Published in final edited form as:

Am J Physiol. 1983 October ; 245(4): H707–H712.

Plasma-soluble marker for intraorgan regional flows

STEPHEN E. LITTLE and JAMES B. BASSINGTHWAIGHTE

Center for Bioengineering WD-12, University of Washington, Seattle, Washington 98195

Abstract

Measurement of regional plasma flow is needed to quantitate the delivery of substrates and drugs to cells. For estimating regional plasma flows an ideal deposition marker should be 100% extracted during transorgan passage and retained until local tissue concentrations can be measured. To escape quickly, the tracer must penetrate capillary endothelial cells rapidly. To be retained, it must bind or be transformed or accumulated by cells. Desmethylinipramine (DMI, mol wt 266.3), a norepinephrine reuptake inhibitor, is suitable. On injection of [³H]DMI and ¹³¹I-albumin simultaneously into the coronary artery inflow of isolated Ringer-perfused rabbit hearts at 37°C, extractions were >99% at plasma flows (F_s) up to 2.3 ml·g⁻¹·min⁻¹ and >94% with F_s up to 5.1. Retention at $F_s < 2.3$ averaged 99.0 ± 0.55% (SD, $n = 6$) at 0.5 min, 98.4 ± 0.5% at 1 min, and 96.6 ± 1.1% or >95% at 3 min. Retentions were similar in two dog hearts in situ. With $F_s > 3$ ml·g⁻¹·min⁻¹, there was greater escape, 4.2 ± 2.7% at 1 min and 6.8 ± 4.2% at 3 min. The fractional escape rates of loss at 2 min or more were about 1%/min at all flows, suggesting that the spatial profiles of deposition did not change thereafter. Thus DMI is nearly ideal as a “molecular microsphere.”

Keywords

regional coronary blood flow; desmethylinipramine; endothelial cells; isolated perfused heart; rabbits; dogs; extraction; retention; adrenergic receptors; 10-11-dihydro-5-[3-(methylamino) propyl]-5H-dibenz[b,f]azepine; multiple indicator dilution; transport functions; residue functions; tricyclic antidepressant

REGIONAL BLOOD FLOWS in the heart are widely heterogeneous, as revealed by studies using microsphere deposition (14,27) and regional O₂ saturation (24,25). That there might be stable patterns of heterogeneity was suggested by the recurrence of similar patterns of NADH fluorescence with each of repeated episodes of hypoxemia in rat hearts (1,20). Direct evidence of the stability of heterogeneity over a few hours was reported by Bassingthwaight (2) using 15-μm microspheres in the coronary circulation of awake baboons; these data lead to a conclusion that “twinkling,” the temporal fluctuation in local flow described by Yipintsoi et al. (27) and by Sestier et al. (19), is not the dominant causative factor in heterogeneity. Such heterogeneity affects regional delivery of substrates; the nonuniformity, unless it is accounted for, strongly affects the estimates of transmembrane fluxes and local metabolism. Thus it is important to quantitate the heterogeneity of plasma flow with a technique as free of artifact as possible.

Microspheres may be subject to local artifacts causing systematic errors in deposition compared with local flows. The smaller the regions examined, the worse the problem. Larger size microspheres deposit preferentially in subendocardial (vs. subepicardial) regions (23,27); presumably they tend to take the straightest path (15) or the high-flow path (10). Endocardial-to-epicardial ratios for antipyrine and potassium were smaller than for microspheres (26), but neither of these tracers is either completely extracted or well retained in the tissue. About 50% of intra-arterially injected water and antipyrine wash out by 1 min at a flow of 1 ml·g⁻¹·min⁻¹. Potassium (21) and thallium (4) are better retained but extractions are only 50–

80s. Extraction is less and washout is faster in high-flow regions. For such reasons, the local depositions of these various solutes are not proportional to flow; this leaves the question of the accuracy of the microsphere technique virtually untested.

The regional flows of blood-borne particles (whether microspheres or red blood cells) may differ from plasma flows. The velocities of red blood cells and plasma do differ: intracapillary hematocrits in the heart are lower than large vessel hematocrits (9,17), and the transorgan transit times are less for red blood cells than for plasma (18,28). Substrate delivery into the capillary-tissue exchange regions is in proportion to plasma flow; O₂ delivery (as observed by NADH fluorescence) is more nearly in proportion to red blood cell flow. Thus, even if microspheres give a good measure of regional red cell delivery, they may not give accurate estimates of regional plasma flows.

The surest indicators of local flow are those that are completely extracted and retained in the tissue, given that they are delivered in proportion to flow and stop in the microcirculation. Indicators that penetrate the capillary wall via only the aqueous channels (principally the clefts between endothelial cells) have permeabilities too low to be completely extracted during single transcapillary passage: this is true even of those such as sodium, potassium or urea, whose diffusion coefficients approach that of water. Therefore, any useful marker must contact or traverse the plasmalemma of the endothelial cell. Thus the requirements for the indicator are as follows.

First, it must be acted on rapidly at the luminal surface of the endothelial cell, i.e., it must either 1) be small and lipid soluble to penetrate the membrane, or 2) be carried across the bilayer via a transporter, or 3) become bound by a receptor on the luminal plasmalemmal surface.

Second, the indicator must have a large volume of distribution to be retained, i.e., it must 1) be transformed rapidly and incorporated into a fixed intracellular substance, or 2) be transported faster into cells than out of cells so that it is concentrated inside cells, or 3) become attached to receptor sites that are abundant and of high affinity.

It is not enough that the molecule be simply small and highly lipid soluble. Although such a molecule can diffuse across plasmalemmal bilayers and so achieve the requisite unidirectional flux out of the capillary lumen, the flux will be equally rapid in the opposite direction; some other phenomenon must be invoked to produce longer retention. Water and antipyrine (26), xenon (3), krypton (12), various monohydric alcohols (6), and ethers don't work, in spite of the rapid transport across the membrane, because there is no process for their retention. Thus for an indicator to be considered ideal, a combination of conditions must be fulfilled, i.e., 1) a large number of high-affinity receptors on the endothelial luminal surface, or 2) a high-capacity transporter on the luminal endothelial plasmalemma with endothelial retention provided by high influx compared with efflux, a concentrative process, or 3) rapid permeation of the capillary wall followed by binding in interstitium or in cells or rapid transformation and incorporation into cell constituents.

In this report we demonstrate the suitability of a proposed marker for regional blood flows, desmethylimipramine, a substance passing rapidly through the endothelial cell membrane and retained strongly. Although it is now available commercially labeled only with a beta-emitter, it and related molecules have the potentiality for labeling with gamma-emitters.

METHODS

Experiments were performed in isolated rabbit hearts to determine the extraction and retention of desmethylimipramine (DMI). The situation chosen was one that should give the most demanding test of the adequacy of this substance, i.e., a situation in which the perfusate flows

could be made very high to test the limits of the conditions under which it might be nearly completely deposited. In isolated-perfused rabbit hearts, flows can be made very high without producing much tissue edema.

For the isolated heart studies, the hearts were removed from 2- to 3-kg New Zealand White rabbits after anesthesia with pentobarbital sodium and mounted on a perfusion system in which the flow was controlled and the perfusion pressure monitored. The perfusate was (in mM) Na^+ 142, K^+ 5.4, Mg^{2+} 1.0, Ca^{2+} 2.0, Cl^- 141, HCO_3^- 12, PO_4^{3-} 0.435, glucose 11.0, and insulin 2 U/l, at 37°C, unless otherwise noted. The coronary sinus flow and Thebesian flow into the right ventricle were collected via a cannula placed through the pulmonary artery, and the total effluent flow was measured with graduated cylinder and stopwatch. Tracers were injected into the aortic cannula, which supplied only the coronary system. Samples of the coronary sinus effluent were taken from the cannulated pulmonary artery at intervals of 1, 2, 4, or 10 s; higher sampling rates were used during the first part of the curves. We calculated the outflow response as the fraction of dose emerging per unit time, $h(t)$, at each time t

$$h(t) = F \cdot C(t) / q_0 \quad (1)$$

where F is the perfusate flow ($\text{ml} \cdot \text{s}^{-1}$), $C(t)$ is the concentration of tracer (cpm/ml), and q_0 is the injected dose of tracer (cpm). The residue function $R(t)$ is defined

$$R(t) = 1 - \int_0^t h(\lambda) d\lambda \quad (2)$$

To estimate extraction of DMI relative to albumin we use the instantaneous extraction, $E(t) = 1 - h_D(t)/h_R(t)$, which is the fractional difference between the albumin reference intravascular curve [$h_R(t)$] and the curve for the permeant DMI [$h_D(t)$] at each moment; $E_{\max}$ is maximum value of $E(t)$, occurring around the time of the peak of $h_R(t)$. The cumulative or net extraction with single transorgan passage is given by $E_{\text{net}}(t) = [R_D(t) - R_R(t)]/[1 - R_R(t)]$, which gives the fraction of DMI retained in the organ compared with the intravascular albumin.

Tracers

^{131}I -albumin was used as the intravascular reference tracer in amounts of 0.5–2 μCi /injection (Squibb, La Mirada, CA). For the deposited tracer we used [^3H]DMI (mol wt 266.3; New England Nuclear no. NET-593; sp act 33.4 Ci/mmol) in amounts of 5–10 μCi /injection. DMI blocks norepinephrine uptake at nerve endings (13,22) and appears to bind at other sites, such as those for serotonin (16).

For comparison, a series of experiments was done with [^3H]water (mol wt 20, New England Nuclear no. NET-001B), making observations of retention $R(t)$ at different flows.

The tracers to be injected were premixed, and 10–15 aliquots were taken for calibration of the dose of injectate. Isotope counting was performed in a 3-channel Beckman LS9000 liquid scintillation counter.

The purity of each batch of DMI was checked using ascending silica gel thin-layer chromatography (TLC) (E. Merck, Darmstadt, FRG) with ethanol: NH_4OH (29:1) as the solvent. The impurities were closer to the solvent front than was the DMI and thus were presumably oxidation products, such as 2-OH DMI, which bind less well to receptors. To diminish degradation, the tracer was refrigerated and shielded from light prior to injection. In four lots the fractions of non-DMI that were separable on chromatography were 3, 6, 4.3, and 1.2%; the latter lot was used in our experiments. For increased accuracy in measuring flow,

high-pressure liquid chromatography (HPLC) or TLC should be used to purify DMI to better than 99%. The fraction of free iodine in the ^{131}I -albumin was found, by electrophoresis, to be less than 0.5%.

Preliminary experiments were done to see how well DMI was extracted by blood-perfused hearts. These were performed in two open-chest mongrel dogs (15 and 24 kg) anesthetized with pentobarbital sodium. $[^3\text{H}]\text{DMI}$ (100 μCi) and ^{131}I -albumin (20 μCi) were injected into the left atrium, and samples were collected from the coronary sinus at 2-s intervals. The analytic methods were those described above.

RESULTS

Sets of dilution curves are shown in Fig. 1 for albumin and DMI. The curves show that at a high plasma flow (right panel) the extraction of DMI is over 99% during the upslope of the albumin indicator-dilution curve. The difference between this and 100% could be explained either on the basis that there is some tracer-labeled impurity in the DMI that does not attach to the receptor site or that there is a small fraction of the DMI that does not escape from the vascular space because of a capillary permeability barrier.

Extractions

At very high flows less DMI was extracted during transcapillary passage. The maximum instantaneous extractions ranged from 99.8 to 99.5% in the physiological range of flows (0.5–2.3 $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$) and were 99.2–93.9% at very high flows (3–5.1 $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$). More importantly from the point of view of measuring regional flows by measuring the local concentrations (by tissue sectioning or by positron tomography), the values of E_{net} at $t = 1$ min were high, 98.8–98.2% at normal flows. This was a much higher retention than for water, whose maximum instantaneous extractions ranged from 97 to 88% at low to higher flows but whose retention was less than 75% after 1 min even at low flows.

Residue functions

Curves of the residual content of the organ at time t [$R(t)$] are plotted in Fig. 2 for several different flows in one heart. At low flows the washout was characterized by a slow smooth curve, and the retention was long. At high flows there was a sudden emergence of a small fraction of the tracer, which represents the loss of intravascular tracer that failed to escape across the vascular wall and so emerged with the intravascular reference tracer. (See also Fig. 1.) These curves were obtained at relatively high flows, i.e., mostly over 1.5 $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ and so represent flows of 2 to 10 times the normal plasma flows in the heart. The normal myocardial plasma flows, given a 40% hematocrit, are usually less than 0.6 $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$.

Residual content at particular times (T)

In anticipation of using the deposition of this indicator at particular times after injection to give a measure of regional myocardial plasma flows, $R(T)$, at particular times, T , is shown in Fig. 3. At flows less than 2.3 $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ the retention at 0.5 min averaged $99.0 \pm 0.55\%$ ($n = 6$) and was greater than 98%. At $T = 1$ min, the average was $98.4 \pm 0.50\%$ ($n = 6$), at $T = 2$ min, $97.2 \pm 0.9\%$, and at $T = 3$ min, $96.6 \pm 1.06\%$, all being greater than 95%. At higher flows, above 3 $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$, the retentions were less: at $T = 1$ min, $95.8 \pm 2.7\%$ ($n = 8$) and at $T = 3$ min, $93.2 \pm 4.3\%$ ($n = 8$). The data show that if the heart were suddenly stopped 1 min after injection, the residue of DMI in the tissue even at flows of 4–5 $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ would be greater than 95% (at 37°C) or 88.6% (at 23°C) and, for plasma flows in the normal physiological range of less than 1 $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$, the extraction and retention would be over 99%. At 1 min there is little retention of albumin; the areas of the two albumin curves in Fig. 1 up to 1 min were 90.7 and 96.1%.

With 95% retention as an arbitrary level of adequacy, these data indicate that DMI deposition at <1 min provides an adequate measure of flow up to 5 (37°C) or 3.5 ml·g⁻¹·min⁻¹ (23°C) and is highly accurate (99% or better) for low to normal plasma flows, up to 1 ml·g⁻¹·min⁻¹.

The tracer initially extracted is washed out slowly, but the rate is not highly flow dependent. A quantitative measure of the rate of loss is the fractional escape rate,

$\eta(t) = - (dR/dt) / R(t) = h(t) / \left[1 - \int_0^t h(\lambda) d\lambda \right]$, which is the fraction of the retained tracer leaving the organ per unit time (2). At early times, the escape rate was higher at higher flows, but after 2 min it diminished to about 1%/min and was almost unaffected by flow. [For example, at $F_s = 0.73 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$, $\eta(t = 2 \text{ min})$ was 0.75%/min and in another run at $F_s = 4.5 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$, $\eta(t = 2 \text{ min})$ was 1.1%/min.]

This behavior is in marked contrast to that of flow-limited tracers (such as H₂O or antipyrine) that wash out in proportion to flow. These tracers have been used to measure flow because their capillary and cell permeabilities are high, and thus these extractions are high, 90% or more, due to their dilution in an extravascular region (cells and interstitial space) whose size is large compared with the vascular pool. The high permeabilities that render their washout flow limited compromise their use as deposited markers. Residue functions for tritiated water (THO) are shown in Fig. 2 along with $R(t)$ for DMI for contrast. At the end of 1 min, the retentions ranged from 75 to 0% for THO, in contrast to 98.5–93.8% for DMI.

Arterial deposition is small

To test whether or not a substantial fraction of the DMI was deposited in the endothelial lining of large vessels, as opposed to the small vessel regions, a heart was stopped at 30 s after injection, and the large vessels were dissected down to about the level of 300- μm diameter arterioles. The fraction of the injected [³H]DMI retained in large arteries was 0.0036; the fraction retained in the aortic wall was 0.012. These results indicate that retention in the large vessels is not a serious problem detracting from the use of DMI as a flow-distributed marker in small pieces of tissue.

Preliminary in vivo studies

Four sets of paired ¹³¹I-albumin, [³H]DMI dilution curves were obtained by the coronary sinus sampling after left atrial injection in two dogs. E_{max} values were 93.6–98.9%, averaging $97.2 \pm 2.4\%$ ($n = 4$); the retentions at 1 min were 84.2–98.0%, averaging $93.7 \pm 6.4\%$. If we omit the only curve, from one of the dogs, with values more than 10 SD away from the data on the other three curves, the means were $98.4 \pm 0.45\%$ ($n = 3$) for E_{max} and $96.8 \pm 1.1\%$ for $R(T = 1 \text{ min})$. These data suggest that DMI will be useful in vivo.

Equilibration between plasma and red blood cells

In a sequence of samples obtained during a dilution curve, the activity was measured in red blood cells and plasma separately. The ratio of DMI concentrations in erythrocytes to plasma averaged 0.355 ± 0.072 ($n = 27$); this ratio is less than the ratio of water contents in erythrocytes and plasma (0.69 to 0.74) and would suggest that there is exclusion from erythrocytes. The dog studies show that exchange with erythrocytes does not prevent high extraction during transcapillary passage; this is true of lipid-soluble substances in general and is also true of the [³H] water in this study. A closely related compound, imipramine, which also distributes similarly between red blood cells and plasma, is highly extracted in the liver (8). The important fact is that carriage by red blood cells does not inhibit high extraction.

DISCUSSION

DMI attachment to, or passage into or through, endothelial cells is demonstrated by these studies. An inert hydrophilic molecule of comparable size traversing only the aqueous channels, such as sucrose (mol wt 342), would show $E_{\max}$ of less than 30%, even at the lower flows.

Water, although having $E_{\max}$ almost as high as DMI, is poorly retained, exhibiting flow-limited washout (Fig. 2; and Ref. 26). Therefore, because of differences in times of entry of [^3H]water into local regions, there is no instant at which the regional tissue [^3H]water concentrations can be proportional to regional flows, whether an impulse or step input is used.

Because extractions are high, tracer will be deposited in higher concentrations at the upstream ends of capillary-tissue units than at downstream ends, in accord with the mathematical descriptions of Goresky, Bach, and Rose (11). At high flows these profiles will be less evident. Such gradients may help in identifying microvascular units in a tissue. Their existence will have little impact on the estimation of regional flows from concentrations in small pieces as the smallest pieces usually contain thousands of capillary-tissue units.

Because of the long retention, the proportionality between the regional DMI concentrations and the regional flows at the time of DMI injection will be maintained for many minutes after deposition in spite of some washout. We infer from the similarity of fractional escape rates at high versus low global flows that fractional escape rates are likely to be the same in high-flow as in low-flow regions. With, for example, an $\eta(t)$ of 1%/min, the percent change in ratios of regional concentrations at the end of a 20-min period would be less than 7.2% when there is a sixfold range of regional flows.

Consequently, the presence of impurities in the DMI, even if they wash out rapidly, will have little effect on the estimation of relative regional flows but would give underestimation of absolute flows. The firm retention would also tend to maintain any axial concentration gradients.

DMI may be useful in other organs in which there are norepinephrine receptors or transport sites. Competition with natural substrates for the receptor in the heart or elsewhere could conceivably reduce retention, but this is unlikely since cardiac receptor affinity is 10^4 higher for DMI than for catecholamines (16).

A comparison of DMI with deposited cations is shown in Fig. 4. The difference between DMI deposition and the ideal deposition is small. Rubidium and thallium deposition are strongly barrier limited rather than flow limited at moderate to high flows.

This is the first step toward a "molecular microsphere," a marker for regional plasma flows rather than blood flows, which when fully developed should serve as a reference standard against which to assess the deposition of other tracers and of microspheres.

Acknowledgments

The use of DMI was stimulated by Dr. Daniel Cousineau's observations of its rapid and enduring effect on norepinephrine uptake in the heart. We are indebted to Craig Althen and Marta Chaloupka for help in the experiments and to Geraldine Crooker and Edith Boettcher for the preparation of the manuscript.

This work was supported by National Institutes of Health Grants HL-19139 and RR-01243.

REFERENCES

1. Barlow CH, Chance B. Ischemic areas in perfused rat hearts: measurement by NADH fluorescence photography. *Science* 1976;3:909–910. [PubMed: 181843]
2. Bassingthwaight JB. Physiology and theory of tracer washout techniques for the estimation of myocardial blood flow: flow estimation from tracer washout. *Prog. Cardiovasc. Dis* 1977;20:165–189. [PubMed: 335437]
3. Bassingthwaight JB, Strandell T, Donald DE. Estimation of coronary blood flow by washout of diffusible indicators. *Circ. Res* 1968;23:259–278. [PubMed: 4874081]
4. Bassingthwaight JB, Winkler B. Kinetics of blood to cell uptake of radiotracers.. In: Columbetti, LG., editor. *Biological Transport of Radiotracers*. CRC Press; Boca Raton, FL: 1982. p. 97-146.
5. Budinger TF, Yano Y, Huesman RH, Derenzo SE, Moyer BR, Mathis CA, Ganz E, Knittel B. Positron emission tomography of the heart. *Physiologist* 1983;26(4):31–34. [PubMed: 6601810]
6. Chinard FP, Thaw CN, Delea AC, Perl W. Intrarenal volumes of distribution and relative diffusion coefficients of monohydric alcohols. *Circ. Res* 1969;25:343–357. [PubMed: 4898398]
7. Consigny PM, Verrier ED, Payne BD, Edelist G, Jester J, Baer RW, Vlahakes GJ, Hoffman JIE. Acute and chronic microsphere loss from canine left ventricular myocardium. *Am. J. Physiol.* 1982;242:H392–H404. *Heart Circ. Physiol.* 11.
8. Erlandsen EJ, Gram LF. Imipramine kinetics in the single pass rat liver perfusion model. *Acta Pharmacol. Toxicol* 1982;50:137–147.
9. Everett NB, Simmons B, Lasher EP. Distribution of blood (Fe^{59}) and plasma (I^{131}) volumes of rats determined by liquid nitrogen freezing. *Circ. Res* 1956;4:419–424. [PubMed: 13330185]
10. Fung YC. Stochastic flow in capillary blood vessels. *Microvasc. Res* 1973;5:34–48. [PubMed: 4684755]
11. Goresky CA, Bach GG, Rose CP. Effects of saturating metabolic uptake on space profiles and tracer kinetics. *Am. J. Physiol* 1983;244:G215–G232. *Gastrointest. Liver Physiol.* 7. [PubMed: 6337505]
12. Hoedt-Rasmussen K, Sveinsdottir E, Lassen NA. Regional cerebral blood flow in man determined by intra-arterial injection of radioactive inert gas. *Circ. Res* 1966;18:237–247. [PubMed: 5323856]
13. Iversen, LL. *The Uptake and Storage of Noradrenaline in Sympathetic Nerves*. Cambridge Univ. Press; London: 1967.
14. Marcus ML, Kerber RE, Ehrhardt J, Abboud FM. Three dimensional geometry of acutely ischemic myocardium. *Circulation* 1975;52:254–263. [PubMed: 1149207]
15. Phibbs RH, Dong L. Nonuniform distribution of microspheres in blood flowing through a medium-size artery. *Can. J. Physiol. Pharmacol* 1970;48:415–421. [PubMed: 5429156]
16. Raisman R, Sette M, Pimoule C, Briley M, Langer SZ. High-affinity [^3H]desipramine binding in the peripheral and central nervous system: a specific site associated with the neuronal uptake of noradrenaline. *Eur. J. Pharmacol* 1982;78:345–351. [PubMed: 7067730]
17. Rakusan K. Vascular capacity and hematocrit in experimental cardiomegaly due to aortic constriction in rats. *Can. J. Physiol. Pharmacol* 1971;49:819–823. [PubMed: 4259220]
18. Roth AC, Feigl EO. Diffusional shunting in the canine myocardium. *Circ. Res* 1981;48:470–480. [PubMed: 7460218]
19. Sestier FJ, Mildenerberger RR, Klassen GA. The role of autoregulation in spatial and temporal perfusion heterogeneity of the canine myocardium. *Am. J. Physiol* 1978;235:H64–H71. *Heart Circ. Physiol.* 4. [PubMed: 677330]
20. Steenbergen C, Deeleuw G, Barlow C, Chance B, Williamson JR. Heterogeneity of the hypoxic state in perfused rat heart. *Circ. Res* 1977;41:606–615. [PubMed: 908108]
21. Tancredi RG, Yipintsoi T, Bassingthwaight JB. Capillary and cell wall permeability to potassium in isolated dog hearts. *Am. J. Physiol* 1975;229:537–544. [PubMed: 1211446]
22. Trendelenburg U. A kinetic analysis of the extraneuronal uptake and metabolism of catecholamines. *Rev. Physiol. Biochem. Pharmacol* 1980;87:33–115. [PubMed: 6999585]
23. Utley J, Carlson EL, Hoffman JIE, Martinez HM, Buckberg GD. Total and regional myocardial blood flow measurements with 25 μ , 15 μ , 9 μ , and filtered 1-10 μ diameter microspheres and antipyrine in dogs and sheep. *Circ. Res* 1974;34:391–405. [PubMed: 4818762]

24. Weiss HR, Neubauer JA, Lipp JA, Sinha AK. Quantitative determination of regional oxygen consumption in the dog heart. *Circ. Res* 1978;42:394–401. [PubMed: 624146]
25. Weiss HR, Sinha AK. Regional oxygen saturation of small arteries and veins in the canine myocardium. *Circ. Res* 1978;42:119–126. [PubMed: 618593]
26. Yipintsoi T, Bassingthwaighte JB. Circulatory transport of iodoantipyrine and water in the isolated dog heart. *Circ. Res* 1970;27:461–477. [PubMed: 5452741]
27. Yipintsoi T, Dobbs WA Jr, Scanlon PD, Knopp TJ, Bassingthwaighte JB. Regional distribution of diffusible tracers and carbonized microspheres in the left ventricle of isolated dog hearts. *Circ. Res* 1973;33:573–587. [PubMed: 4752857]
28. Ziegler WH, Goresky CA. Transcapillary exchange in the working left ventricle of the dog. *Circ. Res* 1971;29:181–207. [PubMed: 4935840]

FIG. 1.

Outflow indicator-dilution curves for desmethylimipramine (DMI) and albumin from a Krebs-Ringer bicarbonate buffer-perfused rabbit heart. *Upper panels:* both scales are logarithmic to cover large spans of normalized concentrations $[h(t)]$ and of times (t) of sampling. Albumin is the intravascular reference $[h_R(t)]$; DMI is the diffusible solute $[h_D(t)]$. *Lower panels:* high extractions indicate rapid exit from vascular space. Flows (F) were 0.73 (*left panel*) and 4.54 $\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ (*right panel*).

FIG. 2.

Residue function $[R(t)]$ curves of DMI, compared with microsphere deposition and residue function curves of H_2O . *Upper panel:* plots of the residue functions for DMI vs. time in one isolated perfused rabbit heart (expt 230383) at varied flows. Flows (F_s , in ml g^{-1} min $^{-1}$) are indicated in figure. Initial extraction is reduced at higher flows causing $R(t)$ to diminish rapidly in early phase of washout; in contrast, at lower flows $R(t)$ remains high for many seconds. A logarithmic time scale is used to encompass the long times of observation. The 'x' symbol gives mean and bars the SD of the retention of 8.8 ± 0.9 - μ m microspheres in dog hearts from Consigny et al. (7) using N_2O anesthesia; with halothane anesthesia 2-min retention was only 0.937 ± 0.030 , off our graph. *Lower panel:* plots of residue functions for $[^3H]$ DMI and for $[^3H]H_2O$ in 2 experiments on isolated perfused rabbit hearts. Perfusate flow (F_s , in ml g^{-1} min $^{-1}$) marked on each curve.

FIG. 3.

Residue function $R(T)$ at particular times, T , after injection, plotted as a function of the flow of solute containing mother fluid, F_s . Lines join points obtained at the same times t after injection at the different flows. Upper panel, at 37°C lower panel at 23°C . Note the difference in ordinate scales between upper and lower panels; retention is lower at the lower temperature.

FIG. 4.

Deposition in heart plotted vs. perfusate or plasma flow, F_s . Deposition, the product of the net extraction at T min after injection times the flow (F_s) is net clearance from blood into tissue during T min. The ideal is the line of identity labeled $E_{\text{net}} = 1$, which would mean 100% extraction and retention in all regions at all flows. Rubidium data are from regression line for blood-perfused dog hearts of Budinger et al. (5). Thallium curve is from Bassingthwaight, Winkler, and Kern (4).