Reproducibility of Results Obtained with Indicator-Dilution Technique for Estimating Cardiac Output in Man

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Recent studies in our laboratory demonstrated strikingly different estimates of "central blood volume" by the indicator-dilution technique when two different arterial sites were sampled simultaneously. The cardiac output values, calculated from the simultaneous dilution curves recorded from the two different sites, often demonstrated unexpected discordant results. The present study was, therefore, undertaken to examine the reproducibility of the indicator-dilution estimate of cardiac output, utilizing simultaneous determinations from two sites.

Methods

Two hundred and seventy-eight simultaneous pairs of indocyanine-dilution cardiac output measurements using two balanced linear densitometers were obtained in 36 subjects. All subjects were young, healthy males, except for two hospitalized patients, one of whom had a femoral arteriovenous fistula, and the other had hypertension and a cerebral thrombosis.

The simultaneous dye-dilution cardiac output determinations were made in the following manner: A no. 6F Cournand catheter was introduced into the superior vena cava, and 1.5 to 2.0 ml. of indocyanine dye solution* (500 mg. per cent in aqueous diluent) were rapidly injected, using a calibrated syringe. The catheter was then flushed immediately by 10 ml. of normal saline, via a three-way stopcock. In three subjects, the dye was injected in similar fashion into a peripheral vein through an indwelling 18-gauge Cournand needle. The resultant dye curves were inscribed simultaneously at the arterial sampling sites by two linear cuvette densitometers† coupled to an Electronics for Medicine photographic recorder. In order to minimize calculation errors, the sensitivity of the densitometers was adjusted so as to give curves of maximum area that could be recorded on paper 17.6 cm. in width. The linear response of each instrument was verified using the method recommended by the manufacturer. The 95 per cent response time of densitometer A was 0.5 second. As an additional check on instrumental accuracy, densitometer A was compared with three different instruments† which had 95 per cent response times of 0.5, 0.5, and 0.9 second. This comparison of different instruments was prompted by the finding, not altogether unexpected, of a slight mean densitometer difference or bias in the various groups. Analysis of the total data from each densitometer, including the one with the slower response time, did not reveal significant or uniform differences in the results attributable to the instruments themselves.

Arterial blood was withdrawn from the indwelling needle through each densitometer by a constant-speed syringe at average flow rates of 0.35 to 0.45 ml. per second. Twelve centimeters of polyethylene tubing with a 1.1 mm. internal diameter connected the cuvette to the arterial needle. The total volume of the sampling tubing, needle, and cuvette was 0.5 ml. and was the same for each densitometer. Flow through the densitometers was carefully monitored, the average withdrawal flow determined for each dye curve, and in the majority of studies, each ml. of blood withdrawn was marked on the record as a further check on

*Cardio-green, Hyson, Waste & Dunning, Inc., Baltimore, Maryland.
†Gilford Instrument Laboratories, Inc., Oberlin, Ohio.

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constancy of flow through the cuvette. In a separate study, it was observed that a significant difference in area between two simultaneous dye curves recorded at the same site did not occur until the flow of blood through one densitometer was reduced below 0.20 ml/sec while maintaining withdrawal flow through the other at 0.40 ml/sec. or greater.

The densitometers were calibrated by passing blood with 1.0 mg. per cent and 1.5 mg. per cent of indocyanine dye through the densitometers connected in series. The dilutions were made by diluting 1.0 ml. and 1.5 ml. of the original indocyanine dye solution to 10 ml. with distilled water, and 0.5 ml. of each of the resulting dilutions was promptly added to 25 ml. of heparinized whole blood, in order to obtain the two desired concentrations for calibration. The instruments were first set on zero with undyed blood passing through the cuvette, and then the deflections produced by the 1.0 mg. per cent and the 1.5 mg. per cent dilutions, thoroughly mixed, were recorded. The two instruments, connected in series, were calibrated simultaneously from the same dilution. Calibrations were repeated 3 to 12 times, and the average deflection (with the 1.5 mg. per cent value proportionately corrected to a 1 mg. per cent dilution) was used as a calibration factor. The two different dilutions provided a constant check on the linearity of the instruments and, because of slight variations with each repetition, 6 to 24 calibration deflections were averaged to obtain the calibration factor. This factor was used to convert dye-cm-ve deflections into concentrations of dye in mg. per cent. Curves in which a recognized factor prevented proper recording (e.g., bubbles or poor flow through the cuvette) were excluded.

The cardiac output was calculated from the dye curves after semilogarithmic extrapolation of the downslope, according to the method of Kinsman, Moore, and Hamilton, using the formula:

\[
\text{Cardiac output (ml/min.)} = \frac{60 \cdot I}{\sum c(t)}
\]

I = mg. dye injected.
\(\sum c(t)\) = sum of dye concentrations in mg. per cent at one-second intervals from appearance time to extrapolated disappearance time.

The measurements and calculations were repeated by a second person. As a further check, the area under the curve was also determined by planimetry and the resulting cardiac output values did not differ significantly from those calculated using \(\sum c(t)\).

The simultaneous dye curves were inscribed at rest and during the following alterations in blood flow, in the upper and/or lower extremity:
1. Reactive hyperemia of the arm or both legs produced by occlusive cuffs maintained at 200 mm. Hg for five minutes and the cardiac output measurement made 30 seconds after release.
2. Three to 15 minutes after 2 mg. of atropine intravenously.
3. During infusion of 2 \(\mu\)g. per minute of isoproterenol.
4. During the Valsalva maneuver.
5. Ten minutes after ice packs were placed around both upper extremities.
6. During hyperventilation.
7. During leg or hand exercise.
8. With cuffs at arterial diastolic pressure on the right upper arm, forearm, or both thighs.

In order to determine the range of error due to instrumentation, 90 simultaneous cardiac output measurements in 11 subjects, designated as group I, were recorded with the two densitometers connected to the same no. 16 Cournand needle in the femoral artery, via a three-way connector (fig. 1). In effect, then, the two densitometers in this group may be considered to be sampling the same dyed blood. In another study, to separate the components of the error due to instrumentation, a single densitometer was connected to two amplifier channels, and in this manner, 38 dye curves were recorded in duplicate. Thus, the effect of differences in densitometer cuvette flow was eliminated in this subgroup, leaving only errors due to mensuration and calculation of the cardiac output from the dye curve.
Group I. The 90 cardiac output estimates obtained from one densitometer (Dens. A) compared with those obtained simultaneously from the other (Dens. B) from the same arterial site (same needle). The center line is the regression line of B on A, and the two heavy lines represent ±2 standard deviations from the regression line. These lines are used for purposes of illustration only since the method of analysis actually employed is based on differences between the simultaneous determinations. See text.

Group II includes 29 simultaneous cardiac output determinations in four subjects in which one densitometer was attached to a no. 18 Cournand needle in the right brachial artery and the other to a similar needle in the right radial artery, i.e., both densitometers may be considered to be sampling different segments of the same arterial stream (fig. 1).

In the remaining 159 simultaneous output determinations in 21 subjects, designated group III, one densitometer sampled from the brachial (144 studies) or radial artery (15 studies) and the other from the femoral artery (fig. 1), via no. 18 Cournand needles. In this group, therefore, the dye curves were recorded from divergent arterial sampling sites, i.e., different arterial streams. In all three groups the densitometers were alternated between the two sampling sites.

In all the calculations, the variance was corrected for densitometer difference or bias; thus, if \( d \) is the difference between a pair of readings on the two densitometers, its variance, for each group of \( n \) pairs of results, was estimated by:

\[
\frac{\sum d^2 - (\sum d)^2/n}{n-1}
\]

and the standard deviation by the square root of this quantity. The densitometer bias was small, varied slightly among the different groups, and can be seen graphically in figures 2 to 4 by the fact that the regression lines, used for illustrative purposes only, differ from a slope of 1 by a slight degree. (The regression lines are calculated on the assumption that densitometer A is not subject to measurement errors, an assumption that may well not be valid).

Results

In order to assess the reproducibility of the indicator-dilution cardiac output method, the values obtained by one densitometer were compared with those obtained simultaneously by the other instrument. In group I, if the cardiac outputs measured by the two densitometers are compared (90 paired determinations), the resulting differences give an indication of the range of error due to instrumentation, including all steps in calcu-
lation of the cardiac output from the dye curve. Graphically, the spread of the points appears to be approximately the same order of magnitude throughout the scale of cardiac output values (fig. 2). The range of differences between the simultaneous determinations in group I is characterized by two standard deviations equal to 430 ml./min. (6 per cent of the mean cardiac output). In the 38 instances in which a single densitometer was connected to two amplifiers, the resulting two standard deviations of the difference between the duplicate dye curves were slightly larger, 486 ml./min. Therefore, for practical purposes, the sole source of error in group I is due to mensuration and calculation of the cardiac output from the curve. The limits of this measurement error may be arbitrarily defined as ± 430 ml./min., i.e., the approximate 95 per cent confidence limits for the difference between simultaneous determinations in group I.

In group II, sampling for the 29 paired determinations was from different segments of the same arterial stream in the arm. Comparison of the cardiac output values obtained by one densitometer with those from the other (fig. 3) yielded an overall value of two standard deviations of the difference equal to 650 ml./min. or 8 per cent of the cardiac output.

In group III, consisting of 159 paired measurements, sampling was done from divergent sites. If the cardiac output, as determined by one instrument, is compared with that from the other, a much wider variation of the differences or scatter of the points than that observed in group I is evident (fig. 4). In contrast to group I, the spread of points in this group appears to be much greater at higher levels of cardiac output, i.e., seems best described by a per cent deviation. An overall value of two standard deviations of the difference equal to 1200 ml./min. or 20 per cent of the mean cardiac output was obtained. This increased disparity between the simultaneous cardiac output determinations of group III over group I is highly significant (P <0.001). Thirty-two per cent of the simultaneous cardiac output measurements in group III differed by amounts greater than the measurement error as defined by the 95 per cent confidence limits obtained for group I, and indicated graphically in figure 5. All except one of the 27 subjects in this group had at least one discrepancy between the simultaneous determinations which was greater than this measurement error. Careful review of all records and data failed to suggest any technical factors which might account for the differences, at times large, between the simultaneous cardiac output measurements. Figure 6 illustrates four paired indocyanine-dilution curves from group III.

A more detailed analysis of the data was performed, including an analysis of the variation of the differences (analysis of variance) among the simultaneous determinations within the same individual, among different subjects,
and in different states. Significant variations in the differences were not expected in group I where each densitometer sampled the same site. However, significant, although small, among-person (i.e., among different subjects) variation of these differences was found in the states of reactive hyperemia of the legs and arm ($P <0.001$ and $<0.05$, respectively); the reasons for this are not at all clear. In addition, the within-person variance of the differences for the higher cardiac output values following isoproterenol (Isuprel) administration was almost four times as large as that for the resting state. This presumably reflected a greater per cent error in the measurement of dye-curve deflections in the smaller dye curves (larger cardiac output) following isoproterenol than in the larger curves obtained at rest. Thus, the measurement error, which includes measurement of dye-curve deflections, tends to be larger, the higher the cardiac output.

In group III, the among-person and within-person variances of the differences are always greater than the comparable values for group I for all states (table 1).

This extra among-person variance is presumably due to the fact that the difference between the blood flow at the two sites varies from person to person. The extra within-person variance in group III presumably reflects fluctuations in relative blood flow to the arm and leg, so that repeated paired measurements on the same individual in the same state differ because of these fluctuations as well as measurement errors. The differences in variance are greater for the states of reactive hyperemia of the legs and arm.

The curves were analyzed in detail as to contour and by measurement of appearance time (AT), extrapolated disappearance time (ET) (arbitrarily taken as the time at which the extrapolated downslope crossed the 1-mm. line on the logarithmic plot), peak concentration time, mean transit time (MTT), and peak concentration (Cp). The extrapolated
disappearance time was used only as a gross indication of the length of the dye bolus at the sampling site. These parameters were examined for possible relationships to the discrepancies between the cardiac output values in group III. Of the 556 curves only eight pairs in one subject, following peripheral venous injection of dye, met Dow’s criteria for suspicious curves with respect to the accuracy of the logarithmic extrapolation in excluding recirculation of dye. The difference between the cardiac output values in these eight pairs of curves did not exceed the measurement error obtained from group I. Particular search was made for differences in the longitudinal dispersion of the dye bolus at the sampling sites (using both AT-ET and AT-MTT intervals), peak concentration of dye, and interrelationships between these two, as related to differences in the cardiac output. Large differences between these parameters of the simultaneous curves were frequently encountered but occurred almost as frequently with nondiscrepant (differences between ±430 ml./min.) as with discrepant cardiac output determinations.

For the 159 pairs of simultaneous dye curves in group III, the larger cardiac output values were associated with the faster (shorter AT and MTT) and slower (longer AT and MTT) curves with approximately equal frequency. In 13 pairs of the simultaneous dye curves in group III, a difference between the MTT of the two curves greater than six seconds was present. Six of these revealed discrepancies in cardiac output greater than the measurement error of group I. However, even with this large difference in MTT, the slower curve did not give a consistently larger or smaller cardiac output than the faster curve.

Of the various maneuvers employed in group III, reactive hyperemia of the arm and legs resulted in significantly greater discrepancies, both in magnitude and number, between the simultaneous determinations. Fifty-two per cent of the paired measurements with reactive hyperemia of the arm and 41 per cent of those with reactive hyperemia of the legs differed by an amount greater than the measurement error as previously defined. The relative magnitude of the discrepancies between the simultaneous determinations with the various maneuvers is indicated by a comparison of the standard deviations of the difference between the two values: resting, 398 ml./min. or 7 per cent of the mean cardiac output (68 paired values); reactive hyperemia of the arm, 536 ml./min. or 10 per cent (29 paired values); and reactive hyperemia of the legs, 776 ml./min. or 12 per cent (40 paired values).

When the cardiac output measured at the arm was compared with that obtained at the leg, the values were not consistently larger or smaller, either for the resting values or for those during reactive hyperemia. In the 15 instances in group III, in which one instrument was sampling from the radial artery and the other from the femoral artery, there was no significant increase in the range of differences between the simultaneous determinations over that obtained for the remainder of the group. Also, there was no consistent effect of sampling at the smaller artery on the cardiac output value.

To assess the influence of sampling site on the reproducibility of the dye-dilution car-

### TABLE 1

**Within-Person and Among-Person Variance of Difference of Groups I and III**

<table>
<thead>
<tr>
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<th>Arm hyperemia</th>
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<th>Isuprel</th>
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<tbody>
<tr>
<td>Within-person variance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>0.026 (44)*</td>
<td>0.016 (12)</td>
<td>0.014 (3)</td>
</tr>
<tr>
<td>Group III</td>
<td>0.122 (47)</td>
<td>0.588 (25)</td>
<td>0.184 (19)</td>
</tr>
<tr>
<td>Among-person variance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>0</td>
<td>0.040</td>
<td>0.084</td>
</tr>
<tr>
<td>Group III</td>
<td>0.038</td>
<td>0.150</td>
<td>0.134</td>
</tr>
</tbody>
</table>

*Degrees of freedom are shown in parentheses.
diac output measurement, variability between successive resting determinations at the brachial artery and femoral artery were compared. The standard deviation of the difference between 24 duplicate successive determinations at the brachial artery was 648 ml./min. (11 per cent of the mean cardiac output) and between 48 duplicate successive determinations at the femoral artery was 514 ml./min. (8 per cent of the mean cardiac output). The difference between these standard deviations at the two sites is not statistically significant.

Discussion
In the present study, it appears most unlikely that the larger discrepancies between the values for cardiac output obtained from divergent sampling sites (group III) can be accounted for solely by errors in instrumentation. The discrepancies between the simultaneous indicator-dilution cardiac output determinations from different sampling sites (group III) were considerably greater than those obtained from the same sampling site (group I). Calculation and instrumentation errors would be expected to occur with equal frequency and magnitude in both groups I and III. Therefore, by exclusion, nonuniform distribution of dye to the divergent sampling sites is implicated to account for this difference. Similarly, nonuniform distribution of dye of lesser degree in the different segments of the same arterial stream is also presumably a factor in the wider range of differences between the paired determinations of group II than of group I.

Postocclusive hyperemia, which alters relative blood flow to the extremities, and which might be expected to accentuate nonuniform distribution of dye, resulted in greater discrepancies, both in magnitude and number, between the simultaneous cardiac output determinations in group III. However, the cardiac output measured at the arm was not consistently larger or smaller than that obtained at the leg during reactive hyperemia of the arm or legs. That is, the effect of blood-flow alterations to the extremities on the differences between the simultaneous cardiac output estimations was not consistent or uniform. In addition, in group III, faster flow to the arterial sampling site, as indicated by the dye curve with the shorter appearance time and mean transit time, was not regularly associated with either the larger or smaller cardiac output value.

For group III, the pathways traveled by the dye bolus to the sampling sites are different only distal to the first branch of the arch of the aorta. Thus, peripheral arterial flow apparently has a marked effect on the result obtained with the indicator-dilution measurement of cardiac output. Detailed analysis of various parameters of the dye curves did not yield any suggestion as to the mechanisms producing the nonuniform distribution of dye to the sampling sites.

Considerable variability in contour between simultaneous brachial artery and femoral artery dye curves was also noted by Lange, Smith, and Hecht, who further stated that the brachial artery curve differed from the femoral artery curve in an unpredictable manner. They concluded that the femoral artery curve showed less distortion than the one from the brachial artery and more closely resembled the curve recorded at the root of the aorta. Cardiac output values were not reported by these authors.

In the present study, the variability between serial cardiac output determinations tended to be less when sampling from the femoral artery than from the brachial artery, but the difference is not statistically significant.

If the discrepancies between the simultaneously measured cardiac output determinations do in fact result from nonuniform distribution of dye, in addition to errors of instrumentation, such discrepancies would presumably also occur unpredictably in successive or any comparative dye-dilution cardiac output study. Therefore, in order to conclude that a difference in two successive cardiac output measurements represents a physiological change in cardiac output, for 95 per cent confidence, a change greater than 20 per cent in the dye-dilution measurement.
would have to be demonstrated. That is, individual differences between two dye-dilution cardiac output measurements of up to 20 per cent can be accounted for by non-uniform distribution of dye and instrumentation errors. The value of 20 per cent of the cardiac output, as defined in this study for 95 per cent confidence, may be looked upon as a measure of the reproducibility of the indicator-dilution method of cardiac output measurement, under a variety of different physiological circumstances. It would appear that the range of error may be smaller than this figure indicates if only measurements at rest are considered, and conversely, if regional blood flow alterations are produced, the value 20 per cent may be too small.

The reproducibility of the indicator-dilution cardiac output estimation, as demonstrated by serial determinations, has been included in a few reports. The variation of the differences between successive determinations in these reports have ranged from 11 to 17 per cent of the cardiac output, slightly larger than that in the present study, for both the simultaneous (7 per cent of the cardiac output) and serial determinations (8 per cent at the femoral artery and 11 per cent at the brachial artery). Serial determinations as a measure of reproducibility have the major disadvantage that unknown changes in cardiac output may occur.

The reproducibility of the dye-dilution estimate of cardiac output can be compared with the reported values for the Fick method only by considering serial resting determinations. The standard deviation of the difference between successive dye-dilution cardiac output estimates sampling at the femoral artery in the present study is 8 per cent of the mean cardiac output. For the Fick method, Thomason reported a standard deviation of the difference of 10.4 per cent of the mean cardiac output, and Richardson et al. obtained a similar value, 11.1 per cent.

From a practical standpoint, the data from this study give some indication of the magnitude of change in cardiac output detectable by the dye-dilution method. If regional blood flow alterations are induced, the indicator-dilution measurement of cardiac output is subject to greater error when sampling is from a peripheral artery. Sampling from the root of the aorta theoretically would obviate the influence of peripheral blood-flow alterations. However, preliminary observations in this laboratory suggest that additional errors may be introduced by the increase in volume of the system when sampling is via a long catheter whose tip is placed at the root of the aorta. Possibly, a sensing device at the catheter tip and placed in the aortic root, by avoiding the effects of peripheral blood flow, might yield more reproducible cardiac output values.

When the indicator-dilution method of cardiac output estimation is considered from the viewpoint of the multiple variables and sources of error, actual and potential, it may indeed seem surprising that the method is as reproducible as the present study would indicate.

**Summary**

The reproducibility of the indicator-dilution estimation of cardiac output was assessed by evaluating pairs of indicator-dilution curves recorded simultaneously from normal subjects and patients by two similar linear densitometers. Measurement error was defined by comparison of 90 simultaneous measurements obtained from the same needle; two standard deviations of the difference equaled 430 ml./min. (6 per cent of the mean cardiac output). A much larger variation resulted when 159 simultaneous cardiac output determinations, sampling from the brachial and femoral arteries, were compared (two standard deviations of the difference equaled 1,200 ml./min. or 20 per cent of the mean cardiac output). Intermediate values were obtained in a comparison of 29 simultaneous measurements from the radial and brachial arteries of the same arm. The larger deviations between the simultaneous measurements from different arteries suggested non-uniform distribution of dye. The discrepancies between the paired cardiac output determinations were larger and more frequent when peripheral blood flow...
flow alterations were produced. However, the nature of dye-distribution abnormalities and the mechanisms whereby peripheral arterial flow influence the dye-dilution cardiac output measurement were not evident.

Acknowledgment
The authors wish to express their gratitude for the helpful criticism and advice of Dr. Eugene A. Stead.

References

Book Reviews

The appearance of the fourth revision of this book marks the 20th anniversary of its first edition. Many medical students, interns, and residents have depended on this book for complete coverage of clinical hematology, particularly the abnormalities in the erythrocytes and leukocytes. Like previous editions, this one has excellent figures, tables, and bibliography, and the text material has been written clearly and in a logical sequence.

The omission of the use of anticoagulants is conspicuous. Hemostasis and coagulation mechanisms are covered in 12 pages. Dicumarol and heparin are included in this limited section, and there is no mention of the other anticoagulants. It is the impression of this reviewer that the topic of anticoagulants should be expanded. If this is done, then all aspects of clinical hematology will be adequately covered.

Many investigators interested in cardiac catheterization will welcome this monograph, which is a summary of the authors' experiences with transbronchial left-heart catheterization. The technique is completely outlined and sample tracings from patients with mitral and/or aortic valvular lesions are included.

For continuity, some readers would probably wish to have a comparison of left atrial and pulmonary arterial wedged pressures. Most of the earlier estimations of the status of the left atrium in patients were derived from recordings of wedged pressure. It will be interesting to await the next edition, when the experience of the authors will include more patients, so that the sections on pathological physiology can be expanded.
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