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Injection method for ocular hemodynamic studies in man.

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The indicator-dilution technique is currently used to study ocular hemodynamics and to measure arm-to-retina circulation time. The passage of injected dyes through the ocular circulation is documented and graphically expressed as a dilution curve. The great variety of injection techniques and the persistent instability of the circulatory system has prevented a systematic study of the relationship between injection methods and dilution curves in the eye.

We recently have been able to record dilution curves from individual blood vessels in the human retina using mutiple injections.[1] We therefore began to study the effects of different injection methods on dilution curves in the eye. Our three main goals were (1) to obtain an injection technique that provides dilution curves of high reproducibility; (2) to use a minimal amount of dye so that several consecutive injections would be possible; and (3) to obtain dilution curves which allow accurate determination of the first appearance time of the dye (t_a), the time of peak dye concentration (t_{max}), and the mean circulation time ($\bar{t}$).

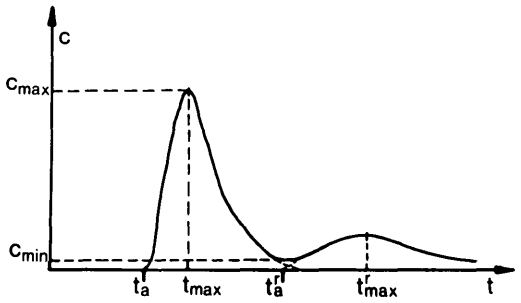

Fig. 1. Unit dilution curve recorded with fundus fluorophotometer from the superior temporal branch of the central retinal artery after injection of 20 mg. of fluorescein during 0.4 second in the superior vena cava. C, dye concentration (proportional to fluorescence intensity in the range of concentration that we use); t_a, time of first appearance of dye bolus; t_{max}, time of maximum dye concentration; t'_a, extrapolated time of first recirculation; t'_{max}, time of peak dye concentration in the first recirculation wave.

In this study, only intravenous injection was used because the intra-arterial method is not suitable for routine clinical work.

Five healthy adult volunteer subjects, two females and three males, served as subjects for the study. Injections of either sodium fluorescein or indocyanine green (ICG) were made by hand through a standard 50 automatic syringe (Socorex ISBA). The duration of injection was recorded graphically using an electrical signal actuated during the injection. The dilution curves were recorded with a fundus fluorophotometer.[2]

A. ***Influence of site of injection on reproducibility.*** We injected 20 mg. of fluorescein diluted in 0.3 c.c. of saline within 0.5 second through a 19 gauge by 24 inch standard catheter (Deseret Intracath No. 3134), which was introduced through the median antecubital vein. The location of the tip of the catheter was varied from the antecubital vein to the superior vena cava near the heart. Ten consecutive injections were made for each location; recordings were obtained from a retinal artery. When the tip of the catheter was located in the antecubital vein, the dilution curves were generally inconsistent in shape, with great variations in t_a and t_{max}. When the tip of the catheter was located in the superior vena cava, successive injections provided highly reproducible dilution curves with relative variations in t_a and t_{max} of less than ± 5 per cent in all five individuals.

B. ***Influence of the duration (τ) and volume (V) of the injection and the amount (A) of dye.*** Using multiple injections in the vena cava, three types of experiments were performed, (1) chang-

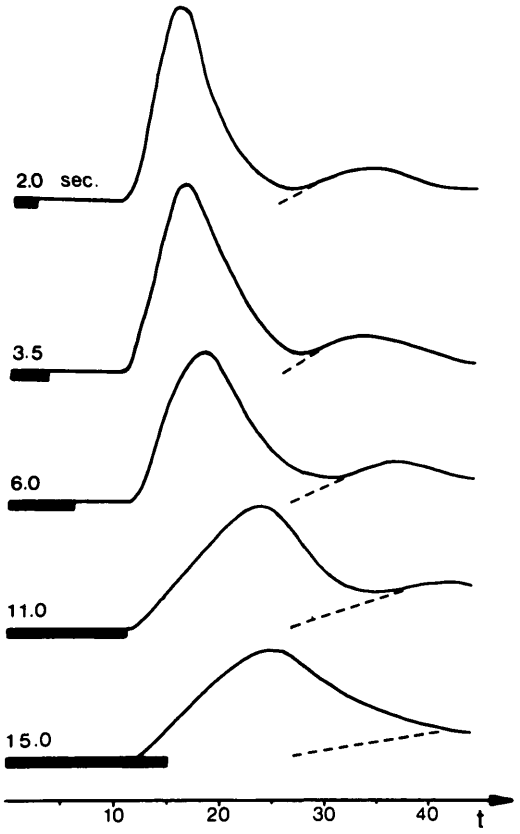

Fig. 2. Fluorescein dilution curves from the superior temporal artery after injection of 20 mg. of fluorescein in 4.0 c.c. of saline in the same subject as Fig. 1. Wide black lines indicate various durations of injection; broken lines represent extrapolated recirculation wave. Note flattening of curve with increased duration of injection.

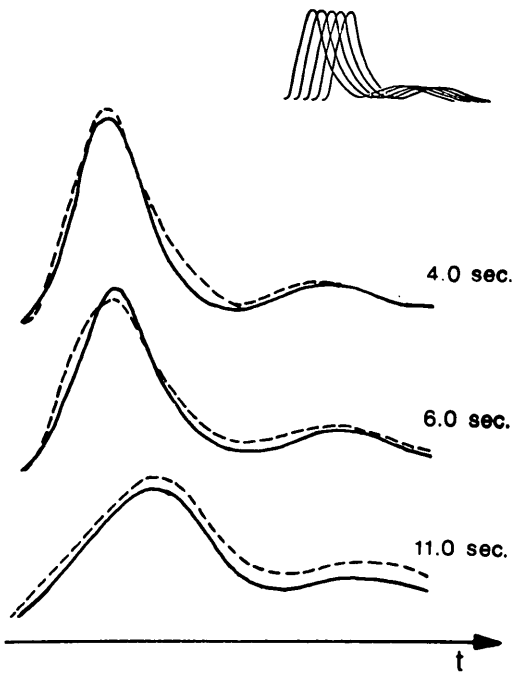

Fig. 3. Comparison of computed curves (broken lines) and actual recorded curves (solid lines) from same subject as in Fig. 1. Duration of injection is listed at right of curves; small inset (top right) illustrates the way in which the top curve (duration, 4 seconds) was obtained by simply summing 5 unit dilution curves (each heart cycle lasts ⅘ second). Similarity of computed curves and actual curves verifies the concept of a unit dilution curve.

ing A, with τ and V constant; (2) changing V, with τ and A constant; and (3) changing τ, with V and A constant.

(1) *Changing amount of dye (V and τ constant).* Five different amounts of ICG, ranging from 5 mg. to 50 mg., were injected. The volume of each injection was 1 c.c. and the duration of injection was 0.7 second. The only observable change was an increase in the height of the curve in proportion to the amount of dye.

(2) *Changing volume of injection (τ and A constant).* Five injections of 25 mg. of fluorescein were made, each lasting 0.5 second. However, the volume of the injections varied from 0.1 c.c. to 2.0 c.c., resulting in a change of concentration by a factor of 20. Within the range of accuracy of the method, the dilution curves were identical despite these variations in volume.

We conclude that the volume of the injection does not affect the dilution curve as long as the duration of the injection and the amount of dye remain constant. Increasing the amount of dye with τ constant results in a proportional increase in the height of the dilution curve.

(3) *Changing duration of injection (V and A constant).* The experiments related to changes of τ can be classified into two categories: $\tau \leqslant$ one heart cycle and $\tau >$ one heart cycle.

(A) $\tau \leqslant$ ONE HEART CYCLE. We injected 20 mg. of fluorescein in 1 c.c. of saline. Ten injections were made with τ varying from 0.4 second to 0.8 second. Nearly identical curves were obtained with relative variations of t_a and t_{max} of less than $\pm$ 5 per cent. A typical dilution curve from this experiment is shown in Fig. 1.

(B) $\tau >$ ONE HEART CYCLE. We injected 20 mg. of fluorescein in 4.0 c.c. of saline. τ varied from 1 second to 15 seconds. Recordings were made from the same retinal artery as above. With increasing τ, there was a flattening in the slope of the front and trailing edge of the curve, an increasing delay in t_{max}, and a decrease in the height of the curve and in the ratio C_{max}/C_{min},

as well as increasing interference of the recirculated dye with the first bolus (Fig. 2).

From our experiments, we conclude that a dilution curve which results from a brief injection (less than one heart cycle) has a characteristic shape determined by the cardiovascular system. This type of curve may be termed a unit dilution curve. Its height depends only on the amount of dye injected.

An injection which lasts longer than one heart beat can be regarded simply as the sum of a series of injections, each lasting for one heart beat. To illustrate this summation principle, which is expressed mathematically in the appendix by Formula 1, we have constructed dilution curves for $\tau = 4$ seconds, 6 seconds, and 11 seconds, using as a unit dilution curve the curve recorded for $\tau = 0.4$ seconds (Fig. 1). The computed curves are very similar in shape to the corresponding recorded curves (Fig. 3).

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Appendix

If we consider the cardiovascular system as a linear system, an injection made in less than one heart beat can be considered as a unit step (brief square wave) input. The output, which is the corresponding dilution curve (Fig. 1) is the admittance function.[3]

Any dilution curve $C_N(t)$ obtained after an injection of an amount A of dye, lasting for N heart beats, can be obtained by summation of N such admittance functions, each corresponding to an amount of injected dye equal to A/N and displaced by one heart beat from the preceding one. That is:

$$C_N(t) = \frac{1}{N} \sum_{n=0}^{N-1} C(t - nT) \qquad (1)$$

where T is the duration of the heart cycle and n is an integer with values: 0, 1, 2, . . . (N-1). C(t) is the admittance function associated with an injection in the vena cava and determined for a particular subject by the characteristics of his cardiovascular system and the recording site.