Duroquinone reduction during passage through the pulmonary circulation

Said H. Audi,1,2 Robert D. Bongard,3 Christopher A. Dawson,1,3,4 David Siegel,5 David L. Roerig,4,6,7 and Marilyn P. Merker4,6,7

1Department of Biomedical Engineering, Marquette University, Milwaukee 53201; Departments of 2Pulmonary and Critical Care Medicine; 3Physiology, 4Anesthesiology, and 5Pharmacology/Toxicology, Medical College of Wisconsin, Milwaukee 53226; 6Zablocki Veterans Administration Medical Center, Milwaukee, Wisconsin 53295; and 7School of Pharmacy, University of Colorado Health Sciences Center, Denver, Colorado 80262

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The lungs can substantially influence the redox status of redox-active plasma constituents. Our objective was to examine aspects of the kinetics and mechanisms that determine pulmonary disposition of redox-active compounds during passage through the pulmonary circulation. Experiments were carried out on rat and mouse lungs with 2,3,5,6-tetramethyl-1,4-benzoquinone [ duroquinone (DQ)], as a model amphipathic quinone reductase substrate. We measured DQ and durohydroquinone (DQH2) concentrations in the lung venous effluent after injecting, or while infusing, DQ or DQH2 into the pulmonary arterial inflow. The maximum net rates of DQ reduction to DQH2 in the rat and mouse lungs were ~4.9 and 2.5 μmol·min⁻¹·g dry lung wt⁻¹, respectively. The net rate was apparently the result of freely permeating access of DQ and DQH2 to tissue sites of redox reactions, dominated by dicumarol-sensitive DQ reduction to DQH2 and cyanide-sensitive DQH2 reoxidation back to DQ. The dicumarol sensitivity along with immunodetectable expression of NAD(P)H-quinone oxidoreductase 1 (NQO1) in the rat lung tissue suggest cytoplasmic NQO1 as the dominant site of DQ reduction. The effect of cyanide on DQH2 oxidation suggests that the dominant site of oxidation is complex III of the mitochondrial electron transport chain. If one envisions DQ as a model compound for examining the disposition of amphipathic NQO1 substrates in the lungs, the results are consistent with a role for lung NQO1 in determining the redox status of such compounds in the circulation. For DQ, the effect is conversion of a redox-cycling, oxygen-activating quinone into a stable hydroquinone. These compounds, as well as their pro- or antioxidant status and propensity for other reactions, with implications for the systemic, as well as pulmonary, effects of such substances. Quinones comprise a class of redox-active compounds having a wide range of physical and chemical properties, and there are a number of mammalian enzymes that have quinone reductase activity. Therefore, compounds can be chosen from this class for properties that are particularly useful for probing processes by which the lungs can affect the redox status of blood-borne redox-active compounds. We carried out the present study to determine the effect of passage through the pulmonary circulation on the redox status of 2,3,5,6-tetramethyl-1,4-benzoquinone (duroquinone, subsequently referred to as DQ), infused or injected into the pulmonary artery of intact lungs from rats and mice, and to examine some aspects of the kinetics and mechanisms that determine the net influence of passage through the lungs on the redox status of DQ exiting via the pulmonary venous effluent. DQ was chosen as the model quinone for this study because it is an amphipathic, nonarylating quinone that has been used extensively in studies of quinone redox metabolism and mitochondrial electron transport in isolated cell and subcellular systems in the past, thus providing direction for studies of the processes involved in DQ disposition in the intact organ.

The results reveal the high capacity of the lungs for reducing DQ to durohydroquinone (DQH2), provide insight into the overall disposition of DQ that determines its redox status in the venous effluent on passage through the lungs, and demonstrate some issues involved in probing plasma-borne substrate redox metabolism in the intact organ.

METHODS

Materials. DQ and other chemicals not otherwise specified were purchased from Sigma (St. Louis, MO). The DQH2 was generated from DQ with potassium borohydride as described.
in Ref. 18. For lung studies, except when oxygen consumption was measured, the perfusate was a salt solution containing (in mM) 4.7 KCl, 2.51 CaCl₂, 1.19 MgSO₄, 2.5 KH₂PO₄, 118 NaCl, 25 NaHCO₃, and 5.5 glucose, with either 5% bovine serum albumin (BSA) or 2.5% Ficoll (average molecular mass 70 kDa). When lung oxygen consumption was measured, the perfusate was Hanks’ balanced salt solution with 10 mM HEPES buffer (pH 7.4), 5.5 mM glucose, and 5% BSA added (referred to as HBSS/HEPES). When the perfusate contained BSA, the sample collection tubes were prepared by adding EDTA to obtain a final sample concentration of 1.5 mM after sample collection.

Lung experiments. Experiments, approved by the Institutional Animal Care and Use Committee of the Veterans Affairs Medical Center, Marquette University, were carried out using lungs from Sprague Dawley rats (Charles River) or CD-1 mice (Charles River). The lung preparation has been described previously for the rat lung (2) and was similar but scaled in size for the mouse lung. Each rat or mouse was anesthetized (pentobarbital sodium, 0.04 mg/g body wt ip). The trachea was clamped, and the chest was opened. Heparin (0.7 U/g body weight) was injected into the right ventricle. The pulmonary artery and trachea were cannulated, and for the rat, the left atrium was also cannulated via the left ventricle. The cannulas were made of polyethylene tubing (1.67 mm inner diameter (ID), 2.42 mm outer diameter (OD) for the rat; and 0.86 mm ID, 1.27 mm OD for the mouse). For the mouse, the heart was cut away allowing the venous effluent to drain directly from the severed pulmonary vein. The lungs were removed from the chest and attached to a ventilation and perfusion system. The single-pass perfusion system was primed with the perfusate maintained at 37°C and equilibrated with a gas mixture of 15% O₂ and 6% CO₂ in N₂ resulting in perfusate P₀₂, PCO₂, and pH of ~105 Torr, 40 Torr, and 7.4, respectively. An initial volume of perfusate (~40 ml for the rat and 4 ml for the mouse) was pumped (Master Flex roller pump) through the lungs until the lungs and venous effluent were visually clear of blood. The flow rate was then set at 10 ml/min for the rat lungs and 2 ml/min for the mouse lungs. The lungs were ventilated with an 8 mmHg end-inspiratory and 3 mmHg end-expiratory pressure at 40 breaths per min for the rat and 80 per min for the mouse and with the same gas composition as used to gas the perfusate. The pulmonary arterial pressure, which was referenced to atmospheric pressure at the level of the left atrium, was monitored continuously during the course of the experiments. For both rat and mouse lungs, the venous effluent pressure was atmospheric pressure. At the end of each experiment, the lungs were weighed dried and reweighed.

To measure the fate of DQ on passage through the lungs, the experiments were either pulse infusion (both rat and mouse lungs) or bolus injection (rat lungs only). For the pulse infusion experiments, the reservoir feeding the perfusion pump was emptied and refilled with perfusate of the composition indicated above and referred to as “control perfusate” or with perfusate of otherwise the same composition but containing one or more of the metabolic inhibitors indicated below, referred to as “experimental perfusate.” After 4–20 min of perfusion with the control or experimental perfusate, the reservoir was refilled with the same perfusate except that DQ or DQH₂ (concentrations dependent on the protocol as indicated in Sample analysis) were included. Samples of the venous effluent were then obtained at intervals during the subsequent 50–120 s of perfusion for the rat lung or 70–150 s of perfusion for the mouse lung. In some pulse experiments, the sampling was continuous, and an additional pulse infusion was carried out with the perfusate containing 4.6 μM fluorescein isothiocyanate dextran (FITC-dex, average molecular wt ~43,200), but without DQ or DQH₂. The FITC-dex was used as a vascular reference indicator to measure the time course for DQ or DQH₂ appearance in the venous effluent had DQ or DQH₂ been simply convected through the pulmonary vessels with the perfusate without interacting with the lung.

For the bolus injection experiments, an injection loop was included in the arterial line to allow introduction of a 0.1-ml bolus into the arterial inflow without altering the flow or perfusion pressure. At the same time that the bolus was introduced into the arterial inflow, the venous outflow was diverted into a sample collector for continuous collection of the lung effluent at a rate and duration depending on the perfusate flow rate. As in the pulse infusion experiments, the reservoir was filled with the desired perfusate, which was recirculated for 15–25 min, after which the reservoir was refilled with fresh experimental perfusate just before the bolus injection. The flow rate was then either left at 10 ml/min or increased to 30 ml/min, and the ventilation was halted. The bolus having the same composition as the perfusate, but with either 0.4 mM DQ or 35 μM FITC-dex or 0.03 μCi [¹⁴C]diazepam and 0.15 μCi [¹⁴C]alfentanil and the FITC-dex added, was then injected.

For the bolus or for the continuous sampling pulse, at the end of each experiment, the lungs were removed from the perfusion system, and an additional FITC-dex bolus or pulse, respectively, was carried out but with the lungs removed and the arterial and venous cannulas connected directly together for rat lungs or with just the arterial cannula for mouse lungs. The data from these “tubing only” experiments were used to obtain the system transit time for calculation of the lung vascular mean transit time and volume. For each kind of experiment, the same procedure was carried out at least once with DQ or DQH₂ bolus or pulse in the tubing only experiment to assess any possible binding of the substrates to the tubing.

Sample analysis. When the perfusate contained BSA, each venous effluent sample from either the pulse or bolus experiments was first centrifuged for 1 min at 5,600 g (model 10MVSS microcentrifuge; Costar, Cambridge, MA). Then, 50–100 μl of each sample were added to each of two microcentrifuge tubes, one prefilled with 10 μl of potassium ferricyanide (1.8–7.2 mM) to oxidize any DQH₂ to DQ, and the other prefilled with 10 μl of deionized water. Ice-cold absolute ethanol (0.4–0.8 ml) was then added, and the samples were mixed and centrifuged at 5,600 g for 5 min at 10°C. One hundred microliters of the resulting supernatant were added to a spectrophotometric quartz microcell, and absorbance at 265 nm was recorded using a Beckman DU 7400 spectrophotometer (Beckman Instruments, Fullerton, CA). When the perfusate contained Ficoll instead of BSA, DQH₂ was oxidized by adding hydrogen peroxide (H₂O₂, 0.1 mM) and peroxidase (7.4 units, Sigma P-8125) to the microcentrifuge tubes, and the absorbance was recorded as above without the addition of ethanol.

Sample concentrations (in μM) of DQ, [DQ], and DQH₂, [DQH₂] were calculated from the absorbances of the fully oxidized (following the addition of potassium ferricyanide or peroxidase/H₂O₂) Abs1 and the original unoxidized (Abs2) venous samples and from the micromolar extinction coefficients at 265 nm of DQ, 0.02164 μM⁻¹ cm⁻¹, and DQH₂, 0.0017 μM⁻¹ cm⁻¹, as follows

\[
\text{Abs1} = 0.02164([\text{DQ}] + [\text{DQH}_2])
\]

\[
\text{Abs2} = 0.02164[\text{DQ}] + 0.0017[\text{DQH}_2]
\]
For a given venous sample, [DQ] and [DQH₂] are given by Eq. 3, which is the solution of Eqs. 1 and 2

\[
\frac{Abs_2 - \frac{0.0017}{0.02164} Abs_1}{0.02164 - 0.0017} = [DQH_2] = \frac{Abs_1}{0.02164} - [DQ]
\]

The FITC-dex concentrations were quantified from the absorbance at 495 nm using a molar extinction coefficient of 93,478 M⁻¹ cm⁻¹, and for venous samples containing [¹⁴C]diazepam and [³H]alfentanil, the ¹⁴C and ³H were measured by liquid scintillation counting.

A perfusate sample that had passed through the lungs but contained no DQ or DQH₂ was treated in the same manner as the rest of the samples and used as the blank for absorbance measurements. In addition, for the bolus experiments, measured volumes of the bolus solution were added to samples that emerged in the venous effluent before the appearance of the bolus contents. These samples were used as standards for determining the fraction of bolus contents per milliliters of venous effluent sample. For the pulse infusion protocol, the lung effluent concentration was compared with the perfusion system effluent concentration measured with the lungs removed from the perfusion system.

**Lung oxygen consumption.** To measure the rat lung oxygen consumption, we prepared the lungs as indicated above except that, after the rat was anesthetized, it was provided with 100% O₂ to breathe for 5 min. The trachea was then occluded before the chest was opened. This resulted in the absorption of all alveolar gas, so when the lungs were perfused, the source of oxygen was that dissolved in the perfusate. The lungs were again connected to the perfusion system supported by the tracheal, arterial, and venous cannulas, but they were not ventilated. The perfusion system was primed with the HBSS/HEPES perfusate and equilibrated with room air.

After the blood was washed from the lungs, the venous cannula was connected to the stirred reservoir, thereby forming a recirculating perfusion system containing a total of 30 ml of perfusate including the lung vascular volume. The PO₂ of the reservoir was monitored continuously on a YSI 5300 Biological Oxygen Monitor (YSI). To begin an oxygen consumption measurement, the perfusate PO₂ was raised to ~160 Torr (a little higher than atmospheric oxygen) by bubbling with oxygen. Then the decrease in reservoir PO₂ (PO₂res) with time was monitored for ~10 min or until it had fallen to ~60 Torr, depending on which came first.

To calculate the lung oxygen consumption, the decrease in PO₂res, with time (t) as oxygen was consumed by the lungs was represented by

\[
\frac{dPO₂res}{dt} = -k_a + k_b(PO₂_{atm} - PO₂_{res})
\]

where kₐ (Torr/min) is the rate of change in PO₂res(t) due to oxygen consumption by the lungs, and kₗ (min⁻¹) is a diffusion coefficient-like term reflecting any exchange of oxygen between the perfusate and atmospheric PO₂ (PO₂atm). The diffusion term was included because no attempt was made to prevent any small amount of oxygen diffusion through the pleural surface or to perfectly seal the reservoir. Equation 5 is the solution for Eq. 4 with PO₂res(t = 0) = PO₂atm.

\[
PO₂res(t) = PO₂_{atm} - \frac{k_b}{k_a} [1 - \exp(-k_a t)]
\]

where kₐ and kₗ were obtained by fitting Eq. 5 to the PO₂res(t) data. The oxygen consumption (µmol/min) of the lung was then kₐ times the perfusate oxygen solubility times the recirculating perfusate volume.

**Lung experimental protocols.** To determine the capacity of the rat and mouse lungs to effect conversion of pulmonary arterial DQ to pulmonary venous DQH₂, we used the pulse infusion method with the lungs perfused with control perfusate at 10 ml/min for rat lungs or 2 ml/min for mouse lungs. Four pulse infusions were performed with sequentially increasing reservoir (pulmonary arterial) DQ concentrations, and then a fifth infusion was a repeat of the low concentration infusion carried out to evaluate the effect of DQ infusion history and or time on the results.

To determine the influence of the metabolic inhibitors on the redox status of DQ in the venous effluent from rat and mouse lungs, we carried out the pulse infusions using the control perfusate and then repeated it with experimental perfusate containing cyanide (2 mM) alone, rotenone (10–20 µM), followed by rotenone plus cyanide, dicumarol alone (20 µM with BSA perfusate, 5 µM with Ficoll perfusate), followed by dicumarol plus cyanide.

The DQ bolus experiments in rat lungs were carried out to evaluate the accessibility of DQ and DQH₂ to their respective tissue volumes of distribution. For DQ, this required first inhibiting DQ reduction, which was accomplished by perfusing the lung with perfusate wherein glucose was replaced by 10 mM 2-deoxyglucose (2-DG), and the flavoprotein inhibitor diphenylenedionium (DPI, 10 µM) was added 15 min before the DQ bolus injection. The [¹⁴C]diazepam and [³H]alfentanil bolus experiments were carried out to determine the rat lung capillary volume and transit time distribution as described in (7).

The oxygen consumption was measured with control perfusate, and the lungs were divided into two groups. In one group, rotenone (17.7 ± 2.9 (SD) µM) and thenoyltrifluoroacetone (TTFA, 17.7 ± 2.9 (µM) were then added to the perfusate, and the oxygen consumption was measured again. Then with the rotenone and TTFA still present, DQ (0.177 ± 0.029 mM) was added. Finally, with rotenone, TTFA, and DQ still present, cyanide (1.77 ± 0.29 mM) was added. In the other group, cyanide alone was added to the perfusate after the control measurement.

Statistical comparisons of effluent [DQ], [DQH₂], or oxygen consumption between control and experimental conditions was by ANOVA, followed by Tukey’s test with P < 0.05 as the criterion for statistical significance.

**Additional measurements.** The DQ (250–1,000 µM) and DQH₂ (125–250 µM) binding to 5% BSA or DQ (50 µM) and DQH₂ (50 µM) binding to 2.5% Ficoll in the perfusate was determined using the Amicon ultrafiltration system as previously described (7, 8). At 5% BSA, 95.6 ± 0.5 (SE) % of the DQ and 76 ± 1.0% of the DQH₂ were BSA bound, and the bound fractions were independent of DQ and DQH₂ concentrations within the concentration range studied. At 2.5% Ficoll, 63% of the DQ and 60% of the DQH₂ were associated with Ficoll. For the model interpretation of the DQ disposition in the lungs described below (see kinetic models), these fractions are converted to α₁ and α₃, defined by α₁ = 1 – (DQ bound fraction/DQ free fraction) and α₃ = 1 + (DQH₂ bound fraction/DQH₂ free fraction), respectively. Thus the values of α₁ and α₃ were set at 25 and 4.2, respectively, for model simulations of experiments carried out with the 5% BSA perfusate and at 2.7 and 2.5, respectively, for the 2.5% Ficoll perfusate.

DQH₂ auto-oxidation was evaluated by monitoring the [DQ] in lung effluent samples (with and without EDTA added to the BSA perfusate) for 50 min following collection. In
EDTA-free BSA perfusate effluent samples, the auto-oxidation rate of DQH2 was <1%/min and undetectable on that time frame in Ficoll perfusate or BSA perfusate with EDTA added.

To confirm the presence of NAD(P)H-quinone oxidoreductase 1 (NQO1) in the rat lungs, we carried out immunoblot analysis as follows. After perfusion, the lungs were weighed, minced, and homogenized in 5 ml buffer/g tissue [buffer composition (in mM): 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 250 sucrose, 3 EDTA, 1 phenylmethyl-sulfonyl fluoride, and 1% protease inhibitor cocktail (Sigma, catalog no. P8340)] at 4°C using a Polytron homogenizer. The homogenate was centrifuged for 65 min at 145,000 g. A portion of the resulting supernatant (41 μg of protein) and purified recombinant human NQO1 protein (0.25 ng) (47) were subjected to electrophoresis using Invitrogen’s (Carlsbad, CA) NuPAGE LDS sample buffer, 4–12% gradient NuPAGE Bis-Tris gel, and MES-SDS running buffer. The proteins were transferred to a nitrocellulose membrane, which was incubated for 1 h in Tris-buffered saline containing 0.1% Tween 20 and 2% BSA, the latter as a blocking agent. The membrane was then incubated sequentially in a 1:10 dilution of tissue culture supernatant containing anti-NQO1 monoclonal antibodies (IgG1) from two mouse hybridoma cell lines (A180 and B771, mixed 1:1) (47), a 1:7,500 dilution of goat α-mouse IgG-horseradish peroxidase (Jackson ImmunoResearch Laboratories), and the Supersignal West Pico Chemiluminescent substrate (Pierce). The signal was captured on CL-Xposure Film (Pierce). As a control, nonspecific mouse IgG1 was substituted for the α-NQO1 monoclonal antibodies.

EXPERIMENTAL RESULTS

Table 1 provides some general information on the lungs and animals studied. In addition, the perfusion pressures at the beginning and end of the experiments were for rats (10 ml/min flow rate) 6.3 ± 0.3 and 6.7 ± 0.4 mmHg, respectively, and for mice (2 ml/min) 6.4 ± 0.2 and 6.8 ± 0.3 mmHg, respectively, and were not significantly different among lungs perfused with the different experimental perfusates. For the rat lungs perfused at 30 ml/min, the pressure averaged 8.9 ± 0.7 mmHg. The wet/dry lung ratios were not significantly different in the lungs perfused only with control perfusate vs. lungs perfused with control and experimental perfusate(s) (5.7 ± 0.1 vs. 5.6 ± 0.1, respectively, for rats; 5.5 ± 0.1 vs. 5.6 ± 0.1, respectively, for mice). Because lung injury is typically accompanied by increased vascular resistance and wet/dry weight, the comparisons between experimental and control conditions suggest that the inhibitors did not cause significant nonspecific injury to the lungs on the time frame of the experiments.

Figure 1, A and B, shows examples of the general form of the effluent concentrations of DQ, DQH2, or FITC (as fractions of the pulmonary arterial [DQ] or [FITC]) obtained with pulse infusions of DQ and FITC with BSA- or Ficoll-containing perfusate in the rat lungs. The FITC curve represents what the DQ curve would have been had DQ simply remained in the perfusate and not interacted with the lung as it passed through the lung vessels. A large fraction of the infused DQ reappeared in the venous effluent as DQH2. When BSA was included in the perfusate, the sum of the effluent DQ concentration ([DQ]) plus DQH2 concen-

![A](image1.png)  ![B](image2.png)

Fig. 1. Examples of the time course for the venous effluent concentrations (as fractions of the respective input concentration) of FITC-dextran (dex), duroquinone (DQ), and duroquinol (DQH2) during a pulse infusion of FITC-dex or DQ into the pulmonary artery of each of 2 rat lungs, 1 perfused with the BSA-containing perfusate (A) and the other perfused with the Ficoll-containing perfusate (B) (flow rates 10 ml/min, 45 μM infusions). The solid lines are model fits to data.
tration ([DQH₂]) was nearly equal to infused [DQ], and the [DQ], [DQH₂], and [FITC-dex] were nearly steady by ~50 s of infusion. When Ficoll was used instead of BSA, the DQH₂ fraction was greater than with BSA perfusate, and it took longer for the DQ and DQH₂ to approach the quasisteady state, reflecting the greater tissue to perfusate partition coefficient in the absence of albumin binding.

Figure 2 is representative of DQ and DQH₂ infusions in the mouse lung. When DQ was infused into the mouse lung, a considerably smaller fraction emerged as DQH₂ than in the rat lung, and when DQH₂ was infused, its oxidation to DQ was also more evident than in the rat lung. As in the rat lung, a nearly steady state was achieved within the infusion period.

The capacity of rat and mouse lungs for DQ reduction was evaluated by varying the infused [DQ]. However, to assess the possibility of residual effects of previous infusions in these experiments, we repeated the low-concentration (~45 μM) pulse after the full concentration range had been covered. The low-concentration infusions produced DQH₂ fractions that were 0.88 ± 0.03 SE at the beginning and 0.87 ± 0.02 at the end of this protocol for the rat lungs and 0.36 ± 0.02 and 0.32 ± 0.01, respectively, for the mouse lungs. Having established that the multiple pulses and high DQ concentrations did not have significant cumulative or irreversible effects, we plotted the steady-state rate of DQH₂ output from rat and mouse lungs vs. the log mean concentration of the infused DQ (Fig. 3). The rate of DQH₂ output is the effluent [DQH₂] times the flow rate. The log mean DQ concentration, namely ([DQ]_{in} – [DQ]_{out}) ln([DQ]_{in}/[DQ]_{out}), where [DQ]_{in} is effluent [DQ] + [DQH₂] and [DQ]_{out} is effluent [DQ], approximates the effective [DQ] within the lung vessels, if one takes into account the fact that the [DQ] decreases as the perfusate passes through the vessels (10). The net rate vs. log mean concentration data have a hyperbolic appearance, with apparent maximum rates of DQH₂ output of 4.9 and 2.5 μmol·min⁻¹·g dry lung wt⁻¹ for rat and mouse lungs, respectively, with a log mean perfusate [DQ] resulting in half the these maximum rates, being 32 μM (free + BSA bound DQ) and 83 μM for rat and mouse lungs, respectively. On the basis of the fraction of DQ bound to BSA, these values are equivalent to 1.3 and 3.4 μM free [DQ], respectively.

Figure 4 summarizes the pulse infusion data obtained from rat lungs with the control perfusate or with the experimental perfusate containing rotenone, cyanide, or cyanide plus rotenone. When DQ was infused, most was reduced to DQH₂, and when DQH₂ was infused, a small fraction was converted to DQ. Cyanide decreased the effluent DQ fraction during DQH₂ infusion. Rotenone treatment resulted in lower effluent DQH₂ fraction during DQ infusion and higher DQ fraction during DQH₂ infusion, both of which were reversed by the addition of cyanide.
fraction during DQH\textsubscript{2} infusion, which, as in the rat lung, was reversed by cyanide.

An initial attempt was made to evaluate the effect of dicumarol in the rat lungs with the BSA-containing perfusate. Dicumarol (20 \textmu M) had little effect on the effluent DQH\textsubscript{2} fraction during the infusion of DQ (89.0 \pm 1.4 SE \% vs. 84.4 \pm 2.1\%, respectively) or the DQ fraction during the infusion of DQH\textsubscript{2} (9.5 \pm 0.6\% vs. 10.0 \pm 0.8\%, respectively). Given the high affinity of dicumarol for plasma albumin (11), we then examined the dicumarol effect with the BSA perfusate replaced by the Ficoll perfusate. Figure 6 is a summary of the effects of dicumarol in the absence of albumin, showing that dicumarol markedly inhibited DQH\textsubscript{2} output during DQ infusion. Interestingly, most of this dicumarol effect was reversible by cyanide.

The bolus experiments were carried out to provide data in which the transient information about DQ disposition on passage through the lungs is emphasized, thus providing insight into the tissue permeation of DQ and DQH\textsubscript{2}. Figure 7 presents the data obtained from bolus injections into the pulmonary artery of a rat lung with the flow rate set at 10 or 30 ml/min and without or with the reduction inhibited by including DPI (10 \textmu M) and 2-DG (10 \textmu M) in the perfusate. The rationale behind the use of 2-DG and DPI to inhibit reduction in the lungs was that 2-DG would decrease production of electron donor NAD(P)H and that quinone reductases are generally flavoproteins inhibited by DPI. This inhibitor regimen was used rather than dicumarol for these experiments because removal of BSA from the perfusate, required for dicumarol inhibition, results in a large DQ tissue-to-perfusate partition coefficient. The resulting low effluent DQ and DQH\textsubscript{2} concentrations during the bolus transient are disadvantageous for the data analysis described in KINETIC MODEL. As in the pulse experiments, the FITC-dex concentration vs. time curves indicate what the
emerged as DQH2. When reduction was inhibited, the DQ and with the control perfusate most of the DQ injected on the reference indicator out fl superimpose the relative concentrations of DQ and DQH2 were re -

Fig. 8 results for the [DQ]/H11001 distribution into the tissue (7, 29). By comparing the dex curves in a manner reminiscent of a shift to the right and more dispersed than the FITC-

B and

C

and the [DQ] curve in Fig. 7C is that they are shifted to the right and more dispersed than the FITC-dex curves in a manner reminiscent of a flow-limited distribution into the tissue (7, 29). By comparing the Fig. 8 results for the [DQ] + [DQH2] curves obtained for the two flow rates with the time scales adjusted to take into account the flow-dependent difference in vascular residence times, we see that the shift appears to be virtually independent of the flow rate, which is also indicative of a flow-limited tissue distribution. The total DQ + DQH2 recovered during the sampling period was >98% for both 10 and 30 ml/min flow rates, and with the control perfusate most of the DQ injected emerged as DQH2. When reduction was inhibited, the relative concentrations of DQ and DQH2 were reversed, but otherwise the shapes of the curves were not much different. However, the total DQ + DQH2 recovered in the venous effluent was only 80%.

These bolus data suggest that, on the time scale of these experiments, there appears to be no significant barrier to the diffusion of DQ or DQH2 into the tissue. As a formal test of this interpretation of the form of DQ + DQH2 curves, the delayed wave transformation developed by Goresky (29) was applied. For this transformation, if the time axis of the flow-limited indicator outflow concentration curve is shifted backward by the common time, t₀, the concentration values are scaled by a factor λ, and the shifted time axis is scaled by 1/λ, then shifting the scaled time axis forward by tₛ should superimpose the flow-limited indicator outflow curve on the reference indicator outflow curve (29). The optimization for t₀ and λ from the FITC-dex reference and the DQ + DQH2 concentration vs. time curves was carried out using the International Mathematics and Statistics Library (IMSL) subroutine DBCLSF as previously described (5). The results shown in Fig. 8, C and D, for 10 or 30 ml/min suggest superposition consistent with a flow-limited distribution into the tissue. The delayed wave transformation was also applied to the [14C]diazepam and [3H]alfentanil data in Fig. 9, thereby allowing for comparison between the transformation applied to the [DQ] + [DQH2] data to the two previously established flow-limited indicators (7), each having a different tissue perfusate partition coefficient (i.e., a different virtual volume of distribution).

Because the reduction was so nearly complete with the control perfusate, one possibility that might be considered is that DQ itself does not actually enter the cells, but instead the reduction occurs on the cell surface followed by cell permeation of the resulting DQH2. This possibility seems to be ruled out by the fact that the DQ + DQH2 retained its flow-limited character even when the reduction was nearly eliminated and the effluent was mainly DQ rather than DQH2 (Fig. 7C). When the reduction was inhibited, some of the DQ also remained in the lungs, and, therefore, the delayed wave transformation was not applicable. However, the flow-limited model was able to account for the data as indicated in the kinetic model results described in KINETIC MODEL. This, along with the fact that the quinones are generally less hydrophilic than their respective hydroquinones, is consistent with both forms having very rapidly equilibrating access to their respective tissue volumes of distribution.

The distribution of pulmonary capillary transit times [hₜ(t)] was estimated from the bolus effluent concentration vs. time curves of FITC-dex and the two additional flow-limited indicators [14C]diazepam and [3H]alfentanil (Fig. 9A), as previously described (5, 7). This was necessary to account for the transit time
dispersion effects on the disposition of a substrate such as DQ or DQH₂ as it passes through the lungs (6). The mean transit time (t₀), variance (σ²), and third central moment (m₃) of the hₙ(t) were estimated from the rat lung FITC-dex, [¹⁴C]diazepam, and [³H]alfentanil outflow curves, as previously described (5, 7). The values of the estimates at 30 ml/min were t₀ = 0.93 ± 0.08 (SD) s, σ² = 0.73 ± 0.12 s², and m₃ = 1.32 ± 0.42 s³. The capillary relative dispersion (RDₙ = √(σ²/t₀)) and capillary coefficient of skewness [SKWₙ = (m₃)/σ²] of hₙ(t) are given in Table 1.

The oxygen consumption provides a measure of the overall rate at which reducing equivalents are being utilized by the lungs under the respective experimental conditions. In addition, the data in Fig. 10 demonstrate that the depression of oxygen consumption by complex I and/or II inhibitors was reversible by DQ and, furthermore, that this DQ effect was inhibited by cyanide. The immunoblot shown in Fig. 11 confirms the presence of NQO1 in the rat lungs. The mouse α-human NQO1 monoclonal antibody does not bind sufficiently to the mouse enzyme to be useful for this evaluation in the mouse lung.

KINETIC MODEL

To aid interpretation of data obtained with bolus and pulse experiments, we expressed the hypothesized fate of DQ on passage through the lungs using the kinetic model presented in Ref. 8 with some modification for the specifics of DQ disposition (see APPENDIX). The model equations (Eqs. A1–A4), for a single capillary element consisting of a capillary volume and its surrounding tissue volume accessible to DQ and DQH₂, become the basis for the whole organ model by taking into account the distribution of hₙ(t). During the transients produced by the bolus injection or the presteady-state phase of the pulse infusion, the identifiable model parameters include the reduction rate parameter (k₉red) and the oxidation rate parameter (k₉ox), both with units of milliliters per second. To put these parameters in perspective, if one were to assume Michaelis-Menten reaction kinetics, they would be approximations to the Vₘₐₓ/Kₘ ratio for the overall DQ reduction and DQH₂ oxidation processes, respectively, that are contributing under a given set of experimental conditions. The other parameters are the DQ sequestration rate constant (k₇,
in μM⁻¹·s⁻¹); the number of DQ sequestration sites (Y₀, in nmol), and the virtual volumes of distribution (VF₁ and VF₂, in ml), which are measures of any rapidly equilibrating tissue interactions of DQ and DQH₂, respectively.

The model fits to the data in Figs. 1, 2, and 7 are included to demonstrate the extent to which the model represented by Eqs. A1–A4 is capable of explaining the transient data over the range experimental conditions studied. The model fits were obtained using all the

Fig. 9. A and B: venous effluent concentration vs. time curves for FITC-dex, [³H]alfentanil (A), and [¹⁴C]diazepam (B) following the bolus injection of these indicators into the pulmonary artery of an isolated perfused rat lung. C and D: results of the delayed wave transformation carried out on the data in A and B.

Fig. 10. Rat lung oxygen consumption. Means ± SE, n = 6 for the control. For 3 of the lungs, the experimental sequence was the control measurement followed by rotenone + thenoyltribluoroacetone (TTFA), rotenone + TTFA + DQ, and then rotenone + TTFA + DQ + cyanide. For the other 3 lungs, the control measurement was followed by cyanide treatment alone. *Significantly lower than control.

Fig. 11. Immunoblot of rat lung homogenate NAD(P)H-quinone oxidoreductase 1 (NQO1). The left lane is a sample of supernatant from rat lung homogenate containing 41 μg of protein. The right lane is 0.25 ng of human recombinant NQO1. The arrows indicate the positions of molecular mass markers. There were no detectable corresponding bands when nonspecific IgG₁ was used as a control in place of α-NQO1 antibody.
data on the respective figures simultaneously, as indicated in Refs. 3, 4, 8, and the APPENDIX. The values of the model parameters for the bolus injection experiments (Fig. 7) were estimated with \( k_{red} \) allowed to change in the presence of 2-DG and DPI but assumed to be flow independent. The values were \( k_{red} = 291 \text{ ml} \cdot \text{s}^{-1} \cdot \text{g dry wt}^{-1}, k_0 = 2.4 \text{ ml} \cdot \text{s}^{-1} \cdot \text{g dry wt}^{-1}, k_2Y_0 = 5.7 \text{ ml} \cdot \text{s}^{-1} \cdot \text{g dry wt}^{-1}, V_{F1} = 162 \text{ ml/g dry wt}, \) and \( V_{F2} = 20 \text{ ml/g dry wt}. \) Treatment with 2-DG and DPI decreased \( k_{red} \) to 13.8 \text{ ml} \cdot \text{s}^{-1} \cdot \text{g dry wt}^{-1}.

For the steady state during the pulse infusion, Eqs. A2–A4 in the APPENDIX reduce to

\[
WV_e \frac{d[DQ]}{dx} = [DQH_2][\frac{k_0}{\alpha_3}] - [DQ][\frac{k_{red}}{\alpha_1}] \\
WV_e \frac{d[DQH_2]}{dx} = -[DQH_2][\frac{k_0}{\alpha_3}] + [DQ][\frac{k_{red}}{\alpha_1}] 
\]

where \([DQ] = [DQ]\alpha_1 \) and \([DQH_2] = [DQH_2]\alpha_3 \) are the total (free + bound) vascular concentrations of DQ and DQH_2, respectively. \([DQ](x,t)\) and \([DQH_2](x,t)\) are the vascular concentrations of free DQ and DQH_2 forms, respectively, at distance \( x \) from the capillary inlet and time \( t \) (see APPENDIX for definitions of other symbols); \( \alpha_1 \) and \( \alpha_3 \) respectively account for the rapidly equilibrating interactions of DQ and DQH_2 with perfusate BSA or Ficoll (see APPENDIX). \([DQH_2](x)\) at any distance \( x \) is equal to the infused \([DQ](x = 0) + [DQH_2](x = 0)\); \( W = L/\tau_c \), where \( x = 0 \) and \( x = L \) are the capillary inlet and outlet, respectively.

The capillary outlet \((x = L)\) steady-state \([DQ]\) during the infusion of DQ is given by Eq. 8, which is the solution of Eq. 6 with the boundary conditions \([DQH_2](x = 0) = 0\) and \([DQ](x = 0) = [DQ]_0\).

\[
[DQ](x = L) = \frac{\beta_1}{\beta_2} + \frac{[DQ]_0 - \beta_1}{\beta_2} \exp\left(-\frac{\beta_2}{F}\right) 
\]

where \([DQ]_0\) is the total (free + protein bound) infused \([DQ]\)

\[
\beta_1 = \frac{[DQ]_0 k_0}{\alpha_3} \\
\beta_2 = \frac{k_0 + k_{red}}{\alpha_1} 
\]

The capillary outlet \((x = L)\) steady-state \([DQ]\) following the infusion of \([DQH_2]\) is given by Eq. 9, which is the solution of Eq. 7 with the boundary conditions \([DQH_2](x = 0) = [DQH_2]_0\) and \([DQ](x = 0) = 0\)

\[
[DQ](x = L) = \frac{\beta_1}{\beta_2} - \frac{[DQH_2]_0 - \delta_1}{\beta_2} \exp\left(-\frac{\beta_2}{F}\right) 
\]

where \( F \) is the total flow rate, \([DQH_2]_0\) is the infused \([DQH_2]\), and

\[
\delta_1 = \frac{[DQH_2]_0 k_{red}}{\alpha_1} 
\]

For a given \( h_c(t) \) or relative flow distribution \( W(f) \), the venous effluent steady-state DQ concentrations following the infusion of DQ or DQH_2 are given by Eqs. 10 and 11, respectively

\[
[DQ] = \frac{\beta_1}{\beta_2} + \sum_{i=1}^{N_f} f_i W(f_i) \cdot \Delta f_i \left(\frac{[DQ]_0 - \beta_1}{\beta_2} \exp\left(-\frac{\beta_2}{F}\right)\right) 
\]

\[
[DQ] = \frac{\beta_1}{\beta_2} - \sum_{i=1}^{N_f} f_i W(f_i) \cdot \Delta f_i \left(\frac{[DQH_2]_0 - \delta_1}{\beta_2} \exp\left(-\frac{\beta_2}{F}\right)\right) 
\]

where \( N_f \) is the number of different transit times through the capillaries \((t_i)\) or flow rates, \( F_i = F \cdot f_i \), where \( f_i = t_i/\tau_c \) is the flow through all capillaries having the i-th transit time relative to the mean flow \((6)\); \( W(f_i) \) and \( \Delta f_i \) are the frequency of occurrence of \( f_i \) and the relative flow increment, respectively, and are related to the \( h_c(t) \) by \( W(f_i) \cdot \Delta f_i = h_c(t_i) \cdot \Delta t_i \), where \( \Delta t_i \) is the capillary transit time increment.

Thus under steady-state conditions, the model parameters decrease to simply \( k_{red} \) and \( k_0 \), identifiable from the steady-state \([DQ]\) following separate infusions of DQ and DQH_2 using Eqs. 10 and 11 in a Levenberg-Marquardt optimization routine (35). The values are given in Figs. 12 and 13 for the various experimental conditions.

DISCUSSION

The results demonstrate the large capacity of the lungs, especially of the rat, to convert DQ to DQH_2 as DQ passes through the pulmonary circulation, and
they establish the basis for a kinetic model of DQ disposition in the intact lung. The model incorporates a set of hypotheses regarding DQ and DQH2 transport and redox interconversions and allows for comparisons of reaction rates under the model assumptions stipulated by those hypotheses.

With regard to DQ and DQH2 transport, the bolus data suggest that DQ and DQH2 freely access their respective tissue volumes of distribution wherein the reactions occur. That is to say, at any time and location orthogonal to the axis of flow through the lung capillaries, the concentrations of DQ and DQH2 at the reaction sites are essentially in equilibrium with their respective perfusate concentrations, without a significant intervening transport barrier. This conclusion, based on the delayed-wave, or flow-limited, behavior, does not in itself mean that reduction of DQ does not occur at the cell surface. In fact, endothelial cell (9) as well as other cell (1, 26, 31, 40, 48, 53) surfaces have quinone reductase activity. It simply means that the concentration at the reaction sites, wherever they are, is essentially in equilibrium with the perfusate concentration. This is consistent with the amphipathic nature of DQ, which has a fairly high water solubility (1.6 mM, Ref. 21) along with a high octanol/water partition coefficient (131, Ref. 42).

A corollary to this conclusion is that the association of both DQ and DQH2 with the plasma albumin (BSA) in the perfusate is rapidly equilibrating (4, 7). Any significantly slowly dissociating albumin-bound fraction would have resulted in throughput that would have degraded the delayed wave transformation. Thus flow-limited access and rapidly equilibrating association with albumin are two of the model assumptions (hypotheses).

The \( h_u(t) \), referred to as the “capillary transport function” (6, 7), is another aspect of the DQ transport through the lungs included in the model. Because this information was not previously available for the rat lung, the method for its determination using the [\(^{14}\)C]diazepam and [\(^{3}\)H]alfentanil in the bolus injection experiment was applied (7). Because the results indicate that \( h_u(t) \), normalized with respect to lung size, for the normal rat lungs was similar to that previously obtained for the rabbit lung (7), application of that method to the smaller mouse lung was not considered to be worth the additional effort. It was assumed that \( h_u(t) \) was similar enough in all the lungs in the study, including the mouse lungs, that after normalization to account for differences in lung sizes (vascular volumes) and flow rates, little error would be introduced by the assumption that they were the same.

Given these aspects of the DQ and DQH2 transport on passage through the lungs, the model also includes representations of the dominant DQ reactions, namely, reduction of DQ, oxidation of DQH2, and sequestration of DQ. Figure 3 demonstrates that the capacity for converting DQ to DQH2 was large. Despite the extensive literature on quinone biochemistry, particularly in fields such as toxicology, oncopharmacology, and mitochondrial electron transport (11, 14, 16–19, 22, 24–27, 30–34, 36, 40, 44–46, 49, 50–55), there is a paucity of comparable studies on any intact organ, much less on the lungs. However, this capacity of the lungs may be appreciated by comparing the apparent maximum DQH2 efflux rate (~4.9 \( \mu \)mol min\(^{-1} \) g dry lung wt\(^{-1} \)) and the rate of oxygen consumption (~2.4 \( \mu \)mol min\(^{-1} \) g dry lung wt\(^{-1} \)) with that reported for slices of rat lungs (148–48 \( \mu \)l min\(^{-1} \) g dry wt\(^{-1} \))

Figure 3 also establishes the concentration dependence of the net reaction, and the rest of the pulse infusion experiments in the study were carried out using inlet concentrations <50 \( \mu \)M, which was considered to be advantageous for measurement, with only minor violation of the linear reaction approximations used in the model.

The sequestration of DQ is the model representation of any mechanism by which the DQ is prevented from returning to the perfusate as either DQ or DQH2 within the time frame of the experiments. DQ is less water soluble than DQH2 (21), which may contribute to a greater propensity for nonspecific associations with other molecules, including the BSA in the perfusate, compared with DQH2. Any slowly dissociating interactions fall into the sequestration category and do not contribute to the effluent DQ and [DQH2] after the transient phase has passed. Likewise, any rapidly dissociating associations within the tissue do not contribute during the steady state except insofar as they...
contribute to the tissue-perfusate partition coefficient, and they are not separable from the physical tissue volume of distribution.

The resulting model (APPENDIX) is similar to that developed in Ref. 8, with one simplification being that because auto-oxidation of DQH2 is virtually nil in the time frame of the experiments, auto-oxidation is not included in the model. The model applied to the bolus or the transient portions of the pulse data involves six free parameters representing the intrinsic rates of DQ reduction: kred, DQH2 oxidation (ko), DQ sequestration (k7), Y0, and the virtual tissue volumes Vf1 and Vf2 accessible to DQ and DQH2, respectively. Although these parameters are independently identifiable from the data from a bolus injection, they are highly correlated, which undermines their robust estimation from the data from a single bolus or pulse infusion. Therefore, the full model (Eqs. A1–A4) was fit to data from multiple bolus (Fig. 7) or pulse infusions (Figs. 1 and 2) carried out under widely varying conditions (4). That is, for the data in Figs. 1, 2, and 7, the model was fit simultaneously to all the data shown.

Given the flow-limited distribution of DQ and DQH2, the model for the steady-state portions of the pulse infusions becomes considerably simplified, with kred and ko the only free parameters. However, they are not independently identifiable from a single pulse. At least two pulses are required, with either the flow rate, the albumin binding, or the ratio of infused DQ to DQH2 varied between pulses to obtain parameter identifiability. Although the full model fit to the transient data is a best least-squares fit to an overdetermined data set, the steady-state model fit, with two parameters and two data points (the venous reduced or oxidized fraction from two pulses), is implicit but exact. The data in Figs. 4–6, showing the effluent fractions of DQH2 during the DQ infusion or the effluent fractions of DQ during the DQH2 infusion, along with reduction and oxidation rate parameters (Figs. 12 and 13) from the steady-state model analysis, support several additional conclusions regarding DQ disposition on passage through the lungs.

The kinetic parameters obtained from the pulse infusion data are presented in Figs. 12 and 13 in a fashion analogous to the concentration data presented in Figs. 4–6. This representation emphasizes the relationships between kred and ko needed to account for the effluent concentration data and the effectiveness of dicumarol inhibition on kred in the rat lungs. Comparison between the rate parameter (Figs. 12 and 13) and concentration (Figs. 4–6) data also helps to expose the impact that even a small ko compared with kred can have on net reduction. This is most obvious for the mouse lungs wherein a ko/kred of only −0.36 can account for the greater net oxidation of infused DQH2 than reduction of infused DQ. This is explainable largely by the different affinities of DQ and DQH2 for BSA. The difference between rat and mouse lungs could be attributed entirely to the lower kred in the mouse lung (note the difference in scale between Figs. 12 and 13). The kreds for the rat and mouse lungs were −242 and 12 ml·min⁻¹·g dry wt⁻¹, respectively, whereas the respective kos were not distinguishable at −4.1 and 3.9 ml·min⁻¹·g dry wt⁻¹, respectively.

The dicumarol effect points to NQO1 (42), which Fig. 11 shows is present in the rat lungs, as the dominant reductase activity involved. For the species for which comparisons are available (rat, mouse, and human, Ref. 20), rat NQO1 has the highest specific activity. However, it is not clear how that observation might factor into the explanation for the species differences in whole organ activity, wherein a number of other factors (e.g., the amount of enzyme) affecting overall activity may be operative.

In each experimental condition studied, the addition of cyanide decreased ko, consistent with a complex III oxidation site for DQH2. In other words, inhibition of mitochondrial complex IV by cyanide would be expected to promote reduction of complex III, thereby closing down complex III as a pathway for DQH2 oxidation. Cyanide also tended to increase kred. This is most obvious in the mouse lungs with their relatively low reduction rate under the control conditions. Cyanide also reversed the effects of both rotenone and dicumarol on kred. The reversal might be related to the overall effect of cyanide on the redox poise of the cells. For example, cyanide has been observed to increase bovine pulmonary arterial endothelial cell NADH concentration severalfold (41). Because dicumarol is a competitive inhibitor at the NAD(P)H binding site of NQO1 (43), an increase in cellular NADH might diminish the inhibitory effect of dicumarol. In addition, or instead, the increase in electron donor concentration may increase the contribution of other reductases. It is notable that, with oxidation markedly suppressed by cyanide, the net reduction returned almost to the control level in the dicumarol inhibition experiments (Fig. 6). This can be explained by recovery of only a very small fractional of the control kred (Fig. 12) along with the relatively large DQ and DQH2 tissue to perfusate partition coefficients resulting from the elimination of BSA from the perfusate. The latter was done to expose the dicumarol effect as indicated above.

The decrease in kred caused by rotenone might reflect a direct inhibition of the DQ reductase activity of complex I (54). Complex I inhibition would also be expected to result in a more oxidized state of complex III, which might increase ko by promoting oxidation of DQH2 via the hydroquinone oxidase-cytochrome c reductase activity of complex III (14, 30, 55). This conjecture is supported by the oxygen consumption data shown in Fig. 10, wherein the decrease in oxygen consumption in response to rotenone and TTFA was reversible by DQ (Fig. 10). In other words, DQ reduction, presumably via NQO1, appears to have provided an electron transport bypass, allowing cytochrome c reduction to proceed and thus restoring oxygen consumption (14, 33). Thus the effects of cyanide apparently indicate that the reoxidation of DQH2 in the lung is predominantly via complex III. This is consistent with the concept that reduction by NQO1 followed by oxidation via complex III, thereby forming an electron.
bypass around complex I, may be a means by which low-molecular-weight amphipathic quinones reverse the effects of complex I deficiency diseases on mitochondrial ATP production (19, 51, 52).

DQ is commonly thought of as a redox-cycling, oxygen-activating quinone via its one electron reduction by various mammalian enzyme systems, which have been studied most extensively in hepatocytes, liver mitochondria, and microsomes (16, 24, 27, 32, 46). This was not directly addressed in the present study. However, the apparent predominance of reduction via NQO1, which is a two-electron reduction (17, 34) and the fact that cyanide largely inhibited the oxygen consumption-stimulating effect of DQ suggest that semiquinone formation is not a dominant reduction product in the lung, and the balance of enzyme activities contributing to the extracellular disposition of DQ on passage through the lungs is two-electron reduction. This would not necessarily be true for quinones in general, since with quinone-hydroquinone pairs that more readily coproportionate and auto-oxidize, two-electron reduction itself could lead to oxygen activation (17, 25, 44, 45, 49). The aerobic DQH2 and DQ-DQH2 mixtures were quite stable on the time frame relevant to the present studies.

Having made an effort to measure h(t) and include the capillary transit time dispersion in the modeling, we provide Fig. 14 to give a sense of its impact on the organ function probed in this study. Figure 14 was obtained from model pulse infusion simulations (Eqs. A1–A4) providing simulated effluent concentration data. The simulations were for a lung with the experimentally determined h(t), a range of kred encompassing the values relevant to this study, and with k, set at constant value of 4.5 ml·s⁻¹·g dry lung wt⁻¹. The curved line is the ratio of the apparent kred that would be estimated if one assumed that there was one common transit time through the lungs to the actual kred used in the simulation. The consequence of ignoring the heterogeneity of the h(t) would be not only substantial but also dependent on the value of the actual kred value. If one thinks of the lung as a chemical reactor determining the systemic arterial redox status of mixed venous DQ, the simulation also demonstrates how the reactor efficiency can be affected by the heterogeneity of the h(t) (28).

In conclusion, this study demonstrates the high capacity of the lungs for reducing DQ as it passes through the pulmonary circulation. The flow-limited tissue access of this amphiphatic substrate allows for a simplified expression for the quasisteady-state disposition in terms of the rates of DQ reduction and DQH2 reoxidation in the intact organ. The reduction appears to be mainly via dicumarol-sensitive two-electron reduction, presumably mediated via NQO1, and the reoxidation mainly via a cyanide-sensitive oxidation, presumably mediated via mitochondrial complex III. The results are consistent with the concept that the net effect of passage through the lungs on this potentially oxygen-activating quinone is a conversion to a stable reduction product. Thus the lungs may have a plasma-detoxifying effect through their capacity for reducing certain classes of plasma-borne redox-active substrates.

**APPENDIX**

**Kinetic Model**

**Stoichiometry.** The free (not plasma protein bound) form of DQ may be reduced to DQH2 via reductase(s) by electron donor(s) EH [e.g., NAD(P)H]. Both DQ and DQH2 may participate in nonspecific, rapidly equilibrating interactions with the perfusate albumin (Pc) and the lung tissue sites of association (Pe). The DQ may also be sequestered [i.e., association with, or binding to, some cellular component(s), which results in removal of the DQ from the perfusate and from participation in the other specified reactions]. Each of these reactions proceeds with a rate constant k in the forward direction and, if reversible, with a rate constant k°⁻¹ in the reverse direction. Each of the stoichiometric equations may potentially represent multiple parallel and/or series processes that are not explicitly specified. For example, intracellular reduction might occur via parallel enzymes contributing to the total quinone reductase activity.

In perfusate (c)

\[ \text{Pc association} \]

\[ \text{DQ} + \text{Pc} \xrightleftharpoons[k_{-1}]{k_1} \text{DQ-Pc} \]

\[ \text{DQH}_2 + \text{Pc} \xrightarrow[k_{-2}]{k_2} \text{DQH}_2-Pc \]

In tissue (e)

**Reduction**

\[ \text{DQ} + \text{EH} + \text{H}^+ \rightarrow \text{DQH}_2 + \text{E}^+ \]

**Oxidation**

\[ \text{DQH}_2 + \frac{1}{2} \text{O}_2 \rightarrow \text{H}_2\text{O} + \text{DQ} \]
rapidly equilibrating associations

\[
\begin{align*}
k_5 & \cdot DQ + P_e \rightleftharpoons DQ \cdot P_e \\
k_6 & \cdot DQH_2 + P_e \rightleftharpoons DQH_2 \cdot P_e
\end{align*}
\]

slowly dissociating or irreversible associations (sequestration)

\[
DQ + Y \rightarrow DQ \cdot Y
\]

where \(E^+\) are the reduced and oxidized forms, respectively, of intracellular electron donor(s); \(Y\) represents cellular sites of sequestration of DQ; and the \(k_i\)s represent the reaction rate constants.

Species balance. These stoichiometric relationships are expressed as species balance equations for an individual capillary element, where the capillary element includes the capillary lumen \(V_c\) and the surrounding tissue volume \(V_e\) accessible to DQ and/or DQH2. The capillary element species balance equations are

\[
\frac{\partial[R]}{\partial t} + W \frac{\partial[R]}{\partial x} = 0
\]

\[
\frac{\partial[DQ]}{\partial t} + \frac{\partial[DQ]}{\partial x} = \left( \frac{k_Y}{\alpha_1} \right) - \left( \frac{k_DQ}{\alpha_1} \right) \left( k_{red} \frac{Y}{\alpha_1} + \frac{Y_{DQ}}{\alpha_1} \right)
\]

\[
\frac{\partial[DQH_2]}{\partial t} + \frac{\partial[DQH_2]}{\partial x} = \left( \frac{k_DQH_2}{\alpha_3} \right) + \left( \frac{DQ}{\alpha_3} \right) \left( k_{red} \frac{Y}{\alpha_1} + \frac{Y_{DQ}}{\alpha_1} \right)
\]

where \(\alpha_1, \alpha_3\) calculated from the fractions of DQ and DQH2 bound to BSA or Ficoll obtained by ultrafiltration, the identifiable model parameters are the reduction rate constant, \(k_{red} = k_3/[EH^-][H^+]^2/V_e\) (in mL/s); the tissue-mediated oxidation rate constant, \(k_5 = V_e k_5/[O_2]^{1/2}\) (in mL/s); the tissue DQ sequestration rate constant, \(k_5\) (in \(\mu\)M\(^{-1}\)s\(^{-1}\)); the number of DQ sequestration sites, \(Y_{DQ} = [Y]/V_e\) (nmol); and the virtual volumes of distribution, \(V_{DF} = V_e \alpha_3\) and \(V_{DF} = \alpha_3\) (in mL), which are measures of the rapidly equilibrating tissue interactions of DQ and \(Y_{DQ}\), respectively. The \([H^+]\), \([O_2]\), and \([EH^-]\) included in some parameter groups are assumed constant during a given sample collection period.

To construct an organ model from Eqs. A1–A4, the \(h_i(t)\), obtained as described in Ref. 7, was represented by a shifted random walk function as previously described (4, 6) using the capillary moments \((\bar{r}_c, \sigma_r^2, \text{ and } m^2)\) obtained from the vascular mean transit time, \(\tau_v\), and the \(\bar{r}_c, \sigma_r^2, \text{ and } m^2\), estimated from the data in Fig. 9.

The \(h_i(t)\), which accounts for the system dispersion outside of the capillaries (i.e., in arteries, veins, connecting tubing, and the injection system), is related to \(h_i(t)\) and the concentration vs. time outflow curve of the vascular indicator, \(C_i(t)\), by \(C_i(t) = (q_2/F) \cdot h_i(t) \cdot h_{se}^m(t)\), where \(*\) is the convolution operator, and \(q_2\) is the mass of the injected vascular indicator. For the bolus injection, \(h_i(t)\) was also represented by a shifted random walk function (8), whereas for the pulse infusion \(h_i(t)\) was represented by: \(h_i(t) = 0\) for \(0 \leq t \leq t_o\), \(h_i(t) = (1/t_0)[1 - e^{-\gamma(t - t_o)}]\) for \(t_o < t \leq t_{sp} + t_o\), and \(h_i(t) = (1/t_0)[e^{-\gamma(t - t_{sp} - t_o)} - e^{-\gamma(t - t_{sp} - t_o)}]\) for \(t_{sp} + t_o < t \leq \infty\), where \(t_o\) and \(t_{sp}\) are the pulse time shift and pulse duration, respectively. The parameters of \(h_i(t)\) (random walk function parameters for bolus injection, and \(t_o\) and \(\gamma\) for pulse infusion) were specified by iteratively convolving \(C_i(t) = (q_2/F)h_i(t)\) with \(h_{se}^m(t)\) until the optimal least squares fit to \(C_i(t)\) was obtained.

To model DQ bolus injections or pulse infusions, given \(h_i(t)\) and \(h_{se}^m(t)\), we solved Eqs. A1–A4 numerically (8). For the bolus the initial \((t = 0)\) conditions: \([DQ] = [DQH_2] = (0, 0)\); \([R] = [H_2O_2] = (0, 0)\), and \(Y_{DQ} = Y_{DQH_2} = 0\), and boundary \((x = 0)\) conditions: \([DQ] = [DQH_2] = (0, 0)\); \([R] = [H_2O_2] = (0, 0)\), and \(Y_{DQ} = Y_{DQH_2} = 0\), where \(q_1\) and \(q_2\) are the masses of the injected DQ and vascular indicator, respectively, and \(P\) is the total flow through the organ. For the pulse infusions, Eqs. A1–A4 were solved with initial conditions: \([DQ] = [DQH_2] = (0, 0)\); \([R] = [H_2O_2] = (0, 0)\), and boundary \((x = 0)\) conditions: \([DQ] = [DQH_2] = (0, 0)\); \([R] = [H_2O_2] = (0, 0)\), and \(Y_{DQ} = Y_{DQH_2} = 0\), where \(q_1\) is the mass of the infused DQ or DQH2. The solution is for a single capillary element. As discussed in Ref. 6, the model solution for a single capillary having the maximum capillary transit time also provides the output for all capillary transit times between the minimum and maximum capillary transit times. To provide the whole organ output for vascular reference indicator and for DQ and DQH2, the outputs for all transit times were summed, each weighted according to \(h_i(t)\). The optimization for fitting the model to the data and parameter estimation was carried out using the IMSL subroutine DBCLSF (IMSL mathLibrary, version 2.0, 1994), which finds the best nonlinear least-squares solution using a modified Levenberg-Marquardt algorithm with finite difference Jacobian.

DISCLOSURES

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