Measurement of Cardiac Output with Indocyanine Green Transcutaneous Fluorescence Dilution Technique

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Background: Cardiac output is an essential parameter for the hemodynamic assessment of patients with cardiovascular disease. The authors tested in an animal model the feasibility of measuring cardiac output by transcutaneous fluorescence monitoring of an intravenous bolus injection of indocyanine green.

Methods: Fluorescence dilution cardiac output was measured in 10 anesthetized rabbits and compared with cardiac output measured with a pulmonary thermodilution catheter and to aortic velocity measured by Doppler ultrasound. Indocyanine green fluorescence was excited with a near-infrared laser and measured with an optical probe positioned on the central ear artery. Measurements were obtained during baseline conditions as well as during short-term decreases and increases of the cardiac output.

Results: The fluorescence of circulating indocyanine green detected transcutaneously varied proportionally to that of arterial blood samples, which allowed for calibration in terms of blood concentration of indocyanine green. Average values of fluorescence dilution cardiac output and thermodilution cardiac output measured in baseline conditions were 412 (± 13) and 366 (± 11) ml/min, respectively. Fluorescence dilution cardiac output showed a close, one-to-one linear relation with thermodilution cardiac output in each animal and in the pooled data from all animals (slope = 0.95 ± 0.03; R = 0.94). Fluorescence dilution cardiac output overestimated thermodilution cardiac output by an average of 46 (± 6) ml/min during baseline conditions. Fluorescence dilution cardiac output was tightly correlated with aortic velocity.

Conclusions: The proposed technique yielded accurate estimates of the cardiac output in experimental animals. This study should provide an initial framework for clinical testing of this new minimally invasive method for measuring cardiac output.

Cardiac output is an essential parameter for the hemodynamic assessment of patients with cardiovascular disease.¹² The standard clinical approach for measuring cardiac output is the thermodilution method, an indicator dilution technique in which a temperature change is sensed in the pulmonary artery after injection of a chilled solution near the right atrium.³ Insertion of a pulmonary artery catheter, however, is time consuming and invasive, and its use is associated with possible complications.⁴⁵

The dye dilution⁶ and pulse dye densitometry (PDD) techniques⁷—which do not require a pulmonary artery catheter—apply the theory of indicator dilution to the transport of a dye, usually indocyanine green (ICG), injected as a bolus in the venous blood stream. These techniques estimate the concentration of ICG in arterial blood from optical absorbance measurements,⁶ which is complicated by the overlap between the absorption spectra of ICG and blood hemoglobin. In addition to absorbing light,⁸ ICG also fluoresces intensely when excited at near infrared wavelengths.⁹,¹⁰ Its peak wavelengths of absorption (775 nm) and emission (830 nm) are in a spectral region where biologic tissues, including blood, are relatively transparent.¹¹ Therefore, the fluorescence of ICG in flowing blood can be excited and measured with optical devices placed on the skin surface. Because tissues do not fluoresce at the wavelengths at which ICG fluoresces, there is no confounding factor to the transcutaneous monitoring of ICG concentration.

The current study tested in an animal model the feasibility of measuring cardiac output by transcutaneous monitoring of the fluorescence of an intravenous bolus injection of ICG. Fluorescence dilution cardiac output (COICG) was compared to pulmonary artery thermodilution cardiac output (COTD) and to ultrasound aortic flow velocity (Vao) during conditions of increased as well as decreased cardiac output. Results of this study should provide a framework for future clinical testing of this new minimally invasive method for measuring cardiac output.

Materials and Methods

Animal Preparation

The Institutional Animal Care and Use Committee (University of Southern California, Los Angeles, California) approved this study, and appropriate guidelines for the use of animals were observed throughout. The experiments were performed in 10 adult, male New Zealand white rabbits (weight, 2.7–3.2 kg). Anesthesia was induced by having the rabbits breathe a 5% halothane in oxygen–air mixture while inside a plastic box. The anesthetized animal was placed supine, and the trachea was intubated through a tracheotomy using a 3.5-mm tube. Thereafter, mechanical ventilation was started, the halothane concentration being kept between 0.8 and 1.2% in an oxygen-air mixture (inspired fraction of oxygen = 0.5). Exhaled carbon dioxide and anesthetic gas concen-
tations were monitored with a capnometer (Ultima; Datex, Andover, MA). Ventilation was adjusted to maintain the end-tidal partial pressure of carbon dioxide between 30 and 34 mmHg. The right external jugular vein was cannulated for continuous infusion of 0.9% saline solution (10 ml/h). The right vagus nerve was isolated and transected. A coiled bipolar electrode was positioned around the distal portion of the transected nerve. In this way, reflex cardiovascular responses were avoided while controllable reductions of the heart rate and the cardiac output could be achieved by stimulating (S48; Grass, West Warwick, RI) the distal vagus nerve with a fixed pulse (1 ms, 5 V) of variable frequency (20–40 Hz). Pilot studies showed this range of stimulation frequencies resulted in substantial reductions (30–60% decrease) of the thermodilution cardiac output COTD. The right femoral artery was cannulated for continuous blood pressure monitoring and arterial blood sampling.

After a sternotomy at the second and third intercostal spaces, a 6-mm-diameter, 20-MHz Doppler ultrasonic cuff probe (ES-20-6; Triton Technology, San Diego, CA) was placed around the ascending aorta for measurement of the instantaneous aortic velocity. The range gate control on the pulsed Doppler flowmeter (model 202; Triton Technology) was adjusted to measure the maximal flow velocity within the aortic cross section. A 4-French thermodilution balloon catheter (AI-07044; Arrow, Reading, PA) was inserted into the right femoral vein and advanced until the thermistor reached the main pulmonary artery. Correct placement of the catheter tip was verified visually through the thoracotomy. The catheter was connected to a cardiac output computer (COM 1; Baxter, Irvine, CA) to measure CO_TTD.

Thereafter, the animal was turned in the prone position and paralyzed with pancuronium bromide (0.1 mg·kg⁻¹·h⁻¹). The right ear was shaved and secured with skin glue (Mastisol; Ferndale Labs, Ferndale, MI) over a servocontrolled electric heater custom designed to fit into the animal’s external ear. Heat-induced vasodilation of the ear vasculature was achieved by raising the ear temperature to 40°–42°C. Body temperature was monitored with a rectal probe and maintained at 40°C with heat lamps.

**Electro-optic Instrumentation**

The illumination source used to excite the fluorescence of the circulating ICG was a fiber-coupled 782-nm laser diode (SRT-F785S; Micro Laser Systems, Garden Grove, CA) whose output was collimated and directed toward a beam splitter (fig. 1). A fraction of the near-infrared excitation light was forwarded to the preparation with a bifurcated fluorescence probe (R400.7; Ocean Optics, Dunedin, FL) comprised of one 400-μm excitation fiber surrounded by six 400-μm emission fibers. The probe tip was positioned flush over the central ear artery (2- to 3-mm diameter after heat-induced vasodilation). The average luminous power at the probe tip was 2.4 mW. The fluorescence emission captured at the skin surface was directed toward an 830-nm interferential filter (079-2230; OptoSigma, Santa Ana, CA) placed in front of a photomultiplier tube (H7732-10; Hamamatsu, Bridgewater, NJ). The photomultiplier output signal was demodulated with a lock-in amplifier (SR 830; Stanford Research Systems, Sunnyvale, CA), which also generated the 2.8-kHz modulation of the excitation light at the level of the laser diode driver (CP 200; Micro Laser Systems). The wavelengths of excitation and detection...
were selected to maximize the intensity of the ICG fluorescence.9,12

A second excitation-emission probe directed a fraction of the laser output toward a magnetically stirred fluorescence cell to measure the fluorescence of blood samples for calibration of the in vivo fluorescence signal in terms of blood ICG concentration.

An eight-channel A/D converter module (Powerlab/8SP; AD Instruments, Colorado Springs, CO) displayed and stored arterial blood pressure, heart rate, expired carbon dioxide concentration, aortic Doppler velocity, thermodilution analog output, and transcutaneous fluorescence dilution curves. In addition, the fluorescence signals measured in vivo and at the level of the fluorescence cell were digitized with an oscilloscope (TDS 320; Tektronix, Beaverton, OR) and transferred to a second computer for online estimation of COICG.

**Calibration Procedure**

In each animal, calibration of the transcutaneous in vivo fluorescence intensity as a function of ICG concentration in circulating blood was performed in two steps. First, a calibration curve was obtained relating in vitro fluorescence intensity to in vitro blood ICG concentration (fig. 2A). ICG solution (0.05 mg/ml in 5% dextrose solution) was added in increasing amounts (5 μl × 6, 10 μl × 7, 20 μl × 7) to 3 ml blood in a fluorescence cell, and the fluorescence intensity was recorded after each addition (21 additions).

Second, the in vitro calibration was applied to derive the relation between the transcutaneous fluorescence intensity obtained in vivo and the in vitro ICG blood concentration giving rise to this signal. A 1-mg dose of ICG (1 ml of 1 mg/ml ICG in 5% dextrose solution) was injected intravenously through the distal port of the thermodilution catheter, the proximal port being reserved for the less concentrated ICG solution (0.03 mg/ml) used for the cardiac output measurements. Five to seven 1.5-ml blood samples were withdrawn from the femoral artery between 2 and 8 min after the injection when the ICG was fully mixed with the animal’s circulating blood (fig. 2B). The transcutaneous fluorescence intensity was measured at the exact times of the blood withdrawals. The blood samples were placed in the fluorescence cell. Their fluorescence was recorded and related to the titrated blood ICG concentration as indicated by the first step of the calibration procedure. Results from the two procedures were combined to derive the in vivo calibration curve used to compute COICG. In six animals, we assessed the variability of this calibration by measuring the relation between in vivo and in vitro fluorescence at three time points during the experiment—at the beginning, the midpoint (after the vagal stimulation), and the end (after the saline infusion).

**Experimental Protocol**

After the surgery, the animal preparation was stabilized during 15 min to allow the ear vasculature to fully dilate by exposure to the heated holder. Thereafter, three baseline measurements of the cardiac output were performed simultaneously with the thermodilution and the fluorescence dilution techniques after bolus injection of 45 μg ICG mixed in 1.5 ml iced dextrose solution (0.03 mg/ml in 5% dextrose solution) through the proximal port of the thermodilution catheter. The ventilator was stopped at end-expiration during the measurement to avoid any variation of the cardiac output with ventilation. We waited approximately 5 min between cardiac output measurements for the transcutaneous ICG signal to return to baseline.

Cardiac output was then measured three times during low-output conditions obtained by vagal stimulation. A 1.5-ml bolus of iced ICG solution was injected when the
heart rate and the peak aortic velocity traces were stable for 10 s. The stimulation was stopped between measurements to let the heart rate return to its baseline level. A series of three baseline measurements of the cardiac output was obtained after the low-output measurements. Some animals were tested with two levels of low cardiac output.

Measurements were then repeated during high-output conditions obtained by rapid infusion (15 ml/min) of 0.9% heated saline solution through the jugular vein catheter. The infusion pump was stopped when the peak aortic velocity increased by approximately 40%. Thereafter, we waited 30 s for the blood temperature to stabilize and then measured the cardiac output with the thermodilution and fluorescence dilution techniques. The procedure was repeated three times with a 10-min pause between saline infusions.

**Data Analysis**

The analysis of the fluorescence dilution curves to derive cardiac output COICG was based on the algorithm used to process thermodilution curves in the cardiac output computer. The fluorescence data were converted to blood ICG concentration using the empirical calibration curve. To eliminate indicator recirculation from the area under the ICG dilution curve, the descending part of the first-pass ICG concentration trace was approximated by an exponential function for data points comprised between 80% and 30% (C30) of the peak ICG concentration deflection. The area under the first-pass dilution curve was computed by adding the area between the beginning of the ICG concentration trace and point C30 to the area under the exponential approximation beyond point C30. COICG was estimated by dividing the amount of injected ICG by the area under the first-pass dilution curve.

The Doppler aortic velocity signal (uncalibrated, in volts) was averaged over 5–10 heartbeats overlapping with the rise of the ICG fluorescence trace to estimate the time-averaged peak velocity in the aorta VAor. Thermodilution cardiac output COTD was obtained from the cardiac output computer display. Because hemodynamic conditions could not be exactly replicated during vagal stimulation and saline infusion, all values of COICG and VAor were analyzed by linear regression analysis. The relations between COICG and COTD and between COICG and VAor were analyzed by linear regression analysis. Values are reported as mean (± SE). Statistical significance was set at P < 0.05.

**Results**

**Calibration of Transcutaneous ICG Fluorescence as a Function of ICG Concentration**

The fluorescence intensity measured *in vitro* as a function of the titrated blood ICG concentration yielded a slightly curved calibration response that could be fit to an empirical parabolic equation over the range of concentrations used in the study (fig. 2C). The coefficients of the parabolic fit were constant across experiments after accounting for a scaling factor that resulted from small variations in the position of the optical probe relative to the fluorescence cell.

Indocyanine green fluorescence measured *in vivo* at the surface of the skin was linearly related to the fluorescence of circulating blood measured *in vitro* in the fluorescence cell (fig. 2D). The ordinate of the regression line was near 0 for all experiments. The slope of the regression line varied between experimental animals with a 33% coefficient of variation (SD/mean). This variability was likely due in part to differences in probe placement between animals. In the six experiments in which ICG fluorescence *in vivo* was measured three times as a function of blood fluorescence *in vitro*, the slope of the regression line remained nearly constant within each experiment, with an average coefficient of variation between the three measurements of less than 5%. For each experiment, multiplying the slope of the regression line by the coefficients of the parabolic relation between *in vitro* fluorescence and titrated ICG blood concentration yielded the calibration equation for transcutaneous ICG fluorescence as a function of circulating ICG blood concentration.

**Characteristics of ICG Concentration and Thermodilution Curves**

During baseline conditions, blood ICG concentration measured transcutaneously at the level of the ear began to increase approximately 2 s after the iced ICG solution was injected in the inferior vena cava (fig. 3A). The delay accounted for the circulatory time between these two anatomical sites. In contrast, the temperature in the pulmonary artery changed almost immediately after the injection (fig. 3B). The ICG trace increased to its peak in approximately 3 s and then decayed briskly. Recirculation of the ICG peaked at approximately 9 s after the peak of the first-pass trace. Decreased cardiac output during vagal stimulation delayed the appearance of the ICG at the level of the ear probe and broadened the ICG first-pass trace. Increased cardiac output after saline infusion resulted in a swifter appearance of the ICG and a briefer first-pass trace.

The injection of iced solution induced a marked slowing of the beat-to-beat heart rate that overlapped with the temperature change in the pulmonary artery (fig. 3C). The beat-to-beat heart rate began to return toward
its preinjection level by the time ICG was detected at the level of the ear. The transient heart rate decrease coincided with a brief decrease of the time-averaged aortic velocity $V_{Aor}$ that was followed by an increase to supranormal values of $V_{Aor}$ and a return to baseline (fig. 3D).

**Relation between $CO_{ICG}$ and $CO_{TD}$**

Average values of $CO_{ICG}$ and $CO_{TD}$ measured in baseline conditions in the 10 animals were 412 (± 13) and 366 (± 11) ml/min, respectively, in the expected range for anesthetized rabbits (250–650 ml/min). In each animal, $CO_{ICG}$ was linearly related to $CO_{TD}$ (table 1). The slope of the regression line (range, 0.74 – 1.25) was not different from 1.0 in 8 studies. In the combined data from all 10 studies (fig. 4), the linear relation between $CO_{ICG}$ and $CO_{TD}$ had a slope (0.95 ± 0.03) not different from 1.0 and an ordinate (77 ± 10 ml/min) that was significantly greater than 0. Cardiac output $CO_{ICG}$ over-estimated $CO_{TD}$ by an average of 46 (± 6) ml/min during

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**Fig. 3.** Representative thermodilution and fluorescence dilution measurements. (A) Transcutaneous fluorescence dilution curve converted in indocyanine green blood concentration (µg/ml). The first-pass dilution curve, observed approximately 2 s after indocyanine green injection, is followed by a discernible recirculation hump. An exponential approximation of the descending part of the trace between 80% and 30% of the peak concentration is used to correct for the recirculation artifact in the computation of the area under the concentration curve. (B) Analog output from cardiac output computer proportional to temperature change in pulmonary artery ($\Delta T_{pa}$, in volts). (C) Beat-to-beat heart rate decreases transiently and markedly immediately after injection of iced solution in inferior vena cava. (D) Time-averaged aortic velocity ($V_{Aor}$, in volts) decreases and then rebounds to supranormal values after the injection of iced saline. Slight shift in timing of heart rate and aortic velocity decays originates in differences in processing techniques used to derive the two signals. Oscillations in heart rate beginning 15 s after time 0 correspond to resumption of artificial ventilation.
related to the simultaneously measured time-averaged sion). Each baseline, vagal stimulation, saline infusion, and all experimental conditions in one animal. Multaneous measurements in one animal. The slope of the regression line varied markedly between the two measurements in each animal. The slope of the regression line was not different from 0 in seven of nine studies, suggesting a proportionality relation between the two measurements in each animal. The slope of the regression line varied markedly between experiments.

**Relation between COICG and VAor**

In each animal, cardiac output COICG was linearly related to the simultaneously measured time-averaged peak velocity in the aorta VAor (table 2). The ordinate of the regression line was not different from 0 in seven of nine studies, suggesting a proportionality relation between the two measurements in each animal. The slope of the regression line varied markedly between experiments.

**Discussion**

The main findings of this study were (1) fluorescence dilution cardiac output COICG showed a close (R = 0.94), one-to-one linear relation (slope = 0.95 ± 0.03) with thermodilution cardiac output COTD and (2) cardiac output COICG was correlated with Doppler flow velocity in the ascending aorta (average R = 0.91).

**Comparison of Transcutaneous Fluorescence and Thermodilution Cardiac Output**

Fluorescence dilution cardiac output COICG was linearly related to thermodilution cardiac output COTD. The slope of the regression line between these variables was near 1.0 for most experiments, as well as for the grouped data from all experiments. In most cases, COICG overestimated COTD such that the intercept of the regression line was greater than 0. We noted that the heart rate decreased transiently immediately after injection of the chilled ICG solution as cooled blood flowed near the sinoatrial node. The temporary bradycardia was likely accompanied by decrease followed by a delayed (approximately 1 s) compensatory increase of the beat-to-beat flow output of the heart as suggested by the variations of the aortic velocity VAor. Consequently, the cold-induced bradycardia had a more pronounced effect on...

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**Table 1. Relationship between Measurements of Cardiac Output with Fluorescence Dilution and Thermodilution Techniques**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Equation</th>
<th>N</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>COICG = 0.94 (± 0.08) COTD + 84 (± 23)</td>
<td>21</td>
<td>0.94</td>
</tr>
<tr>
<td>2</td>
<td>COICG = 1.25 (± 0.17) COTD - 0 (± 39)</td>
<td>17</td>
<td>0.88</td>
</tr>
<tr>
<td>3</td>
<td>COICG = 0.74 (± 0.11) COICG + 122 (± 26)</td>
<td>20</td>
<td>0.85</td>
</tr>
<tr>
<td>4</td>
<td>COICG = 0.90 (± 0.05) COTD + 98 (± 15)</td>
<td>11</td>
<td>0.99</td>
</tr>
<tr>
<td>5</td>
<td>COICG = 1.08 (± 0.11) COTD + 84 (± 47)</td>
<td>14</td>
<td>0.94</td>
</tr>
<tr>
<td>6</td>
<td>COICG = 1.07 (± 0.09) COTD + 16 (± 33)</td>
<td>14</td>
<td>0.96</td>
</tr>
<tr>
<td>7</td>
<td>COICG = 1.15 (± 0.06) COICG + 29 (± 25)</td>
<td>12</td>
<td>0.99</td>
</tr>
<tr>
<td>8</td>
<td>COICG = 0.82 (± 0.09) COTD + 83 (± 37)</td>
<td>12</td>
<td>0.94</td>
</tr>
<tr>
<td>9</td>
<td>COICG = 0.88 (± 0.12) COTD + 98 (± 62)</td>
<td>16</td>
<td>0.89</td>
</tr>
<tr>
<td>10</td>
<td>COICG = 1.05 (± 0.08) COTD - 20 (± 33)</td>
<td>15</td>
<td>0.97</td>
</tr>
<tr>
<td>All</td>
<td>COICG = 0.95 (± 0.03) COTD + 74 (± 10)</td>
<td>152</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Linear regression analysis between fluorescence dilution cardiac output (COICG) (ml/min) and thermodilution cardiac output (COTD) (ml/min) for individual experiments and for group data. Different N values in different studies originate from measurements for two levels of vagal stimulation in first three experiments or from occasional additional measurements during baseline conditions in subsequent studies.

* Not different from 0; † not different from 1.0.

N = number of data points; R = coefficient of regression.

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**Table 2. Relationship between Cardiac Output Measured with Fluorescence Dilution Technique and Aortic Doppler Flow Velocity**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Equation</th>
<th>N</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>COICG = 402 (± 38) VAor - 41 (± 38)</td>
<td>21</td>
<td>0.92</td>
</tr>
<tr>
<td>2</td>
<td>COICG = 360 (± 50) VAor + 47 (± 33)</td>
<td>16</td>
<td>0.89</td>
</tr>
<tr>
<td>3</td>
<td>COICG = 193 (± 32) VAor + 127 (± 28)</td>
<td>20</td>
<td>0.82</td>
</tr>
<tr>
<td>4</td>
<td>COICG = 608 (± 68) VAor - 46 (± 47)</td>
<td>10</td>
<td>0.96</td>
</tr>
<tr>
<td>5</td>
<td>COICG = 926 (± 128) VAor - 116 (± 91)</td>
<td>14</td>
<td>0.90</td>
</tr>
<tr>
<td>6</td>
<td>COICG = 521 (± 45) VAor + 1 (± 25)</td>
<td>14</td>
<td>0.97</td>
</tr>
<tr>
<td>7</td>
<td>COICG = 492 (± 89) VAor - 85 (± 82)</td>
<td>12</td>
<td>0.84</td>
</tr>
<tr>
<td>8</td>
<td>COICG = 627 (± 111) VAor - 159 (± 100)</td>
<td>12</td>
<td>0.87</td>
</tr>
<tr>
<td>9</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>COICG = 489 (± 30) VAor - 67 (± 31)</td>
<td>12</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Linear regression analysis between fluorescence dilution cardiac output (COICG) (ml/min) and peak velocity in the ascending aorta (VAor) (uncalibrated, in cm/s) for individual experiments. Aortic velocity was not measured in experiment 9.

* Not different from 0.

N = number of data points; R = coefficient of regression.
CO\textsubscript{Td}, representing the flow output of the right heart immediately postinjection and a lesser effect on CO\textsubscript{ICG}, which represented right heart and left heart outputs over several heartbeats after the cold ICG injection.

A similar phenomenon has been reported in human subjects in whom cardiac output measured by aortic transpulmonary thermodilution\textsuperscript{4,16} or by PDD\textsuperscript{7} overestimates thermodilution cardiac output measured with a pulmonary artery catheter. The bias is attributed primarily to a slowing of the heart rate caused by the iced injectate, which affects more intensely the right heart output than the left heart output because the former is measured almost immediately after the cold injection.\textsuperscript{4}

The cooling effect of the indicator on the cardiac output is likely to have been more pronounced in rabbits than in humans, given that the volume of injectate (1.5 ml) represented approximately 1.5 times the stroke volume in our animals, compared with approximately 0.15 times the stroke volume in human trials. As a result, a larger overestimation of CO\textsubscript{ICG} relative to CO\textsubscript{Td} was observed in the present experiments (approximately 12% of the mean cardiac output for baseline conditions) in comparison with the human studies (approximately 2.5–10% of mean cardiac output). Note that the ICG fluorescence technique could accommodate smaller volumes of injectate, whereas 1.5 ml was the smallest volume that could be used with the commercial cardiac output computer used in this study.

The difference between CO\textsubscript{ICG} and CO\textsubscript{Td} was the largest during low cardiac output induced by vagal stimulation. Because of the decreased flow rate, the transit time of the cooled venous blood near the sinoatrial node would have increased, which would have amplified the transient reduction in right heart output measured by CO\textsubscript{Td}.

**Comparison of Transcutaneous Fluorescence Cardiac Output and Aortic Velocity**

In each experimental subject, cardiac output CO\textsubscript{ICG} varied in proportion with the peak velocity in the cross section of the ascending aorta VA\textsubscript{aor}. The proportionality between these measurements, which are based on different techniques (indicator dilution and Doppler effect), provides additional evidence of the validity of CO\textsubscript{ICG} as a measure of the central volumetric output of the heart. The proportionality coefficient between the two variables varied between experiments. Pulsed Doppler flowmetry yields the velocity in a small volume within the cross section of the ascending aorta. Because the average blood velocity in the vessel cross section is not readily available with this technique, we adjusted (range gate control) the location of that volume to produce the maximum signal intensity, corresponding to the maximum velocity. Because of the curvature of the ascending aorta, the velocity profile across its cross section changes from near flat at the aortic root to markedly skewed further up the ascending aorta and into the arch, with the highest velocities observed along the inner (caudal) wall.\textsuperscript{17} The slope of the regression line between CO\textsubscript{ICG} and VA\textsubscript{aor} varied between experiments, possibly because placement of the cuff flow probe could not be exactly replicated. However, for any single placement of the probe, cardiac output CO\textsubscript{ICG} was proportional to the aortic velocity.

**Stability of Transcutaneous ICG Fluorescence Intensity**

We showed in six animals that the regression line between transcutaneous ICG fluorescence measured *in vitro* and fluorescence of circulating blood samples measured *in vitro* remained constant throughout the approximately 3-h duration of an experiment. For a fixed position of the optical probe on the skin surface, the effectiveness of the optical probe at capturing the fluorescence emitted by the circulating ICG was stable in time. In addition, generation and attenuation of the fluorescence in the vascular volume under the probe remained unchanged. This was likely because local heating provided stable, maximal vasodilation of the local vasculature. Successful application of the transcutaneous fluorescence cardiac output measurement requires that the measurement site be well perfused and that local vaso-motor control by heating or pharmacologic means for the duration of the fluorescence dilution measurement and calibration procedure.

**Comparison with Pulse Dye Densitometry**

In recent years, PDD has been proposed as another technique for estimating cardiac output from transcutaneous optical measurement of ICG concentration in circulating blood.\textsuperscript{7,18} PDD measures the ratio of ICG to hemoglobin concentration from optical absorption measurements at two near-infrared wavelengths,\textsuperscript{19} whereas the fluorescence dilution technique requires a single wavelength of excitation. In PDD, the contribution of ICG to the light absorption signal must be extracted from that of the far more abundant circulating hemoglobin, which requires simplifying approximations about the propagation and absorption of light in tissue.\textsuperscript{19} By comparison, in the transcutaneous fluorescence dilution technique, ICG is the only source of fluorescence at the wavelength of the measurement. Our study shows that the fluorescence intensity measured at the skin surface can be expressed in terms of circulating ICG concentration using a simple algebraic relation. In addition, PDD only considers changes in optical absorption associated with the arterial pulse to derive the concentration of ICG in blood. We showed that the whole fluorescence intensity signal measured at the skin surface—not just its fluctuations with the arterial pulse—was proportional to the concentration of ICG in circulating arterial blood. This represents a technical advantage in terms of signal-
to-noise ratio in using the more intense fluorescence intensity over its weak pulse-related fluctuations.

**Clinical Significance**

The current standard for clinical assessment of the cardiac output—the thermodilution technique—is associated in a small percentage of cases with significant morbidity, including embolization, infection, and bleeding. Currently, a need exists for a minimally invasive technique, which would provide rapid, serial measurements in patients whose low risk does not justify the use of central monitoring of cardiac function with a pulmonary artery catheter. If the proposed technique can be successfully applied in human subjects, it could lead to the development of a practical device for the monitoring of cardiac output in minimally instrumented patients, especially the pediatric population. A critical step in this development will involve simplifying the technique for calibrating transcutaneous fluorescence intensity in terms of blood ICG concentration. We observed in the rabbit that a linear relation existed between fluorescence measured noninvasively at the skin surface and fluorescence measured in circulating blood. The intercept of the straight line was null because, in the absence of ICG in the bloodstream, the fluorescence signal was null, whether measured in a blood sample or through the skin. The slope of the linear relation could in principle be estimated from a single measurement by dividing the fluorescence at the skin surface by the fluorescence in a blood sample drawn at the time of the transcutaneous measurement. The blood could be obtained from a venous catheter because the calibration technique requires for the dye to be homogeneously mixed in the circulating blood at the time the blood is sampled. If this finding holds true in humans, a single blood sample may suffice to calibrate the transcutaneous fluorescence measurement in terms of blood ICG concentration.

Recent pilot experiments in our laboratory have shown that even when the fluorescence probe is positioned on vasodilated ear tissue away from the central artery, a well-defined fluorescence dilution trace is observed that can be calibrated in terms of circulating ICG concentration. Because this configuration is more practical for clinical application of the ICG fluorescence dilution technique, future studies will aim at validating fluorescence measurements obtained on arterialized tissue for computation of the cardiac output.

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