# Mixing in the Right Ventricle and Pulmonary Artery in Man: Evaluation of Ventricular Volume Measurements from Indicator Washout Curves

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A B S T R A C T  To assess the mixing characteristics of the right ventricle and pulmonary artery, radioiodinated ^{131}I serum albumin and indocyanine green dye were injected simultaneously in 16 subjects. One indicator was injected into the atrium and the other into the ventricle, or both were injected at different sites in the ventricle. Washout curves were obtained by rapid catheter sampling alternately just above or just below the pulmonic valve. The washout of radioisotope was also recorded with a precordial scintillation detection probe.

Indicator washout from the ventricular inflow tract was rapid, while washout from the region of the ventricular apex was quite slow. Protosystolic dips in indicator concentration, noted in curves drawn below the pulmonic valve, suggest that the ventricle emptied sequentially. Flow values computed from curves sampled below the valve, when compared with reference values, suggest that a significant volume of atrial blood passed through the ventricle without mixing, or mixing to only a small extent, with the residual volume of the chamber. Peak concentration of indicator was higher below the pulmonic valve than above. This finding, plus the close agreement between flow values computed from curves sampled above the valve and the reference values, indicates that further mixing occurred above the valve.

Ventricular volumes computed from washout downslopes are systematically falsely high. This overestimate appeared to be larger in normal subjects than in patients with low stroke volumes. Progressive mixing of blood leaving the atrium occurs during its passage through the ventricle, pulmonic valve, and pulmonary artery and permits accurate estimation of flow.

## INTRODUCTION

Indicator washout curves have been used extensively to estimate the volume of the right and left ventricles (1–9) since the studies of Bing, Heimbecker, and Falholt (10) and of Holt (11). Although the existence of homogeneous ventricular mixing is a prerequisite condition for the valid application of this technique, only a few descriptions of ventricular mixing have been reported. While the available information indicates that mixing does not appear to be homogeneous (12–15), the significance of this observation with respect to estimations of ventricular volume has not been characterized adequately. Volume may still be estimated with validity in the absence of perfect mixing if the inhomogeneities are randomly distributed (16); if systematic regional differences in mixing do occur in the ventricle, however, estimates of volume will be systematically in error.

Therefore, we performed the present study to examine the conditions of mixing within the ventricle with particular emphasis on the possible occurrence of systematic regional inhomogeneities

and on the conditions of mixing beyond the semilunar valves (since washout curves of the indicator are usually recorded at this site). The problem was approached by studying the influence of different injection and sampling sites on the ventricular indicator washout curve. Two indicators (a dye and a radioactive gamma-emitting tracer) were injected simultaneously, one into the right atrium and the other into the right ventricle, or both into the ventricle at different sites. We obtained their time-concentration curves by rapid catheter sampling alternately just distal or just proximal to the pulmonic valve. The ventricular washout curve of the gamma-emitting indicator was also recorded, at the same time, with a precordial scintillation detection probe selectively collimated over the right ventricle.

Our findings indicate that an appreciable amount of blood entering the ventricle during diastole does not mix, or mixes only partially, with the residual volume and is ejected from the chamber during early systole. Blood located at the apex of the ventricle is ejected in late systole. A considerable degree of further mixing occurs beyond the pulmonic valve.

## METHODS

### Patients

16 patients were selected for this study. Six had no cardiovascular abnormalities; six had chronic bronchitis and emphysema: of these, two had cor pulmonale with moderate cardiac enlargement, three had atherosclerotic heart disease with degrees of cardiac enlargement varying from slight to marked, and one had rheumatic heart disease and had undergone mitral commissurotomy 1 yr before the study.

### Protocol

The subjects were studied in the nonsedated, postabsorptive, basal state, having received only Lugol's solution in preparation for the procedure. A stethograph was placed around the chest to record the respiratory cycle, and the projection of the right ventricle on the anterior chest wall was determined fluoroscopically for collimation of the scintillation detection probe. A 7 F thin-wall Goodale-Lubin catheter was inserted through a right antecubital vein into the pulmonary artery for sampling purposes, and a 9 F Cournand double-lumen catheter was passed through the left arm to the right ventricular apex for injection of the two indicators.

The injection catheter was then withdrawn about 1 cm from the apex so that a satisfactory pressure tracing could be obtained from the distal lumen; in some cases it

had to be withdrawn further into the low or mid-ventricle because a rapid injection of saline into the distal lumen produced a premature ventricular contraction. The orifice of the proximal lumen was located on the concavity of the catheter's curve and lay in the high or mid-atrium or in the ventricular inflow tract at the level of the tricuspid valve when the distance between the two terminal openings was respectively 12, 8, and 4 cm. In two subjects two 5 F NIH catheters were used instead of the double-lumen. One was placed in the ventricular inflow and the other in the low or in the mid-ventricle. The sampling catheter was next withdrawn so that its tip lay just above the pulmonic valve in 12 patients and just below the valve in 4.

When the catheters were in the desired positions the precordial probe was collimated over the right ventricle, each lumen was preloaded with one of the indicators, and the sampling catheter was connected to the sampling-detecting system. Indicator dilution curves were obtained by flushing both lumens simultaneously with saline. Each series of curves consisted of the dye and radioisotope curves obtained by catheter sampling and the precordial activity recorded by the gamma detector probe. Successive series of curves were obtained at 6–10-min intervals at various combinations of injection and sampling sites as indicated in Fig. 8.

### Injection technique

The double-lumen catheters were 100 cm long; the volume of each lumen ranged from 0.7 to 0.8 ml. The NIH catheters were 100 cm long and had a volume of 0.6 ml. For each set of injections about 100 $\mu$c of radioiodinated $^{131}$I serum albumin (RISA) (Abbott Laboratories, North Chicago, Ill.) and 1.2 mg of indocyanine green dye (Cardio-Green, Hynson, Westcott & Dunning, Baltimore, Md.) were diluted up to 0.5 ml and preloaded in the two lumens by means of two semiautomatic calibrated syringes. Serum albumin was added to the dye solution in an amount of 0.1 g/mg of dye. The injectate was delivered by flushing the lumens with 1.5 ml of saline. In 10 patients the lumens were flushed manually with two identical 2-ml calibrated syringes set to deliver 1.5 ml. The onset and the duration of the injection was recorded by depressing a foot-switch; the duration varied between 0.5 and 0.7 sec. No attempt was made to synchronize the injections with any event of the cardiac cycle so that the timing of the manual injection was completely random. In five patients two automatic injectors (Sage Instruments, Inc.) were used for flushing. They were actuated by a variable delay circuit (Electronics for Medicine, Inc., White Plains, N. Y.) triggered from the electrocardiogram. With the catheters used, 1.5 ml was delivered in 0.4 sec by both injectors. The delay circuit was adjusted to deliver the injectate at the beginning of diastole. In three instances, however, the injection had to be set at mid-systole because of the systematic occurrence of premature ventricular contractions with early diastolic injection. Only in one instance did an extrasystole occur at the time of injection.

## Sampling-detecting technique

The sampling catheter had two lateral holes within 5 mm of its open tip. In eight patients it was 80 cm long and the volume was 1.6 ml, and in the others it was 100 cm long with a volume of 2 ml.

Before each pair of injections the sampling catheter was connected in series to a flow-through scintillation detection probe (FTP), a densitometer, and a sampling syringe. This system, developed earlier for the continuous, simultaneous detection of two indicators (17), comprised a photomultiplier (Tracerlab DRL 2) and a 1.5 × 1.5 inch NaI (Tl) crystal (The Harshaw Chemical Co., Cleveland, Ohio) with a 1/8 inch cylindrical hole through its center. The pulses from the photomultiplier were fed to one channel of a Dual Rate Computer (Picker Nuclear, North Haven, Conn.). This instrument integrated the counts during each 0.1-sec interval and provided an analogue output that was displayed and photographed on a DR8 recorder (Electronics for Medicine). The densitometer was a Gilford Model 103 IR. Dye time-concentration curves were corrected for the time constant of the densitometer (0.14 sec) by inserting a resistance capacitance combination between two dc amplifiers of the recorder (18). We sampled blood manually into a 50-ml syringe, at a rate of 2.5 ml/sec (timed with a metronome). The volume of blood collected in the syringe during the sampling period matched the amount calculated on the basis of the sampling rate and duration of sampling estimated from the photographic record in all cases in which it was checked.

The distortion introduced by the sampling-detecting system on a practically instantaneous input imposed at the tip of the sampling catheter is rather small, and identical for the two indicators (17).

We calibrated all RISA curves by drawing blood through the FTP while recording the counting rate 6 min after each injection; we determined the activity of the injectate and of the blood samples in a well counter. The dye curves were individually calibrated with the pooled sample method (19) in five patients. No calibrations were obtained in the others. All blood samples, together with the standard, were processed and read in a Beckman DU Spectrophotometer within 90 min of their collection.

## Technique for obtaining precordial curves

The precordial scintillation detection probe consisted of a photomultiplier with a 2 × 2 inch NaI (Tl) crystal (Omniprobe, Picker Nuclear) located in a lead collimator with a straight bore of the same diameter. The crystal surface was recessed 20 cm from the external opening of the collimating channel. In order to further increase the selectivity of the collimation, we divided the collimating channel into four equal segments by introducing a longitudinal cross, just in front of the crystal, made from two strips of lead 2 inches wide, 5 inches long, and 1.5 mm thick. The contribution of scattered radiation to the counting rate was reduced by interposing a 1-mm thick lead disk between the crystal and the lead cross.

The pulses from the photomultiplier were fed to the second channel of the Dual Rate Computer, the output of which was recorded on the DR8 recorder.

The precordial counting rate, with this collimation, began to rise only 3–4 beats after an injection of RISA into the pulmonary artery (when the indicator arrived at the left heart). Hence, the contribution of extracardiac activity to the precordial counting rate was negligible during the first 3–4 heart cycles after injection. Because the collimator was relatively wide (5 cm diameter), despite its selectivity, the precordial counting rate was influenced in a compound fashion by changes in concentration at a constant ventricular volume and by changes in volume while the concentration of RISA remained constant.

## Calculations

*Catheter-sampling curves.* The time-concentration curves of dye and of RISA were plotted on semilogarithmic paper and shifted back along the time axis by a time equal to the mean transit time between the tip of the catheter and the corresponding detecting device for an immediate, direct comparison. For a quantitative evaluation of the observed differences in peak-concentration of the washout curves the following ratio was calculated: $I/C_p$ = DVa (where I is the injectate and DVa is the apparent dilution volume in which the injectate would be distributed at a uniform concentration equal to $C_p$). Only when the injectate is evenly distributed in the end-diastolic volume does DVa correspond to that volume.

Flow values ($\dot{Q}$) were calculated from these curves by the Stewart-Hamilton formula in order to evaluate the importance of the observed differences in area under the simultaneous time-concentration curves.

*Precordial Curves.* Cardiac output was calculated from the precordial curves according to the radiocardiographic method of Donato et al. (20–21). In each subject the value of cardiac output ($\dot{Q}_{ref}$) and of stroke volume ($SV_{ref}$), calculated from the first radiocardiogram (RCG) obtained after injection of RISA into the atrium or into the ventricular inflow, was used for reference.

In the curves obtained after atrial systolic or protodiastolic injection, or ventricular inflow diastolic injection, the fraction of indicator ejected during the first systole after injection appeared to be much larger than that ejected during the successive ones. In order to evaluate this difference, end-diastolic volume ($EDV_{RCG}$) was calculated according to the method of Donato et al. (20–21): $EDV_{RCG} = SV_{ref}/R_i$, where $R_i$ is the average fraction of indicator ejected per beat. $R_i$ in turn is calculated as $1 - K$ where $K$ is the average of the ratios $M_3/M_2$ and $M_4/M_3$ ($M_2$, $M_3$, $M_4$, being the average precordial counting rates during the second, third, and fourth cycle after the injection). For the first and second heart cycles, $K$ was obtained as the ratio between the precordial counting rate during a 0.3-sec interval at the end of the cycle after the first ejection and the counting rate during the corresponding period at the end of the cycle in which the injection was made. $EDV_{RCG}$ also corresponds to the

true end-diastolic volume only in the absence of systematic regional differences in mixing within the ventricle.

## RESULTS

Representative records of a set of curves sampled respectively below and above the pulmonic valve, together with the simultaneous precordial curve, are illustrated in Figs. 1 and 2. The washout of indicator from the ventricular inflow tract appeared to be very rapid. When RISA was injected into the atrium during systole or in early diastole, the precordial counting rate rose rapidly at the beginning of the next diastole (when the indicator entered the ventricle), reached a plateau, and presented a very marked decrease with the first ejection (Figs. 1 and 2). The $EDV_{RCG}$ calculated from the fraction ejected with the first systole was 26% smaller (range $-21$ to $-33\%$) than the $EDV_{RCG}$ calculated from the fraction ejected during the second and third beat (Fig. 3), which indicated that under these conditions of injection the largest percentile fraction of the indicator left the ventricle with the first ejection. The precordial curve showed the same behavior when RISA was injected into the ventricular inflow
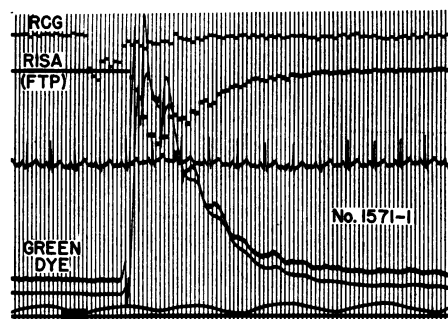


FIGURE 1  Comparison of radioiodinated $^{131}$I serum albumin ($RISA$)/indocyanine green dye curves sampled proximal to the pulmonic valve. RISA was injected manually into the atrium, dye into the low ventricle. From the bottom to top: timing and duration of manual injection; stethographic representation of respiratory cycle (inspiration up); dye curves (continuous, displaced upwards); RISA flow through scintillation detection probe ($FTP$) curve (discontinuous, displaced downwards); precordial RISA curve (discontinuous, displaced downwards). The time lines are 0.1 sec apart. Of note are the dips in dye concentration which are found to occur early in systole during the second, third, and fourth heart cycles after the curve has been "corrected" for the mean transit time between the tip of the sampling catheter and the densitometer. RCG, radiocardiogram.
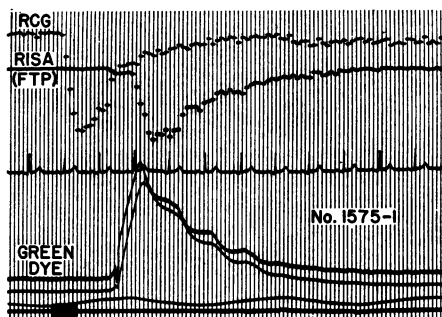


FIGURE 2  Comparison of RISA/indocyanine green dye curves sampled distal to the pulmonic valve. Dye was injected into the mid-ventricle, RISA into the atrium. The tracings are disposed in the same fashion as in Fig. 2. Only a small amount of RISA enters the ventricle before closure of the tricuspid valve (manual injection), and a small fraction is ejected into the pulmonary artery. The precordial counting rate is maximal at the beginning of diastole. The largest percentile decrease of the precordial counting rate occurs during the next diastole. Note the staircase decrease of indicator concentration obtained by catheter sampling and the absence of early systolic dips.

during diastole. In similar fashion, the descending limb of curves obtained under these conditions by catheter sampling proximal to the pulmonic valve was steeper during the first beat after the peak concentration had been attained than it was in the
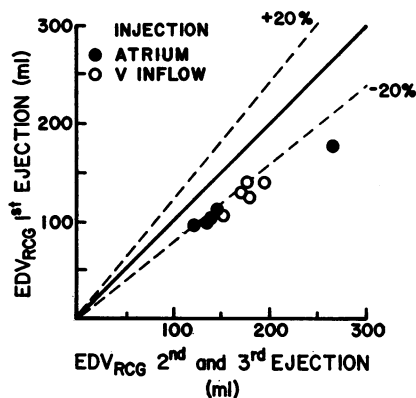


FIGURE 3  Comparison of $EDV_{RCG}$ calculated from the fractional decrease in RISA concentration during the first heart cycle after atrial systolic or protodiastolic (closed circles) and ventricular ($V$) inflow diastolic (open circles) injection, on the ordinate, with $EDV_{RCG}$ calculated from the fractional decrease in RISA concentration during the second and third heart cycle after the same injection, on the abscissa. The values obtained from the fraction ejected during the first cycle are systematically lower.

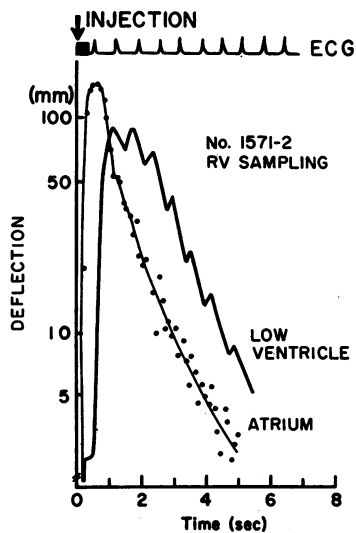*Right Ventricular and Pulmonary Artery Mixing*    851

FIGURE 4 Comparison of time concentration curves sampled proximal to the pulmonic valve after simultaneous manual injection of RISA into the atrium and green dye into the low ventricle. Indicator concentration, in millimeter paper deflection, plotted logarithmically on the ordinate; time in seconds on the abscissa. The largest percentile decrease in RISA concentration occurs during the first heart cycle after injection. The dye curve reaches its peak only in the third cycle and displays protosystolic dips in concentration. RV, right ventricle.

lower part of the curve (Fig. 4). This phenomenon was never observed after mid, low, or apex ventricular injection. The washout of indicator from the apex of the ventricle, in contrast, was particularly slow (Figs. 4, 5, and 6).

The site of sampling markedly affected the peak concentration of the washout curves obtained by catheter sampling, but had no appreciable influence on the washout slope after the first two or three beats. During this initial period, curves sampled beyond the pulmonic valve showed a more rounded peak. The descending limb showed the same rate of decrease, however, although at a lower concentration. Regardless of the site of injection, the peak concentrations of the curves sampled distal to the pulmonic valve were much lower than those of the curves sampled proximal to it within a few minutes, the injectate and the site of injection being unchanged. In five subjects in whom curves were obtained in succession from the pulmonary artery and the ventricular outflow, the values of DVa calculated from the curves sampled below the pulmonic valve were significantly smaller (−48% average, range −25 to
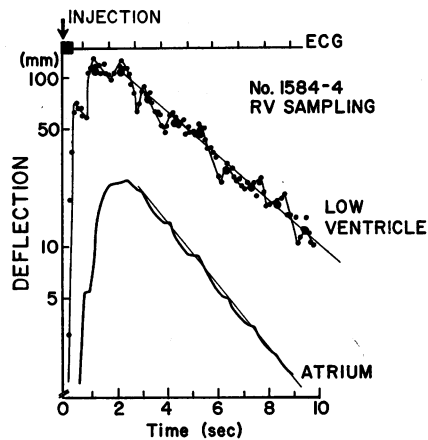


FIGURE 5 Comparison of time concentration curves sampled proximal to the pulmonic valve. In this case RISA was injected automatically in protodiastole into the low ventricle and the dye into the high atrium. The ordinate and abscissa are the same as in Fig. 4. The closed circles on the RISA curve represent the average deflection per heart cycle. The descending limbs of both curves are practically rectilinear after the third heart cycle, but the slope of the atrial curve is appreciably steeper. The RISA curve displays protosystolic dips in concentration on this occasion.

−80%) than those calculated from the curves sampled above the valve (Fig. 7).

The area under the washout curves sampled proximal to the pulmonic valve after ventricular injection was systematically larger than that of the
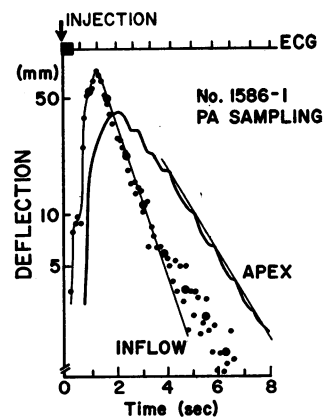


FIGURE 6 Comparison of time concentration curves sampled distal to the pulmonic valve. The ordinate and abscissa are the same as in Fig. 4. RISA was injected into the ventricular inflow, dye into the apex. The washout of indicator from the apex is significantly slower than the washout of indicator from the inflow. The inflow washout becomes slower only after the fifth heart cycle. PA, pulmonary artery.
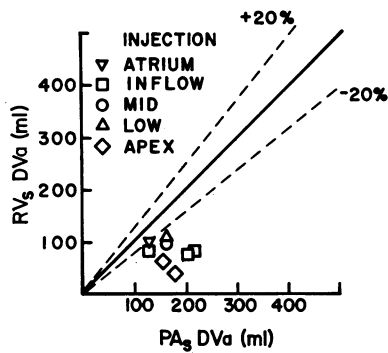
FIGURE 7 Comparison of apparent dilution volume ($DVa$) obtained from curves sampled beyond the pulmonic valve ($PA_s$), on the abscissa, with DVa obtained with ventricular sampling ($RV_s$), on the ordinate. The former values are much larger than those obtained in the same subject with ventricular sampling.

curves sampled beyond the valve. The significance of these differences in area can be better appreciated by comparing the flow values calculated from these curves by the Stewart-Hamilton formula with those obtained with the reference method (Fig. 8). While atrial injection with pulmonary artery sampling yielded cardiac output

values very close to those obtained with the reference technique, $E_s = 6\%$ (standard error of estimate) (Fig. 8 A), ventricular injection with ventricular outflow sampling yielded lower values in all instances (− 53% on the average, range − 20 to − 75%). This underestimate was small in patients with enlarged hearts and low reference cardiac output, but was quite large in patients whose heart size and reference flow values were normal. In all cases the discrepancy was greater after apex or low ventricular injection (Fig. 8 D). When the sampling site was above the pulmonic valve, however, ventricular injection gave cardiac output values significantly lower than the reference method in only 4 instances out of 21 (Fig. 8 C). In three of these curves the indicator was injected into the apex and in one into the mid-ventricle. Atrial injection with ventricular outflow sampling yielded cardiac output values in agreement with those of the reference method in 6 curves out of 7 (Fig. 8 B).

The morphology of the descending limb of curves obtained by catheter sampling was influenced both by the site of sampling and by that of
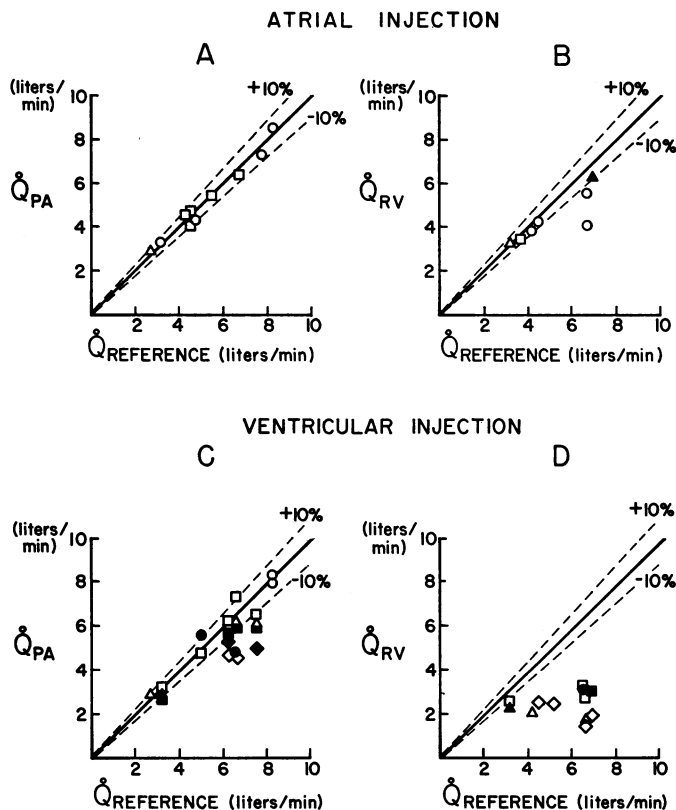


FIGURE 8 Comparison of flow values obtained by catheter sampling of the indicator, on the ordinate, with those obtained by the reference technique, on the abscissa. Injection sites: atrial— □ = high, ○ = mid, △ = low; ventricular—□ = inflow, ○ = mid, △ = low, ◇ = apex; open symbols = RISA; closed symbols = dye. A, Atrial injection and sampling in the pulmonary artery. Agreement is good. B, Atrial injection and ventricular sampling. Agreement is good in six of seven cases. C, Ventricular injection and pulmonary artery sampling. The catheter sampled curves are lower than the reference in only four instances: one case of mid-ventricular injection, and all three cases of apical injection. D, Ventricular injection and ventricular sampling. The values calculated from catheter sampled curves are markedly lower than the reference. The underestimate increases considerably with the level of flow. Apex and low ventricular injection appear to yield the lowest values.

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injection. The curves sampled proximal to the pulmonic valve showed a "staircase" decrease with each diastole, more evident at slow heart rates. In most curves the diastolic "staircase" decrease was systematically preceded by a "dip" in early systole. This pattern was observed with both indicators (Figs. 1, 4, and 5). It was more evident in the 6 curves drawn after injection into the apex, but was also apparent in 5 of 6 curves after injection into the mid or low ventricle, in 2 of 4 curves after injection into the ventricular inflow, and in 3 of 11 curves after atrial injection. Sampling beyond the pulmonic valve also resulted in a "staircase" decrease of indicator concentration with each systole, more evident at slow heart rates (Fig. 2). However, at this site no protosystolic "dip" was noted.

## DISCUSSION

### Mixing in the ventricle and in the pulmonary artery

Our data confirm the existence of systematic regional differences in blood turnover rate in the ventricle with striking uniformity. They indicate, as well, that the ventricle empties in a sequential fashion and that further mixing occurs in the pulmonary artery beyond the pulmonic valve.

### A. REGIONAL DIFFERENCES IN BLOOD TURNOVER RATE IN THE VENTRICLE

The observation that the largest percentile fraction of indicator injected into the ventricular inflow during diastole is ejected from the ventricle with the first systole (Fig. 3) indicates that the blood in this region has a rapid turnover rate. Since the same behavior is observed after either atrial systolic or early diastolic injection (Figs. 1–4) one can infer that an appreciable portion of atrial blood does not mix evenly with the residual volume during diastole, remains predominantly in the inflow tract, and is ejected with the first systole.

At the other extreme, the uniform observation of a slower washout of indicator from the apex, consistent with the findings of Scarpa-Smith (14) and of Carleton, Bowyer, and Graettinger (15) in the left ventricle, indicates that the turnover rate of blood at the ventricular apex is slow. Unless the bulk of the indicator is injected into this region, however, its turnover rate does not

appear to determine the slope of the ventricular washout curve until very late in its course, if ever. The descending limbs of the washout curves were reasonably rectilinear on the semilogarithmic plot, after the first few beats, and their slopes did not differ significantly after atrial, ventricular inflow, or mid-ventricular injection. These findings are reasonable if one considers that during the beat after injection the indicator in the ventricle is in the residual volume and (unless it were specifically injected in a peripheral part such as the apex) it would behave approximately in the same way regardless of the site of injection. On the other hand this linearity can not represent evidence of homogeneous ventricular mixing. In fact, regardless of the site of injection, the concentration of indicator in the regions close to the inflow tract must be very close to zero after the first ejection, and the slope of the ventricular washout curve must then be determined only by changes in concentration of the indicator in those regions with slower turnover rates. The observed slope of our curves therefore repesents the washout of indicator from a region that has a blood turnover intermediate between that of the apex and of the inflow tract.

Some quantitative estimate of the amount of atrial blood which passes through the ventricle without mixing, or mixing only partially, with the residual volume can be derived from the analysis of the washout curves sampled in the ventricle after ventricular injection. When sampling is performed at a constant rate in the presence of pulsatile flow, the concentration of indicator has to be constant during each cycle to permit a correct estimate of flow by the indicator dilution principle (22). While this condition may be met in the pulmonary artery where the concentration may be considered constant, as a first approximation, during each heart cycle, it is not met in the ventricle. It can easily be seen, however, that, in the presence of homogeneous mixing, ventricular sampling would yield a small area and a falsely high value of cardiac output when the indicator is injected into the ventricle at the end of diastole or during systole, but never a large area and a falsely low value of cardiac output even after protodiastolic injection (Fig. 9). The large underestimate of cardiac output observed in those curves sampled proximal to the pulmonic valve
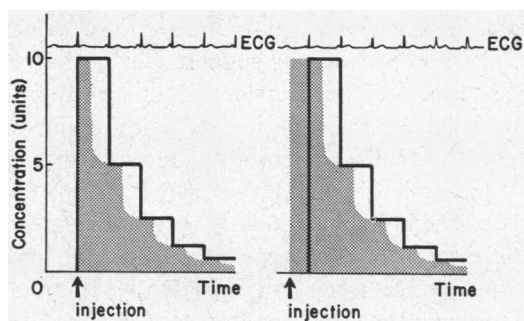
FIGURE 9 A comparison of hypothetical time concentration curves of indicator recorded in the pulmonary artery and in the ventricle after an instantaneous ventricular injection marked by the arrow. It is assumed that mixing in the ventricle is homogeneous, and that no further mixing occurs in the pulmonary artery. Injection is at the end of diastole on the left, and at the beginning of diastole on the right. The area under the curve recorded in the pulmonary artery (enclosed by the continuous line) is independent of the timing of the injection, while the area under the curve recorded in the ventricle (shaded) is not. This latter area can be smaller than that under the curve recorded in the pulmonary artery but never significantly larger.

after ventricular injection (Fig. 8 D) indicates that a large amount of blood containing little or no indicator passes systematically through the ventricular outlet during a fraction of systole. Therefore, the amount of blood passing through the ventricle without mixing, or mixing only partially, with the residual volume is quite large. Since the underestimate was much larger in subjects with higher flow and normal hearts than in patients with enlarged hearts and low flow, the amount of atrial blood traversing the ventricle without mixing seems to be directly related to the flow and inversely related to the ventricular volume. This does not imply that mixing is more homogeneous in the cavity as a whole in large ventricles with small stroke volumes, but only indicates that under these circumstances the indicator is better mixed with blood at the ventricular outlet. On the other hand, as would be predicted from the behavior of atrial blood postulated above, flow values calculated from curves sampled in the ventricle after atrial injection are not systematically lower than those obtained with the reference method (Fig. 8 C).

## B. Sequential Ventricular Emptying

The finding of protosystolic dips in concentration on the downslope of curves sampled proximal to the pulmonic valve suggests that the ventricle empties in a sequential fashion: atrial blood, which passes through the ventricle without mixing, is ejected systematically in early systole; blood from the apex is ejected in late systole. That these dips represent real changes in concentration is demonstrated by their constancy and by their presence either in both curves or only in one, depending on the site of injection. These changes in concentration, which occur in a very short time, must be relatively large not to be completely obscured by the catheter-sampling technique. This phenomenon was first noted by Swan and Beck (13) in 16% of the washout curves recorded by sampling at a very high speed above the aortic valve, after ventricular injection, in dogs. These authors suggested that the "dips" were due to a very low concentration of indicator in the blood ejected at the beginning of systole. According to our findings it appears likely that it is the blood in the inflow tract, where the concentration of the indicator decreases very rapidly, which is ejected in early systole. Further, the fact that the "dips" were more evident after injection into the apex indicates that blood from the apical region is ejected in late systole. The absence, in some cases, of dips after atrial or tricuspid injection is probably due to the fact that the changes in concentration were too small or took place too rapidly to be detected by our catheter-sampling system. The fact that Swan and Beck (13) noted the dips in only 16% of their curves is likely due to the distance of their sampling catheter from the valve (further mixing at this site preventing detection of the phenomenon). The absence of dips in our pulmonary artery curves is probably ascribable to the same cause, as well as to the characteristics of our catheter-sampling system.

The extremely low values of DVa, in two instances even lower than the stroke volume, which were obtained after low ventricular and apical injection (despite the fact that the peak concentration was reached only two or three beats after the injection), are consistent with the concept that the blood from the apex and low ventricle is ejected in the last part of systole. It would be hard to account for the finding of these very high peak concentrations two or three beats after the injection without assuming sequential regional emptying of the ventricle.

### C. Further Mixing above the Pulmonic Valve

The large increase in DVa which occurred when the point of sampling was moved from the ventricle to the pulmonary artery (Fig. 7) indicates that a significant admixture of the stroke volume with blood already present in the pulmonary artery takes place. This conclusion is supported by the closer agreement between flow values obtained by catheter sampling after ventricular injection and the reference values which occur under the same circumstances (Fig. 8 C). Since the descending limb of curves sampled distal to the valve showed the same slope, after the first two or three beats, as the curves sampled in the ventricle, the volume of blood above the valve that is involved in mixing must be small in comparison with its turnover rate, which, in turn, is evidently far more rapid than that of the ventricular regions that determine the slope of the washout curve.

These findings are consistent with the observation of Freis and Heath (23) and of Swan, Knoop, and David (24), that a considerable degree of mixing occurs beyond the aortic valve. However, according to the findings of Freis and Heath (23), Swan and Beck (13), and Rhode, Kines, and Holt (25), as well as the present study, mixing is also not homogeneous above the semilunar valves. The significant underestimation of flow observed in the three curves sampled in the pulmonary artery after apex injection may indicate that blood with a high concentration of indicator coming from the apical region, ejected in late systole, does not mix completely with the blood ejected in the first part of systole and remains just above the valve until the next ejection.

The agreement with the reference method observed in curves sampled from the pulmonary artery after atrial injection, similar to that found in previous comparisons between the radiocardiographic method and systemic arterial indicator dilution methods (26, 27) as well as the Fick principle (4), is consistent with previous reports by Fritts et al. (28, 29), Fox and Wood (30), and Rochester, Durand, Parker, Fritts, and Harvey (31). These authors also found a close agreement between flow values calculated from curves sampled in the pulmonary artery after atrial injection and those obtained with a reference technique. In agreement with independent evidence (32) these observations suggest that an indicator injected into the right atrium is sufficiently mixed with blood, by the time it reaches the pulmonary artery, to yield a correct estimate of cardiac output. They also show that flow can be estimated by indicator dilution methods with reasonable accuracy, even in the absence of a localized perfect mixing site between the injection and sampling points, provided that the indicator attains a sufficiently homogeneous dispersion in blood by the time it reaches the sampling point (33).

A description of the sequence of events which normally occurs during the heart cycle may facilitate evaluation of the results of this study. Atrial blood entering the ventricle at the opening of the tricuspid valve has a greater opportunity and a longer time available for mixing with the postsystolic residual blood than does blood entering during atrial systole. Blood in the central portions of the ventricle has a greater opportunity for mixing with atrial blood than does blood at the apex of the chamber. Furthermore, considering the respective positions of the tricuspid and of the pulmonic valves, it seems likely that blood entering the ventricle in late diastole is distributed adjacent to the tricuspid and below the pulmonic valve. With the onset of ventricular systole the blood below the pulmonic valve and in the middle of the ventricle will be ejected first while a portion of the blood in the low ventricle and in the apex may be ejected only in late systole. The blood ejected in the first part of systole, at higher velocity, is more likely to mix with blood already present above the valve than that ejected at low velocity in late systole. Since it would seem reasonable to expect that blood behaves in a similar way in both ventricles and above both semilunar valves, our findings in the right ventricle and in the pulmonary artery may also be valid for the left ventricle and the aorta.

### Evaluation of ventricular volume measurements by dilution methods

Ventricular end-diastolic washout volume ($EDV_w$) is currently calculated from the stroke volume and the fraction of indicator ejected per beat after a sudden atrial or ventricular injection (1–11). The major assumption of this method is that this fraction equals the fraction of the end-

diastolic volume ejected. While studies on in vitro models substantiate the validity of the mathematical analogue for calculation of volume and of the injection and detecting techniques used (34), proof of the validity of volume measurements in vivo is lacking. Rolett, Sherman, and Gorlin (35) found good agreement between left ventricular $EDV_w$ measured by thermodilution in three dogs and the end-diastolic volume measured at the same pressure after the animals had been sacrificed. However, because of differences in the myocardial distensibility of living and dead animals, this observation is of doubtful significance. Indeed, comparisons of this method with angiocardiographic assessments of ventricular volume indicate that volumes obtained with the indicator washout technique are uniformly and significantly larger (15, 36, 37).

The relative constancy of the fraction of indicator ejected per beat, as indicated by a rectilinear downslope of the washout curve on semilogarithmic paper, and its reproducibility, have been considered the strongest indication of adequate ventricular mixing (1, 3–9, 11, 16, 35, 38). Our findings do not support this interpretation since the downslopes of simultaneous washout curves obtained by catheter sampling were usually rectilinear on semilogarithmic paper after two or three beats, despite the fact that their slopes were markedly different (Fig. 5). Furthermore, these curves were reproducible and their slopes compared well with those obtained over the precordium at the same time. It seems reasonable to conclude that these washout curves are an expression of the turnover rate of the blood in the regions containing the indicator and that their rectilinear downslope on semilogarithmic paper indicates only that the turnover rate of blood in these regions is relatively constant in successive beats.

Information concerning the volume of the regions that determine the downslope of the washout curve after the first or the first few beats may be obtained only from a knowledge of the flow through them. This flow must be only a fraction of the stroke volume to account for the remarkably low values of cardiac output obtained from the curves sampled below the pulmonic valve after ventricular injection, and a significant fraction of the stroke volume must pass through the ventricle without mixing, or mixing only partially, with the

residual volume. Since $EDV_w$ is calculated from the downslope of the washout curve after the first, or the first few beats, its value will obviously be significantly larger than the true end-diastolic volume regardless of the indicator used (be it dye, isotope, or temperature), regardless of the point of injection, and regardless of where or how the washout curve was recorded.

On the basis of these considerations, the indicator-washout method of estimating ventricular volume can only set an upper limit to the value of the end-diastolic volume. The overestimate must be rather large in normal subjects whereas it may be of modest proportions in patients with an enlarged heart and low cardiac output. The application of this method to the study of ventricular responses to various stimuli can only provide information concerning the direction of changes in volume if the pattern of blood transfer across the ventricle remains unchanged. Recent findings suggest that this condition may be met in the absence of marked changes in heart rate or in the ejected fraction (15). Reported alterations in $EDV_w$ in response to physiological manipulations (39–41) should be evaluated with these considerations in mind. Certainly studies of ventricular function derived from the measurement of $EDV_w$ appear to be unjustified at present.

## ACKNOWLEDGMENTS

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