from 9 to 22 kg were injected simultaneously with ICG and radioiron (Fe-59) and plasma volumes were determined using both curves. These data are presented in Table II.

Discussion. Normal values for the majority of clinical tests performed on beagles are not easily found in the literature. The present study was initiated to establish baseline values for ICG clearance in purebred beagles.

The most significant finding in this study was the difference in plasma half time clearance of ICG due to sex. Reports of ICG clearance in the literature do not point out this sex difference. The means reported include the values of both males and females.

In the present study, the longest mean T ½, that of the females, is somewhat longer than those reported by Bonasch *et al,* 8.4 minutes(4), Hunton *et al,* 9.1(6), and Ketterer *et al,* 10.8 minutes(8). None of these investigators reported the sex of their animals, therefore, a possible explanation of these higher half times may be an uneven distribution with more females than males which would result in apparent longer half times. Also, Ketterer performed his tests while the animals were under anesthesia.

In animals with gross liver damage this difference due to sex would not be important because the T ½ would exceed the upper limits of the combined mean; however, the somewhat longer T ½ of a normal female may be diagnosed as slight liver dysfunction when compared with this combined value. It seems essential then, that this male-female difference be recognized.

The mean plasma volume (35.1 ml/kg) found in this study was much lower than the volumes reported by Bonasch *et al,* 59.2 ml/kg(4), Stekiel *et al,* 52.1 ml/kg(7), and Ketterer, 47.4 ml/kg(8). It was in closer agreement with the volume of distribution reported in 2 dogs by Wheeler *et al*(5). In the above reports the volumes of distribution as found with ICG were compared to those using either T-1824 or I-131 labelled albumin and in all cases there was close agreement

TABLE II. Comparison of Plasma Volumes Using Fe-59 and ICG Injected Simultaneously.

Dog No.	Sex	Wt (kg)	T ½ min	ICG plasma vol Total (ml)	ICG plasma vol ml/kg	Fe-59 plasma vol Total (ml)	Fe-59 plasma vol ml/kg
1	♂	13.3	5.3	369	27.7	421	31.7
2	♂	13.5	5.5	563	41.7	541	40.1
3	♀	9.1	6.5	399	43.8	360	39.6
4	♀	12.3	7.6	466	37.9	445	36.2
5	♀	11.9	8.2	363	30.5	452	38.0
6	♀	11.1	8.3	408	36.8	341	30.7
7	♀	9.2	5.8	383	41.6	470	51.1
8	♂	21.5	7.7	672	31.3	624	29.0
9	♂	21.0	6.1	921	43.9	880	41.9
10	♂	20.1	5.8	897	44.6	748	37.2
11	♂	21.9	5.9	771	35.2	809	36.9
			Mean	565	37.7 ± 6.0	554	37.5 ± 6.1

between the results.

Previous studies in this laboratory using Fe-59 indicated plasma volumes comparable to those reported by the investigators previously mentioned; however, in these studies the whole curve over a period of 2½ hours was used. In the present study the early part of the curve (the first 12 minutes) was used and as can be seen in Table II these volumes agreed with those found using ICG. It can, therefore, be concluded that even though the absolute values of the plasma volumes vary between studies, depending on the technique used, the ICG method of estimating the volume of distribution is a reliable one. The sex and T ½ values are included in this Table also to show that the additional data support the sex difference found in this study.

This report also indicates that the single injection method using ICG is a relatively simple and reliable one for evaluating liver function. The somewhat longer plasma clearance time and no evidence of extra-hepatic clearance are decided advantages of the ICG method over the BSP method.

Summary. 1. The mean plasma clearance half time of indocyanine green (ICG) was found to be 5.6 ± 0.8‡ minutes in male beagles, and 7.0 ± 1.48 in female beagles. 2. Female dogs in this study cleared ICG significantly more slowly than the males. 3. Plasma volume estimated using ICG is 37.7 ± 6.0 ml/kg. This figure agrees with 37.5 ± 6.1 ml/kg found using radioiron (Fe-59).

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Relationship of Endogenous Pyrogen and Serum Augmenting Factor To Endotoxin Tolerance.* (30141)

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Following intravenous injection of Gram-negative bacterial endotoxin, a characteristic febrile response occurs in rabbits and in man(1). Repeated daily injection of endotoxin produces a state of tolerance manifested by a decreasing febrile response(2). The mechanism of pyrogen tolerance is poorly understood. Initially tolerance was attributed to more rapid reticuloendothelial (RES) clearance of endotoxin because rabbits made tolerant to typhoid vaccine again became fully susceptible to the pyrogenic action of endotoxin following administration of thorium dioxide, thorotrast, a RES blocking agent(3). The relationship of tolerance to RES clearance has recently been challenged with evidence that endotoxin-tolerant rabbits developed pyrogen tolerance independently of their capacity to clear heterologous colloids from the blood(4). It was also shown that RES blockade did not reverse tolerance to the pyrogenic effects of endotoxin when normal-blockaded were compared with tolerant-blockaded rabbits, and that pyrogen tolerance can be passively transferred(4,5). These studies suggested that some factor present in serum was responsible for endotoxin tolerance. It is conceivable that this substance is related in some manner to the "endotoxin-augmenting" factor (AF) in serum described

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