Manuscript Information

Journal name: Proc S M Mayo Clin
NIHMSID: NIHMS235118
Manuscript Title: Blood level of indocyanine green in the dog during multiple dye curves and its effect on instrumental calibration.
Principal Investigator: James B. Bassingthwaighte (jbb2@u.washington.edu)
Submitter: James B. Bassingthwaighte (jbb@bioeng.washington.edu)

Grant/Project/Contract/Support Information

Name	Support ID#	Title
James B. Bassingthwaighte	R01 HL019139-23	DISPERSION OF DIFFUSIBLE INDICATORS IN THE BODY

Manuscript Files

Type	Fig/Table #	Filename	Size	Uploaded
manuscript		Edwards 60.pdf	712510	2010-09-09 12:36:00

This PDF receipt will only be used as the basis for generating PubMed Central (PMC) documents. PMC documents will be made available for review after conversion (approx. 2-3 weeks time). Any corrections that need to be made will be done at that time. No materials will be released to PMC without the approval of an author. Only the PMC documents will appear on PubMed Central -- this PDF Receipt will not appear on PubMed Central.

Journal Title: Proceedings of the staff meetings of the Mayo Clinic

ISSN:0092-699X

Volume: 35
Issue:
Month/Year: 1960
Pages: ~~747-751~~ 745-751

Article Author: Edwards
Article Title: Blood level of indocyanine green in the dog during multiple dye curves and its effect on instrumental calibration.

Cited In:

Location: Health Serials
Call #: 616.06 M454p Shelved by title

BLOOD LEVEL OF INDOCYANINE GREEN IN THE DOG DURING MULTIPLE DYE CURVES AND ITS EFFECT ON INSTRUMENTAL CALIBRATION*

Anthony W. T. Edwards, M.D.,† Research Assistant, Section of Physiology
James B. Bassingthwaighte, M.D.,‡ Fellow in Medicine, Mayo Foundation
William F. Sutterer, Technical Assistant
Earl H. Wood, M.D., Section of Physiology

When a slowly excreted dye like Evans blue is injected repeatedly into a single subject, a considerable rise in blood level occurs which is maintained between injections and increases with each injection. Attention has been drawn to the effect of this increasing level of background dye on the calibration curves of instruments used to measure its concentration in flowing blood.[1,2] The dye indocyanine green is removed relatively rapidly from the circulatory blood by the liver,[3,4] and consequently during repeated injections it accumulates in the blood to a much lesser extent than does Evans blue. Furthermore, it does not lead to the discoloration of the skin seen with Evans blue. These features have led to its use for multiple determinations of cardiac output in the same subject. It will be shown that with multiple injections, this dye accumulates in the blood to an extent that can be roughly predicted from its initial disappearance curve in a given subject, and that variations of the background level of the dye affect instrumental calibration unless special measures are taken in setting up the instrument.

METHODS

Five dogs weighing from 14 to 26 kg. were used. They were anesthetized with combinations of morphine and chloralose, or morphine and pentobarbital.

In the first dog (fig. 1), dye disappearance was studied at 5, 10, 15 and 30 minutes after a single injection of 2.5 mg. of indocyanine and then after each of three series of seven injections, each series occupying 40 minutes. Injections were into the superior vena cava; samples were collected from the left femoral artery in syringes moistened with heparin and were centrifuged,

*This study was supported in part by research grant G59-14 from the Life Insurance Fund and by research grant H-4664 from the National Institutes of Health of the U. S. Public Health Service.
†In receipt of a Traveling Fellowship from the Post-Graduate Committee on Medicine and the Post-Graduate Medical Foundation, University of Sydney, Australia.
‡Minnesota Heart Association Fellow.

and the indocyanine concentration in the plasma diluted 1:10 with distilled water was estimated in a Beckman DU spectrophotometer at 800 millimicrons. There was no visible hemolysis of the samples.

The second to fifth dogs inclusive received long series of successive injections of dye as part of another project involving simultaneous recording of dilution curves from multiple sites in the circulation. As it is relevant to the question of removal rate of the dye, the procedures on the animals will be described. These four dogs were given large doses of morphine hypodermically to produce bradycardia, so that for the first half of the procedure the heart rate varied from 28 to 60 beats per minute, with a low cardiac output. Midway through each experiment atropine was given intravenously with resultant tachycardia (120 to 230 beats

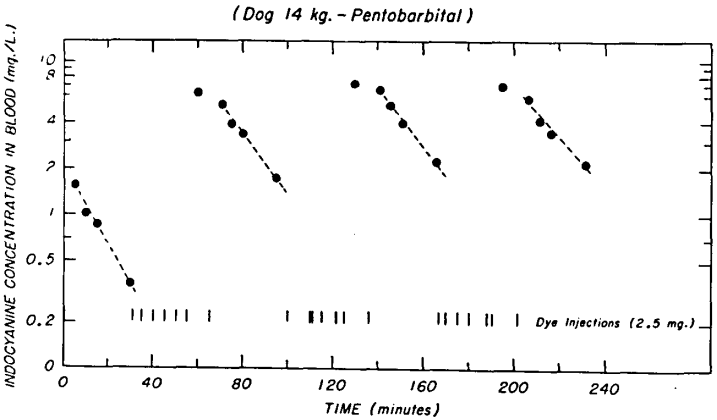

Fig. 1. Concentration of indocyanine green in blood of dog after single and multiple injections of dye. Broken lines represent exponential decrease in concentration. Concentration of dye in plasma x (1-hematocrit reading) is used as the concentration in blood.

per minute) and increase in cardiac output. Details of dye dosage and cardiac output during the procedure on two of these dogs are indicated in figure 2. The concentrations of dye in these experiments were all measured by Waters XC100A interference-filter densitometers described elsewhere.[5] In three experiments the results recorded were the means of readings from two instruments, which were used at constant sensitivity. This permitted serial observations of optical-density changes in the blood from one dye curve to the next, by reference to the original calibration which was determined by using the dye-free blood of the animal being studied. The stability of the densitometers was repeatedly checked during two of these procedures by observations of light transmission through a neutral filter (third dog) or through a standard blood solution (fourth dog). It was found unnecessary to apply

corrections for drift. In the last experiment (fifth dog) the procedure was altered, the instruments being reset to the same full-scale deflection before each curve. In this instance, optical-density changes in the blood were observed in reference to the light transmission through saline solution.

Calibration of Instruments and Testing for Effects of Background Dye.—This has been described in detail elsewhere.[5] Briefly, the calibration procedure was to prepare serial dilutions of indocyanine green in the blood of the animal under study and to run these through the densitometers at a constant rate of flow. The effect of increasing levels of background dye on the sensitivity of the densitometer to added increments in dye concentration was tested by making repeated calibration runs. At the beginning of

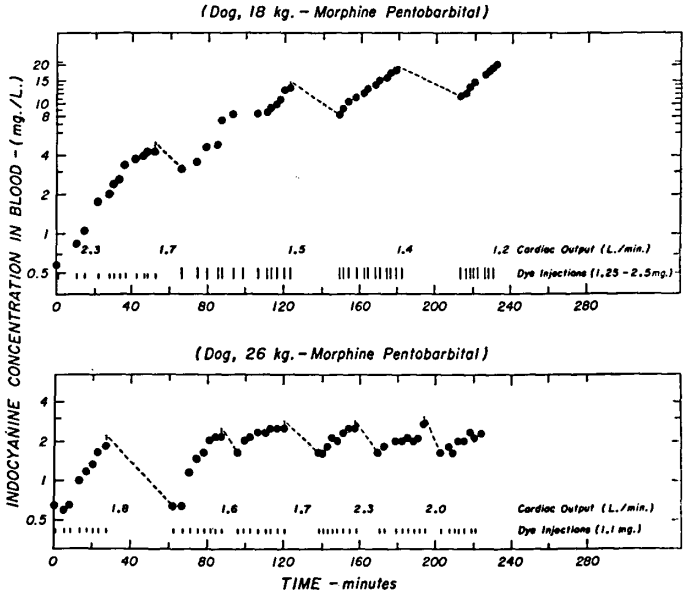

Fig. 2. Concentration of indocyanine in blood of two dogs after multiple injections of dye. Broken lines represent exponential decrease in concentration. In dog represented in lower panel, with smaller injections of dye, level of background dye remained low and was approximately the same at end of each series of injections as it was at the end of the preceding series.

each run, the sensitivity potentiometer of the recording galvanometer circuit was reset so that the full-scale deflection from galvanometer zero position (position when circuit output is zero) to the reading on the "blank" samples (of various known concentrations of background dye) was always the same.

RESULTS AND DISCUSSION

Data on the disappearance and accumulation of indocyanine in the blood of the dog during multiple injections are shown in

figure 1. The first experiment was designed to study the disappearance rate after a single injection, and to investigate whether this rate was modified by a preceding series of injections. The results indicate the usual near-exponential decline[3,4] and a slight reduction in its rate after a series of 21 injections of 2.5 mg. each; the prevailing concentration of dye decreased to half in 10 minutes after the first injection and in 15 minutes after the twenty-second injection. The accumulation of dye during the experiment was about 30 per cent greater than expected on the basis of the first disappearance slope.

The results in the other four dogs show the great variability of dye accumulation depending on the size and frequency of dye injections and also on the basic clearance rate in individual dogs. There was again variation in the clearance rate during each experiment, the rate sometimes decreasing and sometimes increasing as the experiment progressed. The associated trends in cardiac output suggested that variations in indocyanine clearance may have been due to changes in hepatic blood flow.

Only in the fourth dog (fig. 2, upper panel) did the level of background dye exceed 5 mg. per liter, and in this case the level reached was 20 mg. per liter. This followed doubling of the dose to 2.5 mg. after the twelfth injection and administration of this dose at frequent intervals (averaging 5 minutes). Thus, although there were unpredictable variations in the clearance rate of the dye, the accumulation of dye followed a course dictated by the size and frequency of injections. Because of the exponential nature of the disappearance of this dye, it is to be expected that regularly repeated injections would result in attainment of a background concentration of dye that would be approximately the same before each injection until the series was interrupted. This type of phenomenon was seen only in the fifth dog (fig. 2, lower panel), wherein the sequence of injections came closest to being regular.

Effect of Background Dye on Calibration of Waters XC100A Densitometer.—The data shown in figure 3, upper panels, were collected with the galvanometer zero of the densitometer set to coincide with the position assumed by the galvanometer when the light source of the phototube was turned off, that is, the zero-light position. For calibration of the instrument for increments of dye in the presence of background dye in the "blank" blood, the sensitivity of the recording galvanometer circuit was increased so that the galvanometer deflection from the zero-light position for the "blank"-sample reading was identical to the setting for the galvanometer reading used for the "blank" samples containing no background dye. Despite the increase in instrumental sensitivity thus provided in the presence of background dye, there was a loss

of sensitivity to increments in dye concentration as the level of background dye increased. It has been shown elsewhere[5] that the decrease in sensitivity when this procedure is followed can be predicted from the shape of the calibration curve and does not occur with substances that obey Beer's law in the instrument, namely India ink in water. Although the light-transmission characteristics of solutions of indocyanine in plasma obey Beer's law in the Beckman DU spectrophotometer and very nearly do so in the XC100A densitometer, solutions of indocyanine green in whole blood exhibit slight but significant deviation from this relationship when examined in the densitometer. The instrument D_2U was used to obtain the results shown in the upper right panel of figure 3, and provided a striking example of these phenomena, as a leak of white light around the filter caused marked deviation from Beer's law.

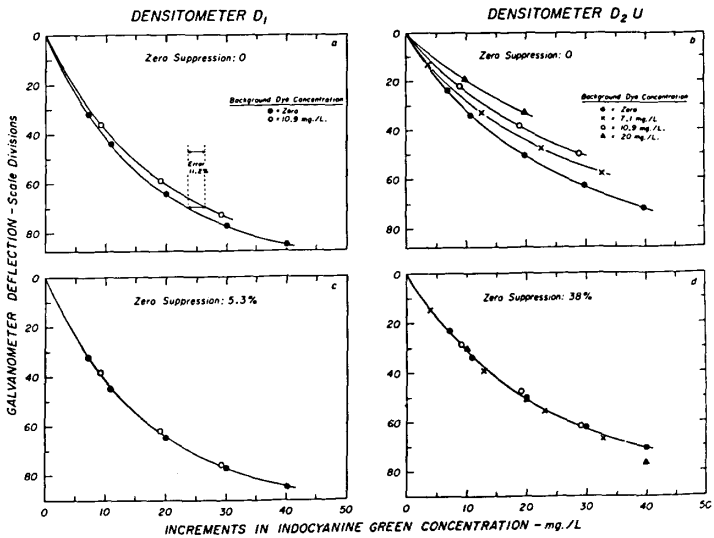

Fig. 3. Calibration curves for increments in concentration of indocyanine green in blood in presence of background dye. Upper panels show calibration curves in densitometers D_1 and D_2U respectively when the galvanometer zero positions were set to coincide with the reading obtained with zero light on the phototube, and the sensitivity of the instruments adjusted to give equal full-scale deflections on the "blank" blood sample pertaining to the respective levels of background dye. In D_1, presence of 10.9 mg. of background dye per liter distorts curve so that use of original calibration curve would give values for increments in dye concentration that would be 11.2 per cent too small. A leak of light around filter in D_2U produced gross differences in calibration at various levels of background dye. Lower panels illustrate excellent compensation achieved by introduction of zero-suppression circuit which causes galvanometer reading with zero light on the phototube to deflect in negative direction from galvanometer zero. Last triangle in lower right panel represents total (background and increment dye) concentration of 60 mg. per liter.

A method has been developed[5] for automatically increasing the sensitivity of the instrument the required degree to compensate for the decrease in slope of the calibration curve for increments in dye concentration that otherwise occurs as the

concentration of background dye in the "blank" samples increases. A negative bias or zero-suppression circuit is incorporated in the circuit on the phototube side of the sensitivity control for the densitometer. This zero-suppression circuit is adjusted so that the reading of the galvanometer with zero light on the phototube is in a negative position from its mechanical zero or zero-current position. A positive deflection is defined for this purpose as the direction of deflection caused by increasing the light on the phototube. The amount of zero suppression required for compensation for background dye differs in various instruments and is determined in the following manner.

A set of serial dilutions of dye in blood is run through the densitometer. The logarithms of the galvanometer deflections, *d*, from the zero-light reading of the instrument are plotted against dye concentration. Plotting the data in this way for solutions whose light-transmission characteristics obey Beer's law will result in a straight line; however, for whole blood a slightly curvilinear relationship is obtained. A constant, *a*, can be found, however, such that the relationship of log *(d-a)* to dye concentration in whole blood is linear over the range of dye concentrations from 0 to 40 mg. per liter. This is equivalent to measuring the galvanometer deflections from a point that is *a* divisions above the zero-light reading of the instrument. Therefore, the galvanometer zero (that is, the zero-current reading) is set *a* units above the zero-light reading by adjusting the zero-suppression voltage mentioned above. This means that light passing through blood in the lumen will produce a positive galvanometer deflection of *d-a* divisions from the galvanometer zero reading, while turning off the light results in a negative deflection of *-a*. With the zero suppression set in this manner, the calibration curve in the range 0 to 40 mg. per liter closely approaches an exponential curve with the galvanometer zero as its asymptote. In this range, therefore, increasing the sensitivity of the instrument to obtain the same full-scale reading on "blank" blood samples containing background dye can be accomplished with no change in the slope of the calibration curve to increments of dye. Thus near perfect compensation for background dye can be accomplished by this simple method.

Its practical application is demonstrated in figure 3, lower panels, which demonstrates effective compensation for background dye by the use of zero suppression coupled with the simple maneuver of increasing the sensitivity of the instrument to the same full-scale deflection for the "blank" blood samples containing various levels of background dye. A high degree of zero suppression (38 per cent of full scale on blood with zero content of dye) was necessary in densitometer D_2U (fig. 3, right

panels), in which a leak of unfiltered light caused marked deviation from Beer's law. In spite of this unusual degree of deviation, the method produced excellent compensation even at concentrations of background dye as high as 20 mg. per liter (fig. 3, lower right panel).

SUMMARY AND CONCLUSIONS

When multiple indicator-dilution curves are determined, indocyanine green accumulates in the circulation in a manner roughly predictable from its disappearance rate and the amount and frequency of injections.

The light transmission of solutions of indocyanine green in blood deviates slightly from Beer's law, in spite of the use of densitometers with linearly responding phototubes and a narrow spectral band of incident light. While such deviations are unimportant at low concentrations of dye, as the concentration background dye increases, as for instance during rapidly repeated multiple determinations of cardiac output, there is a significant decrease in the sensitivity of the densitometer to increments in the concentration of dye in blood.

A method is described for compensating for this decreased sensitivity by the introduction of a zero-suppression circuit. This arrangement automatically results in progressive migration of the zero-light position also away from the blank reading so that the deflection measured from zero light increases slightly and proportionately each time the sensitivity of the system is increased to provide the same "blank" reading in the presence of background dye. The amount of zero suppression required can be estimated from the calibration curve of the densitometer for indocyanine green in blood.

The principles illustrated here are applicable to other instruments recording light transmission of dyes in whole blood, particularly in applications requiring measurements of increments in concentration in the presence of unknown background levels of the dye in question.

REFERENCES

1. Dow, Philip: Estimations of Cardiac Output and Central Blood Volume by Dye Dilution. Physiol. Rev. *36*:77-102 (Jan.) 1956.
2. Edwards, A. W. T., and Korner, P. I.: Factors Determining the Dispersion of Dye in Indicator Dilution Curves in the Normal Mammalian Circulation. Clin. Sc. *17*:265-278 (May) 1958.
3. Fox, I. J., Brooker, L. G. S., Heseltine, D. W., Essex, H. E., and Wood, E. H.: A Tricarbocyanine Dye for the Continuous Recording of Dilution Curves in Whole Blood Independent of Variations in Blood Oxygen Saturation. Proc. Staff Meet., Mayo Clin. *32*:478-484 (Sept. 4) 1957.
4. Wheeler, H. O., Cranston, W. I., and Meltzer, J. I.: Hepatic Uptake and Biliary Excretion of Indocyanine Green in the Dog. Proc. Soc. Exper. Biol. & Med. *99*:11-14 (Oct.) 1958.
5. Edwards, A. W. T., Isaacson, J., Sutterer, W. F., Bassingthwaighte, J. B., and Wood, E. H.: An Interference Filter Densitometer and Its Use For Measurement of Indocyanine Green in Flowing Blood. (Unpublished data.)