been found that the active principle in the proximal area and mid-ileum of the rat small intestine is dialyzable and that its distribution parallels closely that of endogenous vitamin B_{12}. Non-radioactive vitamin B_{12} has been found to be very active in the release of radioactive vitamin B_{12} bound to rat or human intrinsic factor concentrate. This appears to be due to its ability to equilibrate with the bound form. In a complex equilibrium system, endogenous vitamin B_{12} appears to account for substantially all of the activity displayed by the proximal area and mid-ileum of the rat small intestine, and by mucosa homogenates from beef small intestine and various human organ extracts. Vitamin B_{12} bound to hog intrinsic factor on the other hand shows little tendency to equilibrate with unbound vitamin B_{12}.

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Hepatic Clearance of Indocyanine Green in the Beagle. (30241)

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Following its introduction for the measurement of blood flow(1) indocyanine green dye (ICG) was found to be useful for assessment of hepatic activity in dogs(2). Wheeler *et al* (3) found an exponential plasma disappearance rate of 6.6 and 13.3% per minute in 2 dogs; Rapaport and coworkers(4) reported a rate of 18.1% per minute in anesthetized dogs. Other investigators have found plasma disappearance rates of 7.6% per minute, 6.9% per minute and 8.9% per minute (5,2,6).

Because the beagle dog is extensively used as an experimental animal, the purpose of this study was to investigate the rate of ICG clearance from plasma in this breed of dog, and to compare it with values obtained in mongrel dogs.

Methods. ICG clearance was determined by the method of Ketterer *et al*(2) in 24 purebred beagle dogs between one and two years old, maintained within our colony for at least 4 months and 12 mongrel dogs housed for less than one week. Liver function was judged to be normal in all animals based on alkaline phosphatase (AP) and serum glutamic oxalacetic transaminase values (SGOT) determined by standard methods (7,8).

All dogs were fasted overnight. An aqueous solution of ICG* (2.5 mg/ml) was given intravenously, *via* a brachial vein, at a dosage schedule of 0.5 mg/kg. Two or 3 heparinized blood samples were taken from the jugular

* Indocyanine green (Cardio Green), Hynson Westcott & Dunning, Baltimore.

TABLE I. Hematocrit, Alkaline Phosphatase and SGOT Values in Beagle and Mongrel Dogs.

Beagles						Mongrels					
Dog No.	Sex	Wt (kg)	% Hct	Alkaline phospha-tase units	SGOT units	Dog No.	Sex	Wt (kg)	% Hct	Alkaline phospha-tase units	SGOT units
331	♀	11.9	54	4	18	10	♀	7.8	44	13	18
332	♀	8.9	46	6	16	11	♂	8.7	52	9	23
333	♀	7.9	55	4	15	12	♂	8.8	33	6	31
334	♀	5.5	47	4	16	13	♀	8.7	49	4	27
347	♀	6.8	47	13	20	14	♀	8.4	43	7	21
348	♀	8.1	44	8	17	15	♀	5.5	47	13	39
349	♀	6.7	50	3	12	16	♀	9.5	49	5	18
350	♀	9.0	50	5	15	17	♂	10.6	49	5	20
351	♂	7.3	49	8	17	18	♀	7.8	32	4	17
352	♂	8.5	50	4	15	19	♀	10.2	48	6	16
353	♂	9.4	45	6	15	20	♂	13.3	49	2	15
354	♂	9.4	44	4	17	21	♀	11.3	48	5	14
355	♂	9.5	50	7	18						
356	♂	7.2	43	5	20						
357	♂	8.7	47	4	15						
358	♂	5.7	49	3	18						
359	♂	9.1	48	7	20						
360	♀	6.5	48	6	21						
361	♀	5.1	45	7	18						
099	♂	10.0	52	11	17						
111	♂	13.2	43	10	16						
151	♂	4.5	52	8	27						
159	♂	8.4	50	7	20						
262	♂	8.6	43	8	17						
Mean		8.2	48	6	18			9.2	45	7	22
Standard error		.4	.7	.3	.6			.6	2	1	2

vein of each animal by means of either a Vac-U-Tainer® or syringe at intervals between 2 and 10 minutes after ICG administration; exact time of blood sampling was recorded in each instance. Hematocrits were measured, and the plasma separated following centrifugation at 3000 RPM for 15 minutes. A pre-injection blood sample was taken for blank determinations and standard preparation.

The plasma concentration of ICG was determined by diluting 1 ml of plasma with 2 ml distilled water, and measuring the optical density at a wave length of 805 mμ on a B&L Spectronic 20 adapted to the infrared absorption range.

Since plasma disappearance of ICG is exponential(2) the half-life (T-½) was measured from a semi-logarithmic plot (concentration *vs* time) of the plasma concentration and the fractional clearance or percentage removed per minute (k) calculated from the relation:

$$k = \ln 2/\text{T-}½$$

Volume of distribution, plasma clearance rate and estimated hepatic blood flow were calculated according to the formulae of Ketterer *et al*(2). Statistical significance was determined by the use of Student's t-Test as outlined by Snedecor(9).

Results and discussion. Mean and standard errors of animal weights, percent hematocrit, AP and SGOT units are presented in Table I. No significant differences in these parameters were noted between the 2 groups of dogs. All animals were thought to have normal hepatic activity based on the AP and SGOT values which are within the normal range for animals maintained within our colony.

Table II lists ICG clearance data in both beagle and mongrel dogs. In the beagle, k was found to be significantly greater ($P<0.005$) than in the mongrel dogs. Mean values were 13.08 ± 0.56% per minute (range = 8.25 to 19.25% per minute) in beagle dogs, and 9.74% ± 1.18% per minute (range = 4.92 to 16.70% per minute) in mongrel dogs. Plasma T-½ of ICG was significantly greater in the mongrel dogs than in the beagles ($P<0.005$). Mean values were 8.3 ± 0.9 minutes (range = 4.2 to 14.1

TABLE II. ICG Clearance in Beagle and Mongrel Dogs.

Dog No.		Fractional clearance, % per min		Half-life, min		Volume of distribution, ml/kg		Plasma clearance, ml/min/kg		Estimated hepatic blood flow, ml/min/kg‡	
B*	M†	B	M	B	M	B	M	B	M	B	M
331	10	16.90	14.16	4.1	4.9	104	75	17.6	10.6	126	76
332	11	9.62	11.45	7.2	6.1	71	72	6.8	8.2	49	59
333	12	9.36	4.92	7.4	14.1	110	97	10.3	4.8	74	34
334	13	13.86	9.37	5.0	7.4	77	70	10.7	6.6	76	47
347	14	13.32	7.07	5.2	9.8	76	83	10.1	5.9	72	42
348	15	8.25	6.10	8.4	11.4	85	89	7.0	5.4	50	39
349	16	10.66	8.20	6.5	10.0	93	74	9.9	6.1	71	43
350	17	13.58	6.24	5.1	11.1	80	98	10.9	6.1	76	44
351	18	12.15	8.61	5.7	7.3	74	86	9.0	7.4	64	53
352	19	13.07	14.59	4.3	8.1	59	92	7.7	13.4	55	96
353	20	11.74	16.70	5.9	4.8	82	111	9.6	18.5	69	132
354	21	11.74	9.49	5.9	4.2	94	87	10.9	8.3	78	59
355		13.58		5.1		86		11.7		83	
356		13.32		5.2		99		13.2		94	
357		13.86		5.0		95		13.2		94	
358		17.76		3.9		100		17.8		127	
359		16.11		4.3		70		11.3		81	
360		16.90		4.1		80		13.5		97	
361		19.25		3.6		99		19.1		136	
099		11.86		5.8		83		9.8		70	
111		12.39		5.6		86		10.7		76	
151		11.38		6.1		91		10.4		74	
159		12.83		5.4		73		9.4		77	
262		10.39		6.8		73		7.6		54	
Mean		13.08	9.74	5.5	8.3	85	86	11.2	8.4	80	60
Standard error		.56	1.18	.2	.9	2.6	3.6	.7	1.2	4.7	8.2
p§		<.005		<.005		>.05		<.05		<.05	

* Beagle dogs. † Mongrel dogs. ‡ Calculated using an extraction ratio of 0.14 (Ketterer *et al.*). § Statistical significance between mean values of beagles compared to mean values of mongrels.

minutes) in the mongrel and 5.5 ± 0.2 minutes (range = 3.9 to 8.4 minutes) in the beagle. The volume of distribution was found to be the same in both beagle and mongrel dogs (85 ± 2.6 and 86 ± 3.6 ml/kg, respectively). Both calculated plasma clearance rate and estimated hepatic blood flow were greater in the beagle than in the mongrel dog ($P<0.05$).

The differences noted in rate of ICG clearance between beagle and mongrel dogs might be attributed to several factors. These include the breed of animal used and their previous clinical history which may have significantly influenced the health of the animals. All of the beagle dogs were subjected to routine and periodic treatment to insure optimum physical health. Although an attempt was made to select only healthy mongrel dogs for this study, there was no previous clinical history for the animals, nor could the health of the animals be adequately assessed at the time of the experiment.

Two of the mongrel dogs were found to have low hematocrits (Dogs 12 and 18) and one of these (Dog 12) had a slightly elevated SGOT. Two other dogs also possessed above-average SGOT values (Dogs 13 and 15). Dogs 12 and 15 exhibited increased T-½ of ICG. However, the value for dog 15 was no different from that of other animals in that group having high T-½ but average hematocrits and SGOT values. ICG clearance in dog 13 was within normal limits. The low hematocrit of dog 18 did not appear to affect this animal's ICG clearance rate. Only one beagle (Dog 151) exhibited an elevated SGOT; all other values for this animal were within normal ranges.

It appears evident that beagle dogs in our colony exhibit a higher rate of ICG clearance than mongrel dogs whose physical condition is not adequately known.

The estimated hepatic blood flow in un-

anesthetized beagle and mongrel dogs in our studies was higher than reported in the literature(10). This discrepancy might be due to our use of an extraction ratio (percentage of dye removed by liver per unit of time determined by difference between arterial and hepatic venous dye concentration over an approximate 15-second time period) of 14% as reported by Ketterer *et al*(2) in anesthetized dogs. The same investigators have reported that normal, unanesthetized human subjects exhibit an average extraction of ICG of 74%(11). If the extraction ratio in the unanesthetized dog is significantly greater than the 14% used in our calculations, the estimated hepatic blood flow in the present series of experiments may be lower than we have observed.

Summary. The clearance rate of indocyanine green was determined in 24 beagle dogs and 12 mongrel dogs. The results indicate that in the unanesthetized state, the purebred animals exhibited an increased rate of clearance of the dye and decreased T-½ when compared with the mongrel dogs. Several possibilities were discussed to account for the differences noted.

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Penetration of ATP into the Myocardium.* (30242)

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It is generally assumed that nucleoside triphosphates do not enter intact into cells from the extracellular space. This has been shown to be the case for liver(1) and ascites tumor cells(2). During the course of studies on the effect of cardiac glycosides on myocardial nucleotide metabolism, it was of interest to determine whether ATP could penetrate into myocardial cells without chemical change.

The problem was studied by determination of the ratio of the C^{14}- to P^{32}-ATP in the myocardium after perfusion of the isolated guinea pig heart with a mixture of (8-C^{14})ATP and (β, γ, P^{32})ATP of known isotope ratio. Any change in this ratio in the isolated myocardial ATP would indicate that chemical change had taken place during incorporation of the molecule into the cell.

Materials and methods. (8-C^{14})ATP, disodium salt, with a specific activity of 0.75 mC/mM was purchased commercially. (β, γ, P^{32})ATP with an initial specific activity of 7 mC/mM was prepared by the procedure of Pressman(3). Male guinea pigs weighing 200-400 g were maintained on a diet of Purina pellets supplemented with lettuce and carrots. The isolated guinea pig heart was prepared and perfused in the apparatus de-

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