virus inhibitory action of interferon. When the washed cells were again incubated at 37°C before they were infected with virus, they became resistant to virus synthesis without further exposure to interferon. The virus resistance developed by cells under these conditions was blocked by actinomycin D. The significance of these results is discussed in terms of the persistence of resistance to virus infection after interferon treatment and the probable half-life of interferon-induced RNA.

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Effect of Pentobarbital Anesthesia on Plasma Half-Life of Indocyanine Green in Beagles.* (30960)

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Indocyanine green (ICG) has been suggested by several investigators as an agent useful in evaluation of hepatic activity(1-3). Several reports have appeared wherein the plasma half-life (T-½) of this tricarbocyanine dye has been determined while the animals were anesthetized(1,3). Since various investigators have reported that pentobarbital may interfere with the hepatic extraction of ICG(1,3), this investigation was undertaken to determine the effect of pentobarbital anesthesia on the plasma T-½ of ICG in beagles.

Method. ICG clearance was determined in purebred healthy beagle dogs of either sex, ranging in weight from 4.5 to 13.4 kg and between 1 and 2 years of age, by the method of Ketterer *et al*(1). After overnight fasting, ICG (2.5 mg/ml aqueous solution) was given intravenously at a dosage of 0.5 mg/kg. Two or three heparinized blood samples were taken from the jugular vein of each animal at intervals between 2 and 10 minutes after ICG injection; exact time of blood sampling was recorded in each instance. Plasma was separated following centrifugation at 3000 rpm for 15 minutes. A pre-injection blood sample was taken for blank determinations and standard preparation. 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.

Plasma T-½ and fractional clearance were determined as reported previously(4).

* In the manuscript entitled "Hepatic Clearance of Indocyanine Green in the Beagle" by Vogin, *et al.*, Proc. Soc. Exp. Biol. and Med., 1965, v119, 570, an error appears in Table II for the values of volume of distribution, plasma clearance and estimated hepatic blood flow. Since the animals employed in this study are no longer available, it is not possible to determine exact values for these 3 parameters. However, studies performed in other animals indicate that if the value given in the text is multiplied by the factor 0.534, an estimate of the correct value for the above parameters can be obtained. (Example, Volume of Distribution for Beagles: 85 ml/kg × 0.534 = 45.4 ml/kg.)

TABLE I. ICG Clearance in Unanesthetized and Anesthetized Beagles.*

Group	Fractional clearance (%/min)	Plasma half-life (min)
Unanesthetized	12.8 ± .37	5.7 ± .16
Anesthetized	11.0 ± .37	6.6 ± .20
P	<.001	<.001

* Mean ± S.E. of 43 animals.

TABLE II. Percentage Distribution of Values.

	Fractional clearance	Plasma half-life
Unanesthetized >anesthetized	79	16
Unanesthetized =anesthetized	5	5
Unanesthetized <anesthetized	16	79

Anesthesia was induced with veterinary pentobarbital sodium (32.5 mg/kg i.v.) and ICG clearance was performed 15 to 30 minutes after the animals were anesthetized. A cross-over design was used so that ICG clearance could be determined on each dog in both the unanesthetized and the anesthetized states. Results were statistically analyzed by Student's "t"-test for paired comparison(5).

Results. The results of these experiments are presented in Table I. It was found that pentobarbital anesthesia significantly reduced ($P<0.001$) the fractional clearance and elevated the plasma T-½ of ICG by approximately 16%.

The distribution of values found in unanesthetized dogs (*i.e.*, greater than or less than those found in anesthetized animals) for the parameters measured are depicted in Table II. The fractional clearance value in the unanesthetized state was greater than in the anesthetized state in about 80% of the animals; 5% of the animals showed no difference.

Discussion. The use of the cross-over design experiment in which ICG clearance was determined in each animal both with and without anesthesia, decreased the possibility of one set of conditions affecting the results of the other, and increased the sensitivity of the experiment since each animal served as its own control. The values obtained for ICG clearance in this study on the unanesthetized beagle are in agreement with ICG clearance rates previously reported from this laboratory(4).

The present experiments show that the mean of the differences in the plasma T-½ of this group of animals is significantly elevated following pentobarbital anesthesia. The mechanism by which this occurs has not been determined. Gilmore(6) has reported that pentobarbital interfered with the hepatic uptake of bromsulphalein without altering hepatic blood flow. A similar interference with the hepatic extraction of ICG may account for the increased plasma T-½ observed in this investigation.

Although pentobarbital anesthesia resulted in an increased plasma T-½ of ICG in these experiments, the values obtained were not indicative of hepatic damage, since experimental evidence obtained in beagles in our laboratories has shown that gross liver damage is accompanied by marked elevation of the plasma T-½ of ICG. In the present studies, the elevation of the T-½ observed following anesthesia was found to be reversible. However, the fact that pentobarbital anesthesia influences the plasma clearance of ICG may be of importance in the use of this method for assessment of liver function.

Summary. Indocyanine green clearance was determined in 43 beagles in unanesthetized and pentobarbital anesthetized states utilizing a cross-over design experiment. The results show that the plasma T-½ of ICG was elevated by 16% in 80% of the animals following pentobarbital anesthesia. This effect appears to be due to a reduction of the plasma clearance of the dye by the liver.

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