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A comparison of ascorbate and glucose transport in the heart

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Abstract

Multiple indicator-dilution experiments were done to compare the transcapillary exchange of tracer amounts of L - $[^{14}\text{C}]$ ascorbate and D - $[^3\text{H}]$ glucose (against an intravascular reference ^{131}I -albumin) in Ringer-perfused (5 mM glucose) isolated rabbit hearts. The indicator-dilution curves for the two were virtually superimposed over the first 40–80 s. Estimates of the capillary permeability-surface area products, PS_C , were the same, 2.3 ± 0.7 (SD) $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ($n = 18$), in accord with the coincidence of their instantaneous extractions. The similarity of glucose and ascorbate permeabilities is explained by the similarity in molecular weights and passive diffusivity, their lipophobic nature, and the paucity of carrier-mediated endothelial transport for either molecule. The data were analyzed via a model composed of aggregates of spatially distributed capillary-tissue units (capillary blood, interstitium, myocytes) accounting for the heterogeneity of regional flows. The interstitial volumes in this preparation are enlarged, 0.30 ± 0.04 ml/g. There is substantial entry into myocardial cells, the cell permeability-surface area products being approximately 2 – 3 $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ for ascorbate and glucose. The estimated volumes of interstitial and intracellular space, 0.30 and 0.47 $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$, reflect interstitial edema and are very close to measured values, giving reassurance concerning the methods of modeling analysis.

Keywords

myocardial uptake; capillary permeability; sarcolemmal permeability; indicator dilution; isolated rabbit hearts

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ALTHOUGH THERE IS A UNIVERSAL need for ascorbic acid by mammalian cells and its prolonged lack causes degradation of the intracellular matrix, little is known about the kinetics of its exchange. The present studies are designed to provide estimates of the rates of its blood-tissue exchange in the heart, considering it to be exemplary of well-perfused organs. In the course of the initial studies, a close similarity of the capillary permeability-surface area product (PS_C) for ascorbate to that for glucose was observed. Since glucose is not transported across the plasmalemma of endothelial cells in the heart to an extent measurable by the multiple indicator-dilution technique (12), the presumption is that ascorbate also penetrates the capillary wall virtually solely via the intercellular clefts. This raised the

possibility that one might be able to develop a nonradioactive tracer technique for ascorbate dilution curves using detection with a platinum electrode. With such a development, then estimation of the PS_C for ascorbate could be rapidly accomplished and frequently repeated, which would be invaluable for studies in which permeability might be changed or in studies of recruitment of the microvasculature with vasodilation or increased flow, when surface area might be changed. In any case, the proposed methods measure only the product, PS .

METHODS

The experiments were performed, using the standard multiple-indicator-dilution method (7), on isolated non-working, spontaneously beating rabbit hearts weighing 6–10 g and perfused with Tyrode solution [composition (mM): Na 147.4, K 5.4, Ca 1.8, Mg 0.5, Cl^- 133.1, HCO_3^- 23.8, $H_2PO_4^-$ 0.4, ethylenediaminetetraacetic acid 0.01, and glucose 5]. The preparation is similar to that used by Grabowski and Bassingthwaite (9), so that the data on capillary PS_C s should be comparable. The per-fusion rate was controlled with a roller pump on the inflow line. Perfusion pressure, perfusate temperature, coronary sinus pressure, heart rate, and heart weight were monitored and recorded continuously. Experiments were done at room temperature, 22–23°C. Drainage from the coronary sinus, right atrium, and right ventricle was via a short cannula passing from the right ventricle through its wall into a funnel from which the outflow drained into a rotating sampler system with collection periods of 0.4, 1, 2, or 4 s. The small amount of drainage from the left ventricle (the sum of aortic valve leakage and Thebesian vein drainage) was collected via a small cannula through the thinnest part of the apical myocardium. At the end of each experiment the wet weight of the myocardium devoid of obviously fatty tissue was measured.

After stabilization of perfusion pressure, flow, heart weight, and perfusate temperature, samples of venous outflow were removed for measuring background isotopic activities. Then a mixture of ^{131}I -albumin (half-life 8.1 days; 7 μ Ci RISA, Abbott Laboratories, North Chicago, IL), L - $[^{14}C]$ ascorbate (2 μ Ci), and D - $[^3H]$ glucose (4 μ Ci, New England Nuclear, Cambridge, MA) dissolved in saline was injected as a bolus (total vol 0.2–0.3 ml) into the cannulated aortic inflow to the coronary arteries. The duration of the injection was approximately 0.2–0.5 s. Just before the injection, the multiple sampling device was started; for the first 30 samples the coronary sinus effluent was sampled using 1.0-s intervals for studies at low flows and 0.4-s intervals at high flows; for the second 30 samples the collection rate was set at 4- or 2-s intervals. The durations of the curves were 72–150 s. When sampling was completed, the flow was again measured. The samples were weighed, and two aliquots of 0.1 ml were placed in a vial of liquid scintillation fluid for counting in a three-channel Nuclear Chicago Mark II LS counter. For each curve a measured aliquot of the injectate was also counted. Correction was made for isotopic decay of ^{131}I occurring during the counting period. Quench correction curves covering the range of external standard ratios exhibited by the samples were made up, using varied volumes of the perfusate as the quenching agent.

Knowing the time of collection (t), the isotopic activity in each sample $[C(t)]$, the injected dose of each tracer (q_0), and the measured plasma flow (F_s), we calculated the normalized

dilution curves, the pulse response for ^{131}I -labeled albumin [$h_R(t)$] and for the permeant diffusible tracers [$h_D(t)$], using the general equation

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

The primary methods of analysis for deriving parameter estimates from these curves is given below, but some simple calculations were also made. From $h_D(t)$ for each of the permeating tracers, an instantaneous fractional extraction, $E(t)$, at each time point was calculated on the basis that the reference tracer albumin does not leave the capillary blood

$$E(t) = 1 - h_D(t) / h_R(t) \quad (2)$$

The value for E commonly used for the calculation of PS_C is the maximal plateau value ($E_{\max}$), which we chose as the best estimate of the average fractional extraction on the basis that it represented the extraction at approximately the mean of the regional flows to the tissue of the organ (10). This allowed us to compare estimates with the technique of Crone (8)

$$PS_C \text{ (Crone)} = -F_s \log_e (1 - E_{\max}) \quad (3)$$

The molecular weight for ascorbate is 176, compared with 180 for glucose. The diffusion coefficient at 25°C for glucose in 0.15 M electrolyte solution is $0.67 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$ (14).

That for ascorbate would be $\sqrt{176/180} \times D_{\text{gluc}}$ or about 1% less.

Because the heterogeneity of regional flows influences the estimates of capillary and cell PS (4), it was necessary to measure and account for it. Regional blood flows were determined in two of these hearts from the deposition densities of microspheres (^{46}Sc and ^{85}Sr) injected into the aortic root within a few minutes of obtaining the indicator-dilution curves. The microspheres were $9 \mu\text{m}$ in diameter, labeled with ^{46}Sc and ^{85}Sr (3M, Minneapolis, MN). The local tissue deposition density relative to the mean deposition density for the whole heart was measured in each of 60–100 pieces of the heart, each weighing about 0.1 g, and was denoted f_i , which is dimensionless; the mean of the f_i was by definition 1.0. The fraction of the mass of the organ having a relative flow f_i (equal to relative regional deposition) was w_i , so that the w_i describe in histogram form a probability density function of regional flows. The sum $\sum_{i=1}^{i=N} w_i f_i$ is 1.0 where f_i is the width of the i^{th} class and there are N classes; the mean relative flow is $\bar{f}$

$$\bar{f} = \sum_{i=1}^{i=N} w_i f_i \Delta f_i = 1.0 \quad (4)$$

by definition. The fraction of the indicator going to all of the regions having a flow f_i is w_i . The actual flow in the i^{th} region is $f_i F_S$ where F_S is the flow of perfusate ($\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$). The spread of the distribution is summarized by the relative dispersion (RD),

the standard deviation divided by the mean, $\left[\sum w_i(f_i - 1)^2 \Delta f_i\right]^{1/2}/1.0$. The similarity of distributions from one animal to another was also observed in baboon hearts (11a). Since the shapes of the distributions varied so little, a Gaussian distribution with RD = 30% was taken to be representative and was used in the multicapillary analysis by dividing the flows into five groups with approximately this distribution.

Method of Analysis

The multicapillary model defined by Levin, Kuikka, and Bassingthwaight (13) was used for the analysis. The capillary-tissue units of the model were those described by Bassingthwaight and Winkler (5), accounting for cellular uptake and loss and for intracellular consumption of material. Accounting for return flux from the cell is critically important. With axial diffusion set to zero, the single-capillary component of the model reduces to that of Rose, Goresky, and Bach (17). The multicapillary arrangement differs from that of Rose, Goresky, and Bach in that we consider the transport function of large vessels and capillaries to be independent, whereas they assumed linear dependence. The arguments pro and con this choice are given by Bassingthwaight and Goresky (4).

The equations for the three regions are parabolic partial differential equations, which are required to describe the concentration gradients with position along the length of the capillary. Regions are denoted by subscripts, C for capillary, I for interstitial fluid, and cell for myocyte. Transport through endothelial cells was not included, as it was shown that D- and L-glucose have equal capillary PS_C s (12), indicating that there was negligible glucose transport through these cells, compared with diffusive transport between them, and ascorbate appears identical. Radial diffusional relaxation times for intercapillary distances in the heart are short compared with the time constants for capillary exchange, so that interstitial and intracellular concentration gradients in a radial direction are taken to be negligible (2). As a result, the equations do not need to be represented in cylindrical coordinates. The concentration inside the capillary $C_C(x, t)$ at axial position x and time t is then given by the equation

$$\frac{\partial C_C}{\partial t} = -\frac{F_s L}{V'_C} \cdot \frac{\partial C_C}{\partial x} - \frac{PS_C}{V'_C} (C_C - C_I) \quad (5)$$

where V'_C ($\text{ml} \cdot \text{g}^{-1}$) is the volume of distribution for the solute in the capillary, in effect the plasma space [in the heart, about $0.035 \text{ ml} \cdot \text{g}^{-1} \times (1 - \text{Hct})$], and $F_s L/V'_C$ is the velocity ($\text{cm} \cdot \text{s}^{-1}$) in the capillary, and L is an arbitrary capillary length whose value is unimportant since it is cancelled by integration along the length of the capillary. For the reference tracer, albumin, PS_C was set to zero. For the analysis, an arbitrary value of $V'_C = 0.035$ was used; this choice has no effect on estimates of PS .

The equations for the concentrations in the interstitial (C_I) and cell regions (C_{cell}) are similar except that there are no flow terms. The equations are

$$\frac{\partial C_I}{\partial t} = \frac{PS_C}{V_I'} (C_C - C_I) - \frac{PS_{cell}}{V_I'} (C_I - C_{cell}) \quad (6)$$

$$\frac{\partial C_{cell}}{\partial t} = \frac{PS_{cell}}{V_{cell}'} (C_I - C_{cell}) - \frac{G_{cell}}{V_{cell}'} \cdot C_{cell} \quad (7)$$

where G_{cell} is an intracellular clearance ($\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) and G_{cell}/V_{cell}' is equivalent to k_{seq} , a first-order rate constant, s^{-1} , for intracellular sequestration or binding which is irreversible.

PS_{cell} is the sarcolemmal permeability-surface area product. V_I' and V_{cell}' are the interstitial and intracellular volumes of distribution. When there is no binding in the interstitium and no volume exclusion, V_I' equals the actual interstitial water space, V_I , which is about $0.21 \text{ ml} \cdot \text{g}^{-1}$ in intact rabbits (F. Gonzalez and J. B. Bassingthwaighte, published 1990) but larger in Tyrode-perfused hearts. Similarly when the transsarcolemmal transport is symmetrical (equilibrating) and there is no intracellular binding, then $V_{cell}' = V_{cell}$. Given equilibrating transport at capillary and cell barriers, then G_{cell} can be estimated from the arteriovenous difference, when this is small, and the values for PS_C and PS_{cell}

$$G_{cell} = \frac{E' \cdot F_s}{1 - E' \cdot F_s (1/PS_C + 1/PS_{cell})} \quad (8)$$

where $E' = 2(C_{in} - C_{out})/(C_{in} + C_{out})$, an extraction relative to the average of the inflow (C_{in}) and outflow (C_{out}) concentrations. The solutions to the single capillary equation were solved numerically using the same technique as described for a two-region model by Bassingthwaighte (2).

The multicapillary form of the model is that described by Bassingthwaighte and Winkler (5) in which the capillary-interstitial fluid (ISF)-cell units are considered independent of each other; in addition the transport (delay and dispersion) through the large vessels is considered to be independent of that through the capillary-tissue units. The microsphere deposition densities give the amount of tracer going to each of the N regions having relative flow (f_i); in the i^{th} region the impulse response of the capillary-ISF-cell unit is $h_{C_i}(t)$, the form of which depends on the regional flow ($f_i F_s$). The impulse response of the whole organ [$h(t)$] is given by the convolution of the transport function of the large vessels (the arteries and veins together) [$h_{LV}(t)$] with the sum of the different Capillary tissue impulse responses

$$h(t) = h_{LV}(t) * \sum_{i=1}^{i=N} w_i f_i \Delta f_i h_{C_i}(t) \quad (9)$$

Each $h_{C_i}(t)$ has a different transit time, which is therefore defined by the assigned capillary volume ($V_C = 0.035 \text{ ml} \cdot \text{g}^{-1}$) divided by the local flow ($f_i F_s$). Since the model equations are integrated over the length of the capillary, the value of V_C cancels out in the solution, as discussed by Bassingthwaighte and Goresky (4), and the extraction is governed by $PS_C/(f_i F_s)$ and the back diffusion. The model function $h(t)$ was scaled in proportion to the

integrated fraction of dose recovered in the outflow (“Recovery” in Table 1) to correct for variation in the injected doses and their calibrations, thus preventing these two sources of error from influencing the analysis.

Estimates of the transport parameters, PS_C , PS_{cell} , and G_{cell} , and the volumes of distribution, V'_I and V'_{cell} , were obtained by adjusting these values to obtain a best fit of the model to the data set. Values for V'_I for ascorbate and glucose were assumed to be the same, after a first set of analyses in which they were estimated separately they showed no differences. Setting the V'_I 's the same for ascorbate and D-glucose improves the accuracy of the estimates of the PS_{cell} and V'_{cell} which are less directly accessible from the intravascular observation point. The set of three dilution curves (with 50–60 points each) and the microsphere distributions (classified into a 5-class function) therefore provided a large set of information for the evaluation of the other parameters. If it weren't for experimental noise, the nine-parameter estimates (2 PS , 1 G_{cell} , and 1 V'_{cell} for each, plus V'_I) would be overdetermined.

We used an automated parameter adjustment routine (13) in which the adjustment is based on the sensitivities of the model solution to the separate influences of individual parameters at different parts of the recorded dilution curves. The technique gives very close fitting in the regions of the upslope, peak, and early washout phases of the dilution curve; estimating PS_C and V'_I/V_C from these parts gives emphasis on the late portion of the curve for the other parameters. The coefficient of variation (CV) was used only as a measure of the overall goodness of fit and not in the parameter adjustment procedure. We used a linear sum

of squares of the differences: $CV = N \sqrt{\sum (\hat{h}_i - h_i)^2} / [\sqrt{N-1} \sum \hat{h}_i]$. The use of the linear difference rather than logarithmic differences [as used by Rose et al. (17)] gives larger values for CV but is in accord with the methods of obtaining and measuring the actual dilution curves.

No constraints were imposed on the parameter estimates. Because of this the estimates of V'_I and V'_{cell} , whose sum is known fairly precisely from other experiments, will be seen to serve as an independent check on the methods of parameter estimation.

RESULTS

The form of the indicator-dilution curves

Examples of recorded indicator-dilution curves are shown in Figs. 1 and 2. The first part of the dilution curves for ascorbate and glucose have shapes essentially similar to that for albumin, but the peaks are much lower. In addition, for both there is a long low tail of tracer returning to the effluent from the extravascular regions.

The similarity of the first parts of the dilution curves for ascorbate and glucose demonstrates that their rates of efflux from the capillary are essentially similar. This in itself does not attest as to the mechanism of capillary permeation. However, D-glucose and L-glucose have previously been observed (12) to have identical permeability; since less than 0.8% of L-

glucose is transported across the plasmalemma of mammalian cells during the 1st min (unpublished observations), the implication is that it was transported by passive diffusion through the clefts between the endothelial cells. Since ascorbate and D-glucose have almost the same free diffusion coefficient (see METHODS), their permeation through the clefts must occur at the same rate as L-glucose. Since ascorbate efflux is not greater than that for D-glucose, which in turn is not greater than L-glucose, this means that the rate of ascorbate transport into or across endothelial cells must also be very small and that the analysis should not incorporate an attempt to account for transendothelial transport.

The persistence of the close similarity of D-glucose and ascorbate through the downslope and tail of the recorded dilution curves (Fig. 1) has further implications with respect to transport across the myocardial sarcolemma. Following an analogous line of logic, the comparison between D-glucose and L-glucose by Kuikka et al. (12) demonstrated myocardial cellular uptake for D-glucose (and also suggested the return flux of D-glucose from inside the cell to the ISF). The difference between D- and L-glucose was 10–20% of the value of $h_D(t)$ during the late phase of the downslope. D-Glucose and 2-deoxy-D-glucose tails were very similar, as were tails of D-[³H]-glucose and D-[¹⁴C]glucose, both comparisons being consistent with reflux of unmetabolized D-glucose from the cardiac cells and with some metabolism. The absence of a discernible difference between D-glucose and ascorbate therefore is good presumptive evidence that ascorbate is also taken up by myocardial cells (characterized by PS_{cell}) and at approximately the same rate as D-glucose. Since we do not expect the same transport mechanism to be used for both ascorbate and D-glucose, at least not at the same rate, this may simply be regarded as a coincidence. The analysis to follow provides quantitative estimates of the rate constants but does not extend the ideas derived by these considerations of the raw data.

The data on the observed $h(t)$ s for ascorbate and glucose are summarized in Table 1. The recoveries of albumin give a measure of the combined accuracy of the measurements of flow, amount of tracer injected, and concentration of the tracer in the effluent perfusate. The recoveries of the albumin curves were close to 100%, with a standard deviation of 5%, a degree of variability that is about usual for the technique. The high values of recoveries of ascorbate and glucose, being slightly over 100%, which is not physically possible, indicate the greater degree of error in β -counting but nevertheless indicate that most of the ascorbate and glucose had emerged in the effluent by the time the sampling was stopped. Since a small amount of both ascorbate and glucose must have returned still later than 150 s, these high recoveries imply that relatively little tracer was retained by the cells, including that consumed. The recoveries are used as scalars and don't influence curve shape; G_C does affect the shape of the tail and so can be estimated independently.

Maximum values of $E(t)$, E_{max} in Table 1, give an approximate estimate of the fraction of ascorbate or glucose that crosses the capillary wall. When regional flows are heterogeneous or there is return flux from ISF to capillary (i.e., “back diffusion”), then the observed E_{max} gives an underestimate of PS_C . In these studies, the estimates of PS_C from E_{max} for ascorbate and glucose were 1.64 ± 0.54 ($n = 18$) and $1.65 \pm 0.56 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ ($n = 18$). These values were 0.73 ± 0.13 and $0.72 \pm 0.13\%$ of the values obtained by fitting the multicapillary model; the underestimation provided by Eq. 3 is almost 30%.

The values for $\bar{t}_{alb}$ are the average transit times taken for intravascular indicator from the site of injection to the sampling vials. The volumes of the systems, calculated as $F_s \cdot \bar{t}_{alb}$, were $0.35 \pm 0.12 \text{ ml} \cdot \text{g}^{-1}$ ($n = 18$). The intracapillary volumes are only about $0.035 \text{ ml} \cdot \text{g}^{-1}$ in dog hearts (6), and the total vascular volumes in the intact rabbit heart are only $0.15 \pm 0.04 \text{ ml} \cdot \text{g}^{-1}$; these mean transit time volumes for albumin include volume outside of the myocardium, i.e., the 0.08 ml aortic cannula, the right atrial and ventricular cavities, and the 0.10 ml in the venous sampling tubing.

Heterogeneity of regional flows

Data on microsphere deposition densities were not obtained in all hearts. In the two hearts given microspheres the distributions obtained were similar to those reported elsewhere for rabbit hearts (12; F. Gonzalez and J. B. Bassingthwaighte, published 1990), dog hearts (13), and baboon hearts (11a). The distributions were approximately symmetrical (skewnesses were 0.12 and 0.01) but were a little leptokurtic (values for kurtosis were 1.89 and 3.89 vs. 3.0 for a Gaussian distribution). The most important feature is the spread of the distribution, which we report as the relative dispersion (RD); the RDs were 23 and 30% for these two hearts. For the analysis we used a distribution having $\text{RD} = 30\%$, with skewness $\beta = 0$ and kurtosis of 1.8. (For 5 pathways, the values of $w_i f_i$ were 0.01, 0.14, 0.50, 0.31, and 0.04, at f_i , of 0.25, 0.62, 1.0, 1.38, and 1.75.)

Estimation of transport parameters

Estimates are listed in Table 1. Outflow dilution curves fitted by the model are shown in Fig. 2. Note that the curves in the right panel (*expt 15*) have almost the poorest fit of any curves but in fact are not bad fits, and the high CV is a reflection of noise in the data rather than systematic fitting error. Capillary PS_C s averaged 2.27 ± 0.71 ($n = 18$) $\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ for ascorbate and $2.31 \pm 0.69 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ for D-glucose. Initially the curves were fitted without assuming that PS_C (ascorbate) and PS_C (glucose) were similar. The paired estimates are shown in Fig. 3. The conclusion, inasmuch as the regression line is indistinguishable from the line of identity, is that their PS_C differ only randomly. Then the dilution curves were refitted with the model solutions assuming the two PS_C s to be identical but unknown, thus reducing the number of free parameters by one; the purpose of this is to improve the accuracy of all of the parameters estimated. These final best estimates of PS_C are reported in Table 1; for three sets of curves (*expts 4, 8, and 10*, all from the same animal) the initial portions of the glucose and ascorbate curves were sufficiently unlike that the independence of the PS_C s was retained. [$PS_C(\text{ascorbate})/PS_C(\text{glucose})$ was 0.98 ± 0.05 , $n = 18$.]

The Crone-Renkin expression (Eq. 3) predictably gave underestimates compared with the full analysis. The values from Eq. 3 (PS'_C) averaged 1.64 ± 0.50 and 1.65 ± 0.60 for ascorbate and glucose; the ratios (PS'_C/PS_C , (that from Eq. 3 divided by that from the full analysis) averaged 0.73 ± 0.13 and 0.72 ± 0.13 for ascorbate and glucose.

Interstitial volumes of distribution (V_I) averaged 0.30 ± 0.04 ($n = 18$) $\text{ml} \cdot \text{g}^{-1}$. Thus these Tyrode-perfused hearts exhibited larger values for V_I than the values of 0.21 ± 0.03 found

for interstitial space in rabbit hearts in vivo (F. Gonzalez and J. B. Bassingthwaighte, published 1990).

PS_{cell} averaged 3.3 ± 3.1 ($n = 18$) $\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ for ascorbate and 3.1 ± 3.2 ($n = 18$) for D-glucose. The wide scatter is in accord with a right-skewed distribution of estimates. The pairs of values were more consistent, the ratios of PS_{cell} for ascorbate to that of glucose averaging 1.23 ± 0.64 . If none of the glucose or ascorbate that entered the cell returned untransformed to the effluent, i.e., all that entered was metabolized, then, from Eq. 8 with $G_{\text{cell}} = \infty$, under average conditions of flow and capillary permeability ($F_s = 3.3$ $\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$; $PS_C = 2.30$ and $PS_{\text{cell}} = 3.2$ $\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$), the arteriovenous (AV) difference would be 33%. Because this is greater than the observed AV difference, the implication is that there is reflux from the cell to the ISF (see below). This in turn implies that the reflux should be apparent in the fitting of the model to the data and that the intracellular volume of distribution (V'_{cell}) and rate of the first sequestering intracellular reaction (G_{cell}) can also be estimated.

The total water space in these saline-perfused hearts is $0.84 \text{ ml} \cdot \text{g}^{-1}$. The sum $V'_I + V'_{\text{cell}} = 0.78 \pm 0.025$ ($n = 17$, excluding the outstanding odd value for *expt 13*); adding this to the estimated $0.035 \text{ ml} \cdot \text{g}^{-1}$ used for V'_C gives a total of $0.815 \text{ ml} \cdot \text{g}^{-1}$. Since the $0.84 \text{ ml} \cdot \text{g}^{-1}$ certainly included perfusate in small arterioles and venules, reasonably estimated at $0.025 \text{ ml} \cdot \text{g}^{-1}$, the total value of the estimates appears remarkably close to the measured $0.84 \text{ ml} \cdot \text{g}^{-1}$ in such heart. This closeness, which is so highly dependent on the estimates of V'_I and V'_{cell} , gives reassuring confirmation of the results of the model analysis. The values $0.30 \text{ ml} \cdot \text{g}^{-1}$ for V'_I and $0.47 \text{ ml} \cdot \text{g}^{-1}$ for V'_{cell} therefore directly reflect the interstitial edema in the absence of a change in cell water content.

In the accompanying study (1), an ascorbate solution (11 mM) was infused continuously into the perfusate at a rate of $0.36 \text{ ml} \cdot \text{min}^{-1}$. The flow to the hearts (wt 6.2–7.6 g) ranged from 3.0 to $7.8 \text{ ml} \cdot \text{min}^{-1}$ (while the glucose level was 5 mM). The AV difference was $2 \pm 2\%$ or $E' = 0.02 \pm 0.02$ (SD, $n = 4$). Assuming that there is no binding in the interstitium or cells and using F_s and the values of PS_C and PS_{cell} obtained from the analysis, we obtain via Eq. 8 an estimate of G_{cell} of $0.10 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. The average value of G_{cell} from the data in Table 1 was $0.67 \pm 0.37 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ ($n = 18$), which is sevenfold larger. Even though G_{cell} and V'_{cell} are mathematically independent, their values have some degree of interdependence in that their influences on the form of the tail of the outflow dilution curve are similar, increasing values causing lowering of the tail of the model function (3). We think that the data were not recorded for long enough to have the tail of the curve dominated by return flux from the cell, even though the curves were recorded for 90–150 s, and therefore estimates of G_{cell} are likely to be substantially overestimated by this analysis.

DISCUSSION

The overall result that L-ascorbate and D-glucose have similar transcortary transport characteristics is not merely coincidental and gives rise to some potential future uses.

A question that arises concerning the accuracy of the indicator-dilution curves is whether or not the tails of the curves include tracer-labeled metabolites in addition to the injected tracer-labeled substrate. The evidence that the tracer in the outflow is solely untransformed solute is partially indirect and partially direct.

1) For ascorbate, the companion paper (1) demonstrates the synchrony of the tracer and electrode curves over the first 30 s. This is reassuring but the data are neither accurate enough nor continued long enough to be conclusive.

2) For glucose in brain, Sacks (19) reports that the first metabolites appear at 3 min after injection. This is CO_2 , probably from the third and fourth carbons, since Hawkins et al. (11) identified the appearance of some labeled CO_2 after 5 min but none from carbons 1 or 6 until 10 min. Given the small amount of metabolism of the tracer in these hearts, estimated from G_{cell} and an arteriovenous extraction of less than 5%, a delay of 3 or more minutes in appearance is suitable for the known volumes of distribution (water space and bicarbonate space) of the metabolites H_2O and CO_2 . CO_2 in the samples is also fairly likely to be lost with our technique of open sample collecting and pipetting prior to adding scintillator fluid. Our conclusion, not proven but rather likely, is that the dilution curves are composed of untransformed ascorbate and glucose.

The similarity in capillary PS_{CS} is attributable to the combination of the similarity in molecular weights and the negligible degree of passive or carrier-mediated permeation of the luminal surface of the endothelial cells. Combining the observations of this study with those of Kuikka et al. (12), one can say that in the Tyrode-perfused rabbit heart the capillary permeabilities for D- , L- , and 2-deoxy- D- glucose, and L- ascorbate are essentially the same. Since the two forms of D- glucose are normally transported into parenchymal cells, the absence of a difference from L- glucose, which is not transported by a facilitating transporter and enters only very slowly by presumably purely passive mechanisms, implies that ascorbate, like D- and 2-deoxy- D- glucose, does not cross the luminal surface of endothelial cells at more than a negligible rate compared with the rate of diffusional traversal of the clefts between the cells. Thus endothelial cell transport appears negligible even though parenchymal cell uptake is finite. This is the most secure statement to be made from these data.

Estimates of PS_{C} are higher than would be expected in blood-perfused hearts. These Tyrode-perfused rabbit hearts also have an interstitial space expanded by a factor of more than 2 compared with that of intact rabbits (F. Gonzalez and J. B. Bassingthwaighte, published 1990). Both effects are artifacts of the preparation and are partially preventable by using perfusate containing albumin, even at levels less than normal. Nevertheless, this abnormality should not have affected endothelial utilization of glucose, which should be maximal under these conditions in which other substrate is not provided. The endothelial cell volume is very small, in the neighborhood of $0.015 \text{ ml} \cdot \text{g}^{-1}$ or 1.5% of the heart (calculated from $500 \text{ cm}^2 \cdot \text{g}^{-1}$ capillary surface area times a mean cellular thickness of $0.3 \mu\text{m}$). The combination of a small volume and a low rate of entry into the endothelial cells, relative to the high flow, renders the endothelial PS unmeasurable when it is small.

The potential virtue of the absence of substantial endothelial cell transport means that ascorbate may be useful to provide a measure of capillary PS_C under various physiological circumstances. Assuming that surface area (S_C) in a vasodilated preparation does not change, then changes in PS_C might be used to ascertain changes in permeability (P), for example, with changes of albumin concentration in the perfusate. Contrarily, in blood-perfused organs with reactive vascular regulation, if local permeabilities are constant, then changes in PS_C may provide a measure of changes in S_C , i.e., recruitment or derecruitment with reactive hyperemia of hyperoxia. This has particular appeal if the polarographic ascorbate electrode presented by Arts et al. (1) is sufficiently stable in vivo to be used for lengthy periods in the coronary sinus with repeated injections of ascorbate being made into the coronary inflow. In such experiments only PS_C needs to be estimated, which is relatively easy and accurate.

The consistency of the underestimation of PS_C from the extraction maximum [Eq. 3 from Crone (8) and Renkin (16)] invites the possibility of finding a factor to predict the true PS_C from the E_{max} . This might take the form of a multiplier of PS_C (Crone) or of E_{max} , as was found suitable by Guller et al. (10). Either might allow rapid calculation of PS_C and changes in it, without full modeling analysis accounting for flow heterogeneity and back diffusion.

Two simple approximations by which one could estimate PS_C have been tested: 1) $PS_C(k_1) = k_1 PS_C$ (Crone) and 2) $PS_C(k_2) = -F_s \ln(1 - k_2 E_{max})$. In all cases values of $\pm$ were obtained which gave the correct mean estimate, i.e., mean $PS_C(k) = \text{mean } PS_C$. Best values were $k_1 = 1.39$ and $k_2 = 1.20$. But the variances differed little; the CV, using k_1 and k_2 , were 34 and 35%. Our recommendation from this study is to use, for small hydrophilic solutes in rabbit hearts, the expression using k_1 , with its value set at 1.39

$$PS_C = 1.39 F_s \ln(1 - E_{max}) \quad (10)$$

Although the prime purpose of this study was the evaluation of PS_C , estimates of PS_{cell} were also obtained, because the cellular uptake must be accounted for in the analysis. The estimated mean PS_{cell} listed for glucose in Table 1 is $3.1 \pm 3.2 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ ($n = 18$), a high value, but the distribution is skewed greatly by a few exceedingly high values. This mean of the tabulated values is almost surely erroneously high. When PS_{cell} reaches a moderately high level, increasing its value has only a little further effect on lowering the tail of the curve between the 10th and 30th seconds and on raising the tail after 50th second. Thus when there is error in the data or the analysis giving an abnormally high PS_{cell} , this error may be large. The mean PS_{cell} for the 14 curves with the lowest values for PS_{cell} (glucose) is 1.84 ± 0.94 ($n = 14$) $\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$, which is comparable to the values of 1.9 ± 1.3 ($n = 23$) estimated by Kuikka et al. (12) in Tyrode-perfused rabbit hearts, but more than double that found in blood-perfused dog hearts [0.6 ± 0.15 ($n = 11$) $\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$]. While there may be error in the estimates, it would appear safe to conclude that PS_{cell} is actually relatively high in isolated perfused rabbit hearts.

Do errors in estimates of PS_{cell} affect the estimation of PS_C ? The shapes of the dilution curves are considerably more sensitive to PS_C than to PS_{cell} , by a factor of about 40 for the

dog heart studies of Kuikka et al. (Fig. 1 of Ref. 12) and by an approximate factor of 80–200 in the rabbit studies. Estimates of PS_{C} are really totally uninfluenced by errors in PS_{cell} , not just because of the difference in sensitivities, but because the peak of the dilution curve is not influenced by cellular uptake.

The value of PS_{cell} cannot be translated into kinetic parameters of a carrier transporter unless data on PS_{cell} are obtained over a wide range of extracellular glucose concentrations. Following the derivation given by Rosenberg and Wilbrandt (18), the simplified expression for the situation when the intracellular glucose concentration is zero is

$$PS_{\text{cell}} = \frac{P_{\text{CG}} C_{\text{T}} S_{\text{cell}}}{K_{\text{eq}} (1 + P_{\text{CG}}/P_{\text{C}}) + C_{\text{isf}}} \quad (11)$$

where P_{C} and P_{CG} are permeabilities ($\text{cm}\cdot\text{s}^{-1}$) for free carrier and for carrier combined with glucose, C_{T} is the total concentration of all forms of the carrier in the membrane, S_{cell} is cell surface area, K_{eq} is the dissociation constant for the glucose-carrier combination, and C_{isf} is glucose concentration in the interstitium. When $C_{\text{cell}} > 0$, the expression has a second set of similar terms. Since countertransport with 3-*O*-methylglucose has been demonstrated (15), it is clear that P_{CG} is more than P_{C} , perhaps much higher, and the apparent K_{m} and V_{max} , if the process is expressed in terms of first-order MichaelisMenten kinetics, are

$$K_{\text{m}} = K_{\text{eq}} (1 + P_{\text{CG}}/P_{\text{C}}) \quad (12)$$

$$V_{\text{max}} = P_{\text{CG}} C_{\text{T}} S_{\text{cell}} \quad (13)$$

Thus PS_{cell} appears first order, but the K_{m} is higher than the concentration for 50% occupancy of the transporter sites. An appropriate range of perfusate glucose concentrations over which to determine PS_{cell} would be about 0.2–20 mM. PS_{cell} by Eq. 11 must diminish as the concentration of nontracer glucose increases, while the glucose flux rises toward a plateau.

Insulin stimulation would be expected to increase PS_{cell} and to increase transport to such an extent that phosphorylation by hexokinase becomes the rate-limiting step. While this would result in the total tissue glucose concentration approaching the plasma concentration, an increase in V'_{cell} would not be expected. Proper evaluation of PS_{cell} must await future experiments with longer data acquisition times, with plasma glucose levels covering a wide range, with measurement of CO_2 production and the time course of effluent concentrations of tracer-labeled metabolites including CO_2 , including data on glucose labeled at the different carbon positions.

That ascorbate should be taken up by myocytes is hardly surprising since it is involved in a number of intracellular reactions including the provision of hydroxytrimethyllysine for carnitine synthesis. Estimates of PS_{cell} for ascorbate are probably reasonably correct but suffer the same sources of insecurity as do the estimates for glucose. The major function is in oxidative reactions for production of collagens and ground substances such as the “intercellular cement” of the capillary endothelium (20). Thus it is interesting that a rapid

detection system for ascorbate may prove useful for examining the effects of ascorbate deficiency on the integrity of the endothelial layer.

The close similarity between L-ascorbate and D-glucose over the whole of these outflow curves merits further exploration. It is not likely that they use the same sarcolemmal transporter, and even if they did the similarity of rates would seem an unlikely coincidence. Studies with varied concentrations of nonradioactive ascorbate and glucose will be needed to uncover the spectrum of possibilities.

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FIG 1. Coronary sinus outflow dilution curves from a Tyrode-perfused rabbit heart for L- $[^{14}\text{C}]$ ascorbate and D- $[^3\text{H}]$ glucose. ^{131}I -albumin is the intravascular reference marker (*expt I*).

FIG. 2. Outflow dilution curves for L - $[^{14}\text{C}]$ ascorbate and D - $[^3\text{H}]$ glucose fitted with multicapillary aggregates of capillary-interstitial fluid-cell models. Because curves for ascorbate and glucose are so nearly identical, glucose curves have been shifted down by half a decade for this display. *A: experiment 1; B: experiment 15.*

FIG. 3.

Capillary permeability-surface area products (PS_c) for ${}^L[{}^{14}\text{C}]$ ascorbate and ${}^D[{}^3\text{H}]$ glucose in Tyrode-perfused rabbit hearts. Best-fit regression line (assuming equal error) is $y = 1.005x \pm 0.0032$ ($n = 18$, $r = 0.90$).

TABLE 1

Experimental data and estimates of capillary and cell PS products for ascorbate and glucose

Expt	No.	F_s $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	$\bar{t}_{\text{alb}}$ min	% Recovery		E_{max}		PS_C (Crone)		PS_C $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$		V_l , $\text{ml}\cdot\text{g}^{-1}$		PS_{pec} $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$		V_{pc} , $\text{ml}\cdot\text{g}^{-1}$		G_{pec} $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$		CV	
				Alb	Ascorb	D-gluc	Ascorb	D-gluc	Ascorb	D-gluc	Ascorb	D-gluc	Ascorb	D-gluc	Ascorb	D-gluc	Ascorb	D-gluc	Ascorb	D-gluc	Ascorb
A1	1	3.47	0.099	99.1	98.2	99.1	0.36	0.35	1.54	1.47	1.89	1.89	0.38	0.92	0.98	0.45	0.57	0.48	0.53	0.11	0.07
A2	2	2.26	0.087	98.3	97.9	97.7	0.49	0.51	1.50	1.59	2.28	2.28	0.32	2.36	1.81	0.47	0.45	1.06	0.76	0.16	0.15
A3	3	3.76	0.066	104.4	107.0	107.4	0.47	0.51	2.40	2.70	3.30	3.30	0.30	3.34	2.97	0.48	0.48	0.65	0.42	0.15	0.15
B1	4	3.71	0.096	98.8	95.4	97.9	0.39	0.39	1.83	1.82	2.50	2.49	0.34	2.71	1.86	0.45	0.48	0.82	0.71	0.18	0.17
B2	5	2.50	0.105	98.4	99.6	98.9	0.48	0.46	1.62	1.53	1.92	1.92	0.31	0.81	0.90	0.48	0.48	0.38	0.41	0.16	0.14
C1	6	2.84	0.107	87.6	99.6	98.6	0.39	0.34	1.42	1.17	1.46	1.46	0.22	0.99	0.63	0.50	0.63	0.67	0.27	0.36	0.39
C2	7	3.18	0.101	108.5	102.0	100.5	0.52	0.50	2.30	2.22	3.07	3.07	0.30	2.69	1.82	0.48	0.48	1.08	0.87	0.13	0.11
D1	8	2.97	0.117	99.6	99.0	98.5	0.31	0.36	1.08	1.34	1.79	2.21	0.32	1.84	2.79	0.47	0.47	0.76	0.63	0.13	0.15
D3	9	2.87	0.113	100.0	97.8	97.5	0.37	0.39	1.33	1.40	2.03	2.03	0.29	1.83	3.27	0.44	0.51	0.00	0.17	0.27	0.15
D4	10	2.82	0.114	100.0	100.0	99.7	0.32	0.34	1.10	1.18	1.69	1.92	0.30	6.90	2.69	0.47	0.46	0.58	0.48	0.11	0.18
E1	11	1.79	0.152	102.4	107.1	105.8	0.48	0.46	1.19	1.11	1.95	1.95	0.34	13.55	5.06	0.46	0.47	0.82	0.85	0.07	0.08
F1	12	5.07	0.098	110.6	109.5	109.7	0.32	0.33	0.99	1.02	1.11	1.11	0.31	0.27	0.29	0.47	0.47	0.55	0.22	0.19	0.19
G1	13	2.58	0.107	104.0	134.0	117.0	0.46	0.45	1.51	1.44	1.96	1.96	0.23	1.72	5.39	0.39	0.41	0.00	0.00	0.27	0.17
G2	14	2.42	0.124	100.3	113.4	113.3	0.26	0.26	0.72	0.72	1.67	1.67	0.28	5.50	14.35	0.47	0.43	0.77	0.63	0.15	0.14
H1	15	3.79	0.111	101.0	97.7	100.0	0.42	0.41	2.07	2.00	3.09	3.09	0.30	1.76	1.55	0.48	0.48	0.37	0.37	0.30	0.29
H2	16	3.90	0.086	103.0	104.0	106.0	0.43	0.42	2.20	2.09	2.94	2.94	0.30	3.22	2.71	0.48	0.48	0.71	0.56	0.36	0.19
H3	17	3.79	0.184	97.7	101.0	99.6	0.48	0.47	2.48	2.43	3.75	3.75	0.30	5.97	5.30	0.47	0.47	1.60	0.96	0.22	0.11
I1	18	5.07	0.101	95.1	96.6	97.0	0.37	0.39	2.31	2.47	2.51	2.51	0.31	2.62	1.51	0.48	0.48	0.73	0.69	0.25	0.20
Means		3.27	0.109	100.4	103.3	102.4	0.41	0.41	1.64	1.65	2.27	2.31	0.30	3.28	3.10	0.47	0.48	0.67	0.53	0.20	0.17
±SD		±0.89	±0.026	±4.9	±9.1	±6.0	±0.075	±0.071	±0.54	±0.56	±0.71 ±0.69		±0.036	±3.14 ±3.20		±0.023 ±0.048		±0.37 ±0.26		±0.08	±0.07
Paired <i>t</i> test *																					
Difference				0.025				0.18				−0.017				0.14					
SD of difference				0.097				3.33				0.044				0.19					
Student's <i>t</i> test				1.068				0.23				−1.63				3.1					
Significant of test				NS				NS				NS				S at $\alpha = 0.005$					

Alb, albumin; ascorb, ascorbate; D-gluc, D-glucose; other abbreviations as in text.

*. Paired *t* test for differences between ascorbate and glucose.