Exchange of Oxygen Across the Epicardial Surface Distorts Estimates of Myocardial Oxygen Consumption

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ABSTRACT The rate of oxygen consumption of isolated, Langendorff-circulated, saline-perfused hearts of guinea pigs, rats, and rabbits was measured using the classical Fick Principle method. The heart was suspended in a glass chamber the oxygen partial pressure, PO_2, of which could be varied. The measured rate of oxygen consumption was found to vary inversely with the ambient (heart chamber) PO_2. This result prevailed whether the chamber was filled with air, saline, or oil, and whether the pericardium was present or the heart was wrapped in Saran. The effect varied inversely with heart size both within and across species. It is concluded that the epicardial surface is permeable to oxygen which will diffuse either into or out of the heart as the PO_2 gradient dictates. In either case the classically measured rate of oxygen consumption will be in error. The error can be large in studies of cardiac basal metabolism. A simple model is developed to describe the observed rate of oxygen consumption as classically measured. The measured rate is partitioned into two components: the true rate of oxygen consumption of the heart, and the rate of loss of oxygen by diffusive exchange across the epicardial surface. The latter component is proportional to the gradient of oxygen partial pressure from myocardium to environment and to the diffusive oxygen conductance of myocardial tissue. Application of the model allows the true rate of oxygen consumption of the heart to be recovered from measured values which may be considerably in error.

INTRODUCTION

The fundamental goal of cardiac energetics is to assign a rate of energy expenditure to each of the manifold electrical, chemical, and mechanical processes that constitute the cardiac cycle. Requisite to the achievement of this goal is the ability to accurately measure the oxygen consumption of the heart. Since the pioneering paper of Barcroft and Dixon (1906), this has traditionally been performed by use of the so-called Fick Principle. In this method, the rate of oxygen consumption is simply the product of the rate of flow of perfusion fluid through the coronary circulation and the arteriovenous difference in content of oxygen of the perfusate. With only a few

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recent exceptions (Follert, 1971; Wilkman-Coffelt et al., 1983; Loock and Schmidt, 1986), this procedure has been generally assumed to be completely accurate in the steady state. (Indeed, even the oxygen capacitance of cardiac tissue has been typically ignored in non–steady-state formulations.)

An implicit assumption underlying this use of the Fick Principle is that the epicardial surface of the heart is impermeable to oxygen. Hence, it is to be expected that the measured rate of oxygen consumption of the heart (or, equivalently, the measured oxygen content of the venous perfusate) would be independent of the ambient PO_2. The experiments detailed below show that this is not the case. The measured rate of oxygen consumption of the heart varies inversely with the PO_2 of its surrounds. As a consequence, many reported rates of myocardial oxygen consumption may be substantially in error.

A preliminary account of some of this research was presented to the Physiological Society of New Zealand, Massey University, Palmerston North, NZ, May 1987.

GLOSSARY

Definition of Symbols and their Units

a	Measured oxygen content of arterial perfusate	ml $O_2 \cdot ml^{-1}$
v	Measured oxygen content of venous perfusate	ml $O_2 \cdot ml^{-1}$
v^*	True oxygen content of venous perfusate	ml $O_2 \cdot ml^{-1}$
V	Volume rate of flow of perfusate	$ml \cdot g^{-1} \cdot min^{-1}$
P	Perfusion pressure	torr (760^{-1} atm)
VO_2^M	Measured rate of oxygen consumption	$\mu l\ O_2 \cdot g^{-1} \cdot min^{-1}$
VO_2^H	True rate of oxygen consumption	$\mu l\ O_2 \cdot g^{-1} \cdot min^{-1}$
VO_2^L	Rate of diffusive loss of oxygen	$\mu l\ O_2 \cdot g^{-1} \cdot min^{-1}$
A	Surface area for diffusive exchange	cm^2
δ	Diffusion distance	cm
PO_2	Partial pressure of oxygen	atm
P_B	Barometric pressure	atm (760 torr)
FO_2	Fraction of oxygen (PO_2/P_B)	(%)
p_{v^*}	True venous PO_2	atm
p_c	Chamber PO_2	atm
βO_2^H	Solubility of oxygen in heart tissue	$ml\ O_2 \cdot ml^{-1} \cdot atm^{-1}$
DO_2^H	Diffusivity of oxygen in heart tissue	$cm^2 \cdot min^{-1}$
g_H	Diffusive oxygen conductance of the heart‡	$\mu l\ O_2 \cdot g^{-1} \cdot min^{-1} \cdot atm^{-1}$
D_H	Diffusive oxygen capacity of the heart‡	min^{-1}

Defining Identities and their Units

$VO_2^M = V(a - v)$	$\mu l\ O_2 \cdot g^{-1} \cdot min^{-1}$
$VO_2^H = V(a - v^*)$	$\mu l\ O_2 \cdot g^{-1} \cdot min^{-1}$
$VO_2^L = g_H(p_{v^*} - p_c)$	$\mu l\ O_2 \cdot g^{-1} \cdot min^{-1}$
$VO_2^L = D_H(v^* - c)$	$\mu l\ O_2 \cdot g^{-1} \cdot min^{-1}$

$PO_2 = FO_2 \cdot P$	atm
p_c = chamber $FO_2 \cdot P_B$	atm
$v^* = \beta O_2^H \cdot p_{v^*}$	ml $O_2 \cdot ml^{-1}$
$c = \beta O_2^H \cdot p_c$	ml $O_2 \cdot ml^{-1}$
$g_H = DO_2^H \cdot A/\delta^{\ddagger}$	μl $O_2 \cdot g^{-1} \cdot min^{-1} \cdot atm^{-1}$
$D_H = g_H/\beta O_2^{H\ddagger}$	min^{-1}

‡Normalized per gram wet heart weight assuming a specific gravity of 1.05 $g \cdot ml^{-1}$.

METHODS

Hearts of guinea pigs, rats, and rabbits were Langendorff-perfused within a glass chamber. This chamber was filled with fluid (i.e., either gas or liquid) and its oxygen partial pressure maintained constant. Animals were killed by decapitation. The heart was quickly removed, immersed in chilled Krebs solution to induce arrest, and gently massaged to empty the chambers of blood. The aorta was dissected free, severed proximal to the brachiocephalic artery, and tied to the aortic perfusion cannula with braided suture (size 000, Ethicon Inc., Somerville, NJ). Except for a single set of experiments, the pericardium was removed. When the pericardium was left in place, a minute incision was made in its apex to prevent cardiac tamponade.

The Perfusion System

The perfusion apparatus is shown schematically in Fig. 1. Perfusion was Langendorff in nature in that aortic flow was retrograde distal to the aortic valves. Constant perfusion pressure was achieved by a roller pump (Masterflex, Cole Parmer Instrument Co., Chicago, IL). The perfusate was drawn from oxygenated (either 95% O_2/5% CO_2 or 20% O_2/5% CO_2/75% N_2) glass reservoirs immersed in a 20-liter water bath. The temperature of the water bath was maintained constant (at either 37 ± 0.5°C or 5 ± 0.5°C) by a pump (model E3, Haake Buchler Instruments, Inc., Saddle Brook, NJ) which circulated water through a series of water-jackets enclosing the perfusion line and the heart chamber. A thermistor, placed in the perfusion line immediately proximal to the aorta, measured the inlet temperature of the perfusate. A second thermistor recorded the temperature of the air within the enclosed glass heart chamber. The latter was typically 0.25°C lower than the former.

Perfusion pressure was measured by a manometric pressure gauge (Nissei, Kyoto, Japan) placed in parallel with the perfusion line 41 cm vertically above the base of the heart. Hence the true coronary perfusion pressure, P, was 30 torr in excess of the reading on the gauge. P was maintained constant at 60 torr throughout the duration of an experiment.

To enhance viability, the perfusate was filtered (Robinson et al., 1983). The filter was resident in-line and consisted of a stainless-steel housing (Millipore/Continental Water Systems, Bedford, MA) supporting a glass fiber prefilter and a 0.45-μm membrane filter (No. 401112, Schleicher & Schull Inc., Keene, NH). Except for 30 cm of tygon tubing (No. 6408-43, Cole Parmer Instrument Co.) in the roller pump, the entire perfusion line was either glass or stainless steel. This arrangement was found to be essential to minimize loss of oxygen from the perfusate before it reached the heart.

The coronary circuit was completed by passing a catheter into the right ventricle via the pulmonary artery. The catheter was glass except for a 5-cm tygon segment on its proximal tip. When in place, only 1–2 cm of tygon was exposed to the air inside the heart chamber. Preliminary experiments, with the full 5-cm tygon segment attached directly to the aortic cannula (in the absence of a heart), indicated a negligible loss of oxygen through the perfusion line at

flow rates commonly encountered during an experiment. The distal tip of this catheter was situated 5 cm below the apex of the heart, thereby ensuring suction drainage of the right ventricle. Flow from the right ventricle (V) was recorded volumetrically.

The left ventricle was aspirated by a catheter passed through the left atrial wall and atrioventricular valve to reside in the apex of the left ventricle. This catheter was connected to a second roller pump (Masterflex) to achieve aspiration of the left ventricle. The catheter was tied in place by a ligature that completely encircled the left atrium at the level of the atrioventricular annulus. This procedure both prevented perfusion of the left atrium and obviated any possible loss of right atrial (coronary sinus) flow through a patent foramen ovale. Aspiration of the left ventricle is preferred to the usual practice of venting the ventricular wall. The latter procedure necessarily causes a loss of perfusate (of unknown volume and content of

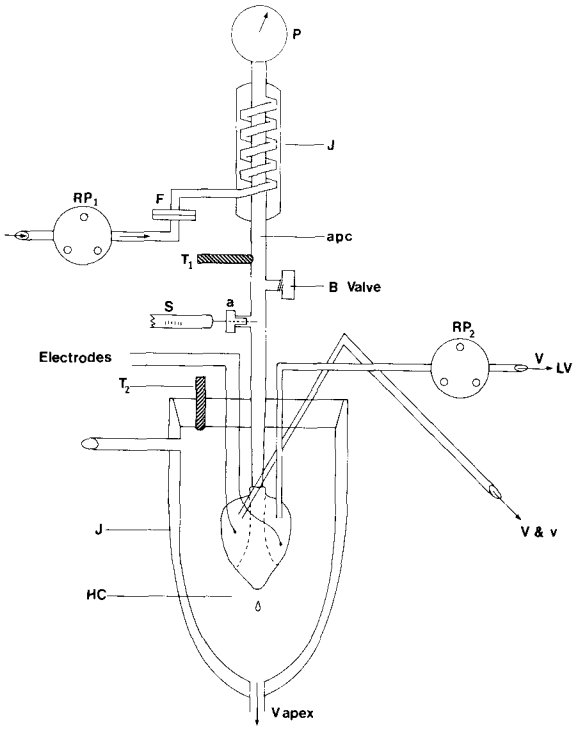

FIGURE 1. Schematic drawing (not to scale) of perfusion system. *RP*, roller pump; *F*, in-line filter; *J*, outer water jacket; *apc*, aortic perfusion cannula; *B*, bleeding valve; *T*, thermistor; *HC*, heart chamber; *s*, 50 μl Hamilton syringe shown drawing an aortic sample for O_2 analysis (see text). Measured variables: *P*, perfusion pressure; *a* and *v*, aortic and venous oxygen content, respectively; *V*, volume rate of flow.

oxygen) from the ruptured coronary capillary bed (Loiselle, 1985). Flow of aspirated left ventricular perfusate (V_{LV}) was recorded volumetrically.

Finally, a single loop of suture was placed around the six atrial inlets: the pulmonary veins on the left side and the inferior and superior vena cavae on the right. With this tie in place the coronary circuit was complete; perfusate returning from the coronary veins to the coronary sinus of the right atrium could not leak out of the right atrial inlets.

Despite completion of the coronary circuit, a small amount of perfusate continually appeared on the surface of the heart and dripped off the apex. This flow (V_{apex}), which is presumed to arise from epicardial lymphatic vessels (Lorber, 1953), was likewise measured volumetrically.

The Heart Chamber

The heart was enclosed by a double walled glass chamber of volume 70 ml. The top of the chamber was formed by a rubber stopper of 2 cm thickness through which passed the cathe-

ters and electrodes. When gas-filled, the chamber was held at constant PO_2 by a stream of gas entering through the upper side-arm inlet (see Fig. 1); apex flow (V_{apex}) was collected from the lower outlet. When the chamber was liquid (saline or oil) filled, it was oxygen-equilibrated by vigorous aeration via the lower inlet. In this case V_{apex} was collected from the upper side-arm. In both cases the chamber was aerated with either 0, 20, 40, 68, or 95% O_2 in 5% CO_2 with the balance N_2.

Heart Rate

Experiments were usually done with the heart in the arrested state. The period of arrest was preceded and followed by brief (10–15 min) periods of spontaneous activity during which the heart rate was monitored as an index of viability. Platinum wire electrodes were placed in contact with the surface of the heart near the base of the right ventricle and on the anterior apical aspect of the left ventricle (see Fig. 1). The electrodes were connected to a low-noise microvolt amplifier. The output of the amplifier passed through a Schmidt trigger to a microprocessor (Microprocessor Developments Ltd., Auckland, New Zealand) via an 8-bit A/D converter. A Fortran computer program determined mean heart rate averaged over 6- and 60-s intervals. By operating a switch distal to the electrodes, the system could be used in stimulation (pacing) mode (using a model S-8 or S-11 Stimulator [Grass Instrument Co., Quincy, MA]). In one series of experiments, guinea pig hearts were paced throughout at a frequency of 4.5 Hz.

The Perfusate

The standard, low-K^+ perfusate was a modified Krebs-Henseleit solution of the following composition (millimoles·liters^{-1}): NaCl, 118; KCl, 4.75; $MgSO_4$, 0.71; $NaHCO_3$, 24.8; KH_2PO_4, 1.18; $CaCl_2$, 2.5; glucose, 10; and insulin (20 U·liters^{-1}). The perfusate contained 20 ml·liters^{-1} of the colloidal plasma substitute Haemaccel (Hoeschst, Behringewerke AG, Marburg, FRG), a concentration that has been shown to enhance the viability of isolated, saline-perfused hearts (Armitage and Pegg, 1977). For the high-K^+ (arresting) solution, additional KCl was added to achieve a final concentration of 20 mmol·liters^{-1}.

The Measurements

For a fixed set of perfusion conditions, oxygen consumption was measured under five different values of FO_2 in the heart chamber: 0, 20, 40, 68, and 95%. Measured oxygen consumption (VO_2^M) was calculated as the product of flow from the right ventricular catheter (V, i.e., the flow that has unequivocally traversed the coronary circulation) and the difference in content of oxygen between arterial and venous samples ($a - v$). The oxygen content of 50-μl samples of perfusate was determined in a Lexington ($LexO_2$ CON-TL, Cavitron Corp., Anaheim, CA) oxygen analyzer calibrated not less than four times daily with 20-μl samples of saturated room air. The arterial sample was drawn into a 50-μl Hamilton syringe through a thick rubber septum (Lexington) placed in the aortic perfusion line 2 cm above the aorta (see Fig. 1). The venous sample was drawn by placing the syringe needle 2 cm into the end of the glass catheter draining the right ventricle. In this manner contamination of samples by exposure to air was completely avoided.

Flow of perfusate from the three sources (RV, LV, and apex) was collected in graduated cylinders for a period of 2 min. Collection began immediately after the drawing of samples for oxygen analyses. A second set of arterial and venous samples was drawn (in the reverse order) immediately after the flow collection period. Thus all arteriovenous differences were determined in duplicate but referred to a single, interposed flow measurement. Typically, duplicate determinations were identical; they rarely differed by >5%.

For any given set of experimental conditions, every attempt was made to keep V (flow from the right ventricular catheter) and a (arterial oxygen content) constant while the chamber FO_2 was varied. Hence any variation of v (venous oxygen content) or VO_2^M (measured oxygen consumption) with chamber FO_2 was not due to differences in perfusion conditions. The measured rate of oxygen consumption was calculated as

$$VO_2^M = V(a - v). \quad (1)$$

This paper reports experiments designed to measure v as a function of chamber FO_2. Because V and a were kept constant this means that the apparent rate of oxygen consumption, VO_2^M, could be determined as a function of ambient (chamber) conditions.

At the completion of the experiment the heart was blotted and its wet weight determined. It was then dried for 15 h at 70°C to determine its dry weight. The mean water content of hearts is reported for each set of experiments. The measured rate of oxygen consumption, corrected to standard temperature and pressure (0°C and 760 torr), is expressed per gram wet weight of the heart.

Experimental Design and Statistical Analyses

In most of the experiments reported below, a period of normokalemic perfusion both preceded and followed a period of high-K^+ arrest. The periods of low-K^+ perfusion permitted assessment of the degree of recovery of metabolic function after arrest. In any set of experiments each heart was subjected to every treatment the order of presentation of which was dictated by a Latin square. This design permits an analysis of the effect of time which is completely independent of the effect of the treatment of interest. The treatments were presented at precisely timed intervals (20–30 min but fixed for any given set of experiments) during the final 10 min of which measurements were made. For each condition the coronary flow, V, as well as the arterial, a, and venous, v, content of oxygen was measured separately for each value of FO_2 in the chamber. In this manner each heart acted on its own control. Results were analyzed by Analysis of Variance using the SAS (Statistical Analysis System) package (Ray, 1982) on a model 4341 computer (IBM Instruments, Inc., Danbury, CT). Differences among means were examined for statistical significance (at the 95% confidence level) using the methods of Rodger (1975).

RESULTS

Effect of Ambient PO_2 (Air Immersion)

Experiments were performed at 37°C on five arrested guinea pig hearts. The hearts weighed 1.17 ± 0.026 g (mean ± SEM); their fractional water content was 0.791 ± 0.001. Each heart was continuously perfused at constant pressure (60 torr) with Krebs solution equilibrated with 95% O_2/5% CO_2. The heart chamber was continuously flushed with a stream of gas containing 0, 20, 40, 68, or 95% O_2 in 5% CO_2 with the balance N_2. The results are shown in Fig. 2.

The measured rate of oxygen consumption, VO_2^M, fell progressively and linearly as the fraction of gaseous oxygen in the chamber increased (Fig. 2 *A*). This decline was entirely attributable to a progressive increase in v, the venous content of oxygen, as both a, the arterial oxygen content, and V, the flow of coronary perfusate, remained constant independent of chamber PO_2 (Fig. 2 *B*). The importance of properly controlling for a possible time-dependent decline in measured rate of oxygen consumption after arrest (for review see Loiselle, 1985) is shown in Fig. 2 *C*.

VO_2^M fell significantly over the first 40–60 min of arrest. Had chamber PO_2 values been progressively (i.e., nonrandomly) increased over this period, the observed effect of chamber PO_2 (Fig. 2 *A*) would have been obscured.

The result of this simple experiment is clear cut. The rate of oxygen consumption of isolated, arrested, saline-perfused hearts, as classically measured, depends on the

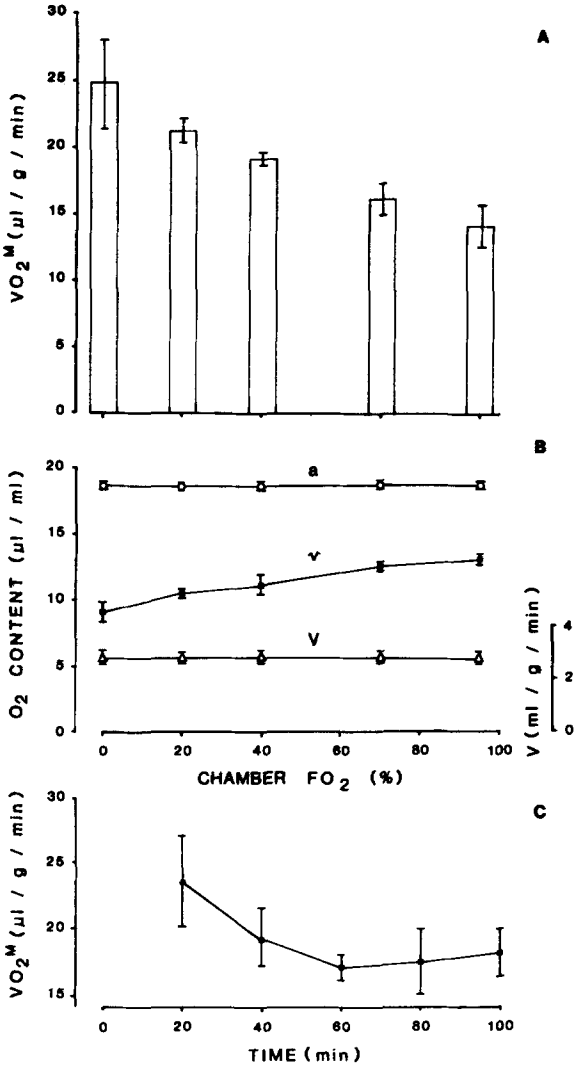

FIGURE 2. Effect of varying oxygen fraction (FO_2) of air-filled chamber at 37°C. (*A*) Measured rate of oxygen consumption (VO_2^M) vs. FO_2. (*B*) Arterial (a) and venous (v) content of oxygen, left-hand ordinate; coronary flow (V), right-hand ordinate. For individual hearts, $V(a - v) = VO_2^M$. (*C*) Measured oxygen consumption vs. time after cardiectomy; $n = 5$ guinea pig hearts.

ambient PO_2. The classical Fick formulation is incomplete and a new model is demanded.

A Model of Oxygen Exchange in the Perfused Heart

Formulation of the model. It is desired to measure the oxygen consumption of the heart, VO_2^H. This is classically given, via the Fick Principle (Eq. 1), by the prod-

uct of the volume rate of flow through the coronary circulation, V, and the difference in oxygen content of aortic (a) and sinus venosus or pulmonary arterial (v) perfusate. This calculation makes two implicit assumptions: (a) fluid mass is conserved in transit through the coronary capillaries, and (b) the only oxygen sink in the system resides within the myocardial tissue.

The current model retains the first assumption but elaborates the second: a further oxygen sink is recognized. Oxygen may be exchanged with the environment across the epicardial surface. That is, oxygen may be lost to the environment from the heart or from the environment to the heart. This loss, denoted by VO_2^L, contaminates the estimate of v and hence distorts the estimate of the true myocardial oxygen consumption, VO_2^H. The model for oxygen exchange in the heart thus becomes:

$$VO_2^M = VO_2^H + VO_2^L, \tag{2}$$

where $VO_2^M = V(a - v)$ is the oxygen consumption as measured classically (Eq. 1). The measured oxygen consumption, VO_2^M, equals the true oxygen consumption, VO_2^H, if and only if VO_2^L is zero. (The glossary gives a definition of symbols and their units.)

To develop the model further it is necessary to introduce the concept of a true mean venous oxygen content, denoted by v^*. This may be thought of as the venous oxygen content that would prevail in the absence of oxygen exchange with the environment or, equivalently, as the venous content that yields the true oxygen consumption of the heart:

$$VO_2^H = V(a - v^*). \tag{3}$$

If there is no exchange of oxygen with the environment across the surface of the heart, then $v^* = v$, where v is the measured oxygen content of the mixed venous (pulmonary arterial) perfusate, so $VO_2^H = VO_2^M$.

Fig. 3 presents the theoretical model (upper enlargement) as well as a highly schematic diagram of the experimental arrangement used to test it. The excised heart was isolated within a glass chamber, the oxygen content of which was precisely controlled by vigorous flushing with a stream of gas of known PO_2. In the upper panel of Fig. 3, a slab of epicardium of thickness δ and surface area A is shown. Oxygen diffuses across this surface driven by the difference between the true venous partial pressure, p_{v^*}, and the partial pressure within the chamber, p_c. That is,

$$VO_2^L = g_H(p_{v^*} - p_c), \tag{4}$$

where g_H is the diffusive oxygen conductance of the heart. By analogy with the concept of the diffusive conductance of the lung, long in use by respiratory physiologists (see for example, Dejours, 1975),

$$g_H = \beta O_2^H \cdot DO_2^H \cdot A/\delta, \tag{5}$$

where βO_2^H and DO_2^H are the solubility and diffusivity of oxygen, respectively, in the slab of heart tissue. (Note that the product of solubility and diffusivity is frequently given the symbol K to denote Krogh's permeation constant. Note further that δ includes the thickness of any unstirred fluid layer on the epicardial surface across which oxygen must diffuse.)

The model for the true oxygen consumption of the heart, VO_2^H, can thus be presented in two equivalent forms:

$$VO_2^H = VO_2^M - VO_2^L \tag{6a}$$

$$V(a - v^*) = V(a - v) - g_H\,(p_{v^*} - p_c). \tag{6b}$$

In Eq. 6b (which follows from Eqs. 2 and 4), V, a, and v are measured. The partial pressure of oxygen in the fluid (gas or liquid)-filled chamber is given by

$$p_c = FO_2 \cdot P_B, \tag{7}$$

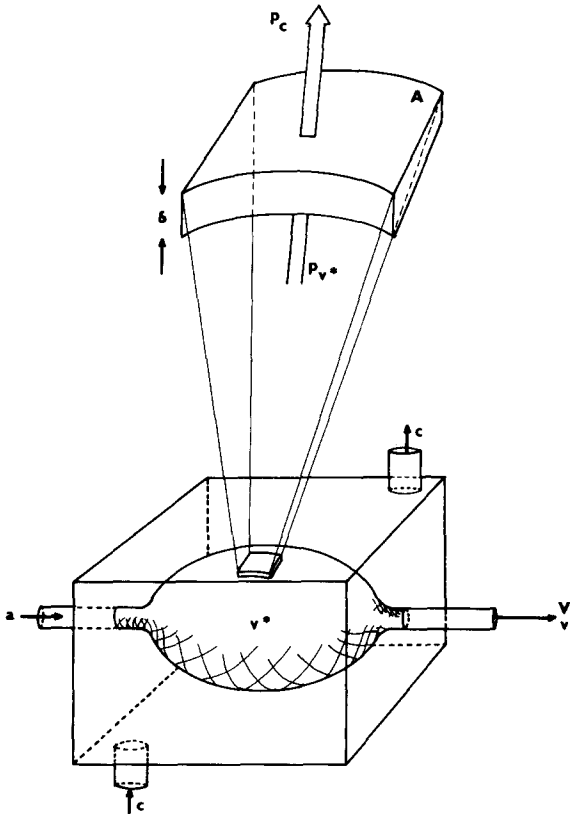

FIGURE 3. (*Bottom*) Schematic diagram of heart in glass perfusion chamber. Oxygen content of arterial perfusate = a, of venous effluent = v, of myocardial tissue = v^*. V = volume rate of flow of perfusate. Fluid-filled chamber is aerated to achieve oxygen partial pressure of p_c. (*Top*) Enlargement of epicardial slab of surface area A and thickness δ.

where FO_2 is the fractional oxygen content of the stream of gas aerating the chamber and P_B is the barometric pressure. The unknown variables v^* and p_{v^*} are related by

$$v^* = \beta O_2^H \cdot p_{v^*}. \tag{8a}$$

Finally, for convenience define

$$c = \beta O_2^H \cdot p_c, \tag{8b}$$

where c connotes "chamber," and

$$D_H = g_H / \beta O_2^H = DO_2^H \cdot A/\delta, \tag{9}$$

where the second part of Eq. 9 follows from Eq. 5. Substitution of Eqs. 7–9 into Eq. 6b yields:

$$V(a - v^*) = V(a - v) - D_H(v^* - c). \tag{10}$$

Cardiac oxygen consumption is usually normalized for wet heart weight. That is, both VO_2^H and VO_2^M are expressed per gram of wet tissue. In order that Eqs. 6 and 10 be dimensionally consistent, it is necessary to express VO_2^L (net loss of oxygen across the heart surface) per gram wet weight as well. When this is done, all three terms of the basic model (Eqs. 6 or 10) have units of $\mu l\ O_2 \cdot g^{-1} \cdot min^{-1}$. When normalized per gram (wet weight) of tissue, the diffusive oxygen conductance of the heart, g_H, has units of $\mu l\ O_2 \cdot g^{-1} \cdot min^{-1} \cdot atm^{-1}$, whereas the diffusive oxygen capacity, D_H, has units of $minutes^{-1}$. In the following, volume rates of oxygen consumption ($\mu l\ O_2 \cdot g^{-1} \cdot min^{-1}$) were corrected to standard temperature (0°C) and pressure (760 torr or 1 atm).

Eq. 10, which describes the model, is the exact equivalent of Eq. 6. Its solution is treated as a problem in the regression of the measured variable v upon the two other measured variables (a and V) and the independent variable c. Rearrangement yields v as a function of c, a, and V:

$$v = v^*(1 - D_H/V) + D_H/V \cdot c. \tag{11}$$

Rearrangement of Eq. 3 yields:

$$v^* = a - VO_2^H/V. \tag{12}$$

Substitution of this expression for v^* into Eq. 11 yields:

$$v = (a - VO_2^H/V)\ (1 - D_H/V) + D_H/V \cdot c. \tag{13}$$

Eq. 13 is to be viewed as a regression equation in which the measured variable v is a function of the two measured variables a and V and the independent variable c. VO_2^H and D_H are estimation parameters that minimize the sum of squares of deviations of these observations from the line of best fit described by Eq. 13. In this formulation, it is implicit that the true oxygen consumption of the heart, VO_2^H, remains constant throughout the measurement period which is therefore made as brief as possible. The explicit value of v^* may be retrieved via Eq. 12; the values of A and δ remain unknown.

Solution of Eq. 13 was achieved using the nonlinear regression procedures available in the SAS computing package (Ray, 1982). In practice, the fit of Eq. 13 to the experimental data was excellent; the proportion of the total variance accounted for by regression ranged from 96.32 to 99.99%.

Solubility of oxygen in cardiac muscle. Eq. 13 implicitly contains (via Eqs. 8 and 9) βO_2^H, the solubility of oxygen in cardiac muscle. This was estimated as the sum of the separate solubilities of oxygen in its three major myocardial components: isotonic saline, protein and fat. The following empirical estimate was used:

$$\beta O_2^H = 0.79\ \beta O_2^{saline} + 0.16\ \beta O_2^{protein}/1.35 + 0.05\ \beta O_2^{lipid}/0.94. \tag{14}$$

The numerical coefficients are the proportions, by weight, of the three tissue components. They arise from the values reported for canine left ventricle by Armiger et

al. (1984). The average specific gravities of protein and lipid are assumed to be 1.35 and 0.94, respectively (Mahler et al., 1985). The specific gravity of cardiac muscle is assumed to be 1.05 $g \cdot ml^{-1}$ or the same as that of skeletal muscle (Hill, 1964).

The solubility coefficient of oxygen in isotonic saline was determined using a Lexington oxygen analyzer. Krebs-Henseleit solution was equilibrated with gas of

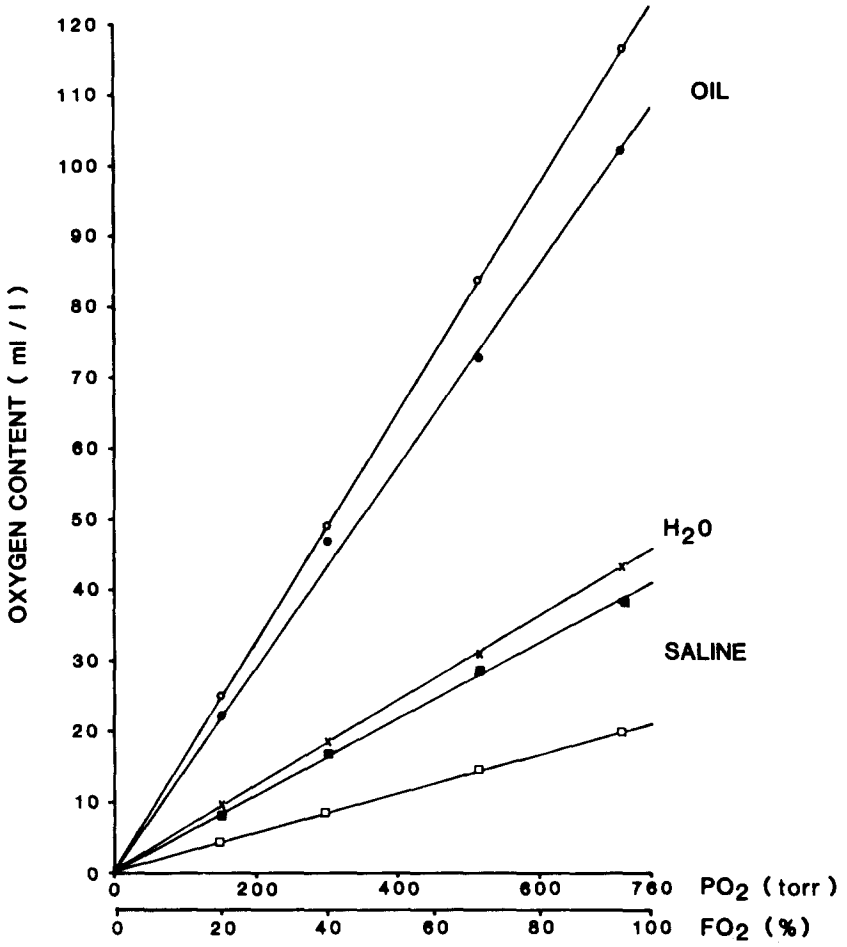

FIGURE 4. Oxygen content of (from top down): soya oil at 37°C (*open circles*), soya oil at 5°C (*solid circles*), distilled water at 5°C (*crosses*), saline (Krebs-Henseleit solution) at 5°C (*solid squares*), and saline at 37°C (*open squares*) as a function of oxygen partial pressure (PO_2) or oxygen fraction (FO_2). Note measured depression of aqueous O_2 solubility with increased salinity (Dejours, 1975).

known oxygen fractions and the oxygen content of 50-μl samples determined at 37 and 50°C. A similar procedure was used to estimate the oxygen solubility of lipid except that soya oil was used instead of saline. The results are shown in Fig. 4, where the solubility is given by the slope of the appropriate line. From this graph the solu-

bility of oxygen in lipid, βO_2^{lipid}, was taken to be 122 ml·liters^{-1}·atm^{-1} at 37°C with a Q_{10} of 1.04. The solubility of oxygen in isotonic saline, βO_2^{saline}, was taken to be 22.7 at 37°C with a Q_{10} of 0.83. After Mahler et al. (1985), the solubility of oxygen in protein, $\beta O_2^{protein}$, was assumed to be 1.6 times that of saline and, in the absence of knowledge, was arbitrarily assigned a Q_{10} of unity.

Predictions arising from the model. Either Eq. 6 or 10 fully defines the model of oxygen consumption of the heart. Each suggests a number of testable hypotheses that arise from the proposition that the net diffusive exchange is proportional only to the difference in partial pressure of oxygen between myocardium (p_{v*}) and chamber (p_c) and to the diffusive oxygen conductance of myocardial tissue (g_H). These hypotheses are as follows.

Hypothesis 1. Oxygen can be lost from the heart to its surrounds when the arterial PO_2 is high with respect to the ambient PO_2; it can be gained by the heart from its surrounds if the arterial PO_2 is low with respect to ambient PO_2.

Hypothesis 2. The net exchange of oxygen is independent of the nature of the fluid medium in the chamber.

Hypothesis 3. The diffusive exchange can be altered by the use of natural or artificial barriers to gaseous diffusion.

Hypothesis 4. The diffusive exchange is independent of metabolic rate.

Hypothesis 5. The diffusive exchange is larger the greater the surface area to volume ratio; i.e., it varies inversely with heart size.

Experimental Tests of Hypotheses Arising from the Model

Effect of the arterial-ambient PO_2 gradient. Hypothesis 1 is, of course, already supported by the results presented in Fig. 2 from which the model arose. These results are amplified in the first row of Table I, where the true rate of oxygen consumption of the heart, VO_2^H, extracted from the measured rates by solution of Eq. 13, is presented. Also presented are the rates of loss of oxygen from the heart, VO_2^L, at each value of chamber FO_2. Note that these values progressively diminish from positive (loss) to negative (gain) as ambient (chamber) PO_2 increases. From these values it is possible to calculate the error that would result from equating the true rate of oxygen consumption with the rate measured classically (Eq. 1). These errors are presented, as mean values, within parentheses in the body of the table.

In row 2 of Table I are shown data from hearts perfused at 5°C with 20% O_2 such that the transepicardial PO_2 gradient was much reduced. The same pattern is seen; net exchange goes from positive to negative as ambient PO_2 increases. Consider the results when the chamber is O_2-free (i.e., the 0% column). The net loss of O_2 is much less, in absolute terms, in 20% than in 95% arterial oxygen, as predicted. (Note, however, that the relative error is much larger.) At the other extreme of chamber FO_2 (95%), the net gain of O_2 by the heart is larger under the lower arterial PO_2, again as predicted by the model.

Effect of saline immersion. If hypothesis 2 is correct, then the ambient PO_2 dependency of the rate of oxygen consumption measured in air (Fig. 2 and Table I) should be unaffected by immersion of the heart in saline. To test this, the heart chamber was filled with Krebs-Henseleit solution and the entire system (perfusate, heart, and chamber) maintained at either 37 or 5°C. At 37°C the perfusate was

equilibrated with 95% O_2. Because the solubility (and hence, content) of O_2 in saline increases with decreasing temperature (see Fig. 4), whereas the rate of oxygen consumption of the arrested heart falls, the FO_2 of the perfusate at 5°C was reduced to 20%. Five hearts were examined in the 95% O_2/37°C combination and another five in the 20% O_2/5°C combination. After 1 h of saline immersion, the fractional water content of the hearts was 0.783 ± 0.003 and 0.796 ± 0.002 at 37 and 5°C, respectively. In this mode, aeration of the chamber was via the lower outlet (Fig. 1). The "surface oozing" that normally gave rise to the "apex drip" (V_{apex}, see Methods) appeared as an overflow from the side-arm outlet.

TABLE I

True Rate of Oxygen Consumption (VO_2^H), Rate of Diffusive Loss (VO_2^L), and Resulting Error of Measurement as a Function of Ambient FO_2 in KCl-arrested Guinea Pig Hearts Immersed in Various Fluids

Chamber fluid	T °C	a	n	VO_2^H	VO_2^L and error				
					0%	20%	40%	68%	95%
Air	37	95	5	20.0 ± 2.1	+4.5 ± 2.2 (+22)	+1.0 ± 0.9 (+5)	−1.0 ± 1.3 (−5)	−4.2 ± 0.8 (−21)	−6.0 ± 1.1 (−30)
	5	20	5	2.2 ± 0.3	+1.5 ± 0.4 (+68)	+0.6 ± 0.1 (+27)	−2.1 ± 0.3 (−95)	−4.2 ± 0.3 (−190)	−7.0 ± 0.1 (−320)
Saline	37	95	5	18.5 ± 3.0	+5.5 ± 2.2 (+30)	+1.8 ± 1.9 (+10)	+0.8 ± 2.8 (+4)	−3.4 ± 1.3 (−18)	−6.2 ± 1.6 (−34)
	5	20	5	2.2 ± 0.2	+1.5 ± 0.3 (+20)	−0.1 ± 0.2 (−5)	−2.0 ± 0.5 (−90)	−6.2 ± 0.6 (−280)	−8.8 ± 1.0 (−400)
Oil	37	95	6	16.6 ± 1.5	+2.3 ± 0.6 (+14)		−1.6 ± 0.5 (−10)		−5.3 ± 0.6 (−32)
	5	20	6	2.3 ± 0.1	+1.5 ± 0.1 (+65)		−2.0 ± 0.3 (−87)		−6.6 ± 0.5 (−290)
Saran (air)	5	20	5	2.6 ± 0.3	+0.3 ± 0.2 (+12)	+0.1 ± 0.2 (+4)	−1.0 ± 0.2 (−38)	−2.1 ± 0.2 (−58)	−3.6 ± 0.2 (−140)

T = temperature; a = arterial FO_2; n = number of hearts; VO_2^H = true rate of oxygen consumption of the heart (mean ± SEM; $\mu l\ O_2 \cdot g^{-1} \cdot min^{-1}$); VO_2^L = rate of loss of oxygen from heart to chamber (+) or from chamber to heart (−) (mean ± SEM; $\mu l\ O_2 \cdot g^{-1} \cdot min^{-1}$); values in parentheses = mean error of measurement calculated as $(VO_2^M/VO_2^H - 1) \times 100$ where $VO_2^M = VO_2^H + VO_2^L$ is the rate of oxygen consumption measured according to the classical Fick principle (see Eq. 1, text).

The results are presented in Fig. 5 and Table I. Immersion of the hearts in saline failed to prevent the PO_2 dependency of the measured rate of oxygen consumption. For both temperature-arterial PO_2 combinations, VO_2^M declined linearly as chamber FO_2 increased (Fig. 5 *A*). This was exclusively due to variation in the measured venous content of oxygen (Fig. 5 *B*) because coronary flow remained constant over time in each group: 3.43 ± 0.31 and 4.00 ± 0.50 $ml \cdot g^{-1} \cdot min^{-1}$ at 37°C/95% O_2 and 5°C/20% O_2, respectively.

At 5°C in 20% O_2, the oxygen content of the coronary arterial perfusate was 7.80

± 0.25 μl/ml. In Fig. 5 *B* it can be seen that this arterial value was less than that measured in the coronary venous effluent when the chamber was aerated with either 68 or 95% O_2. As a consequence, at both of these values of chamber FO_2, the measured rate of oxygen consumption became negative, consistent with the behavior shown in Fig. 5 *A*.

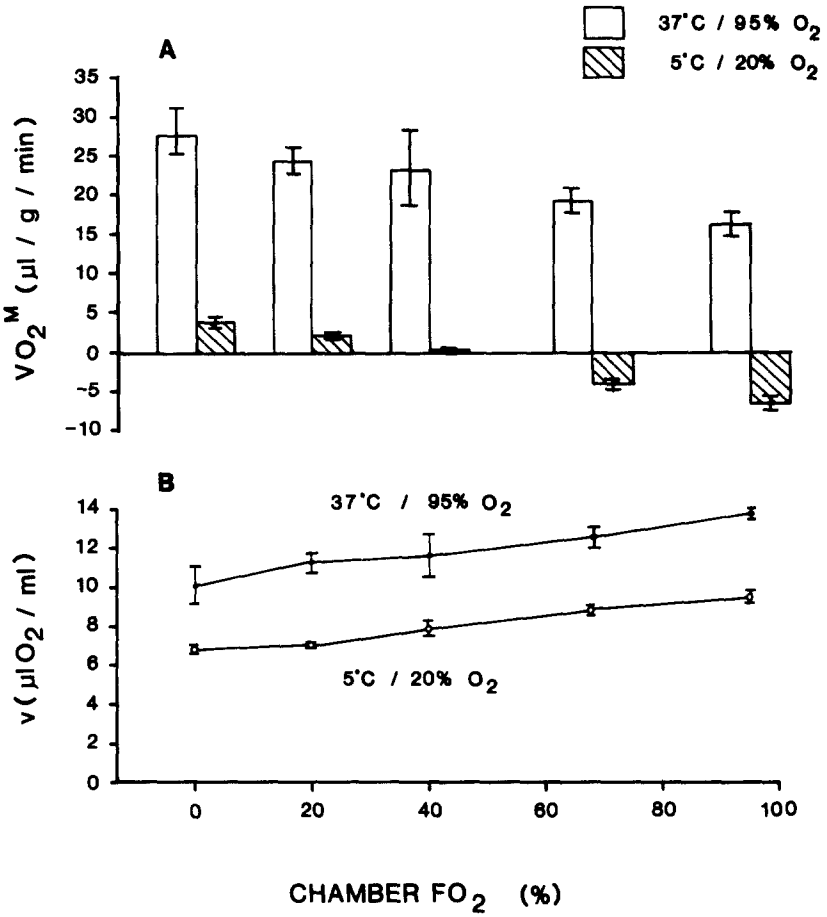

FIGURE 5. Effect of varying oxygen fraction (FO_2) of saline-filled chamber. n = 5 guinea pig hearts perfused and immersed under each of the arterial FO_2-temperature combinations indicated. (*A*) Measured oxygen consumption (VO_2^M); (*B*) Venous oxygen content (v) as a function of ambient oxygen fraction (FO_2).

Effect of oil immersion. An even more rigorous test of hypothesis 2 is achieved by immersion of the heart in oil. 12 hearts were saline-perfused in the usual manner but with the heart chamber filled with ordinary, supermarket-grade soya oil, the oxygen solubility characteristics of which are shown in Fig. 4. Only three values of chamber FO_2 were examined: 0, 40, and 95% O_2. Six hearts (mean weight, 1.20 ± 0.02 g and 1.25 ± 0.09 g, respectively) were studied at each of 37°C/95% O_2

and 5°C/20% O_2. After 1 h of immersion in soya oil, the fractional water content of the hearts was 0.790 ± 0.001 and 0.796 ± 0.002, respectively, in the two groups.

As can be seen in Fig. 6, immersion of the hearts in oil again failed to prevent the ambient PO_2-dependence of VO_2^M. As with both gas (Fig. 2) and saline (Fig. 5) immersion, when the chamber FO_2 was increased, the measured oxygen consumption decreased linearly becoming negative at high ambient PO_2 in the 5°C/20% O_2 group. Again, this result was entirely due to variation in v, the measured content of oxygen in the pulmonary arterial exudate (Fig. 6 *B*). When the oil-filled chamber

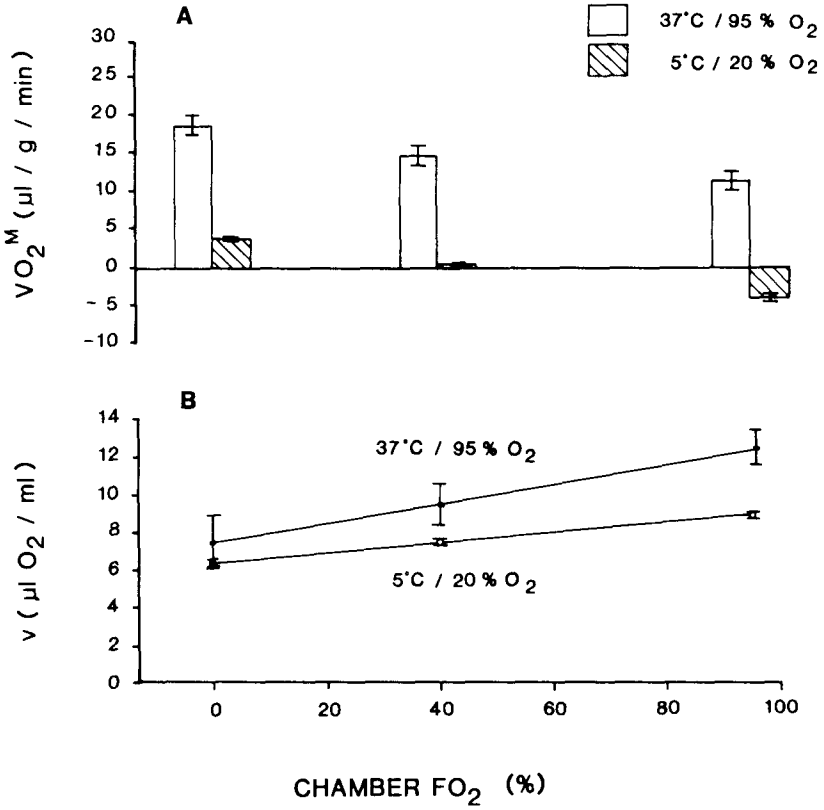

FIGURE 6. Effect of varying ambient oxygen fraction (FO_2) on measured oxygen consumption (*A*) and venous oxygen content (*B*) of six guinea pig hearts immersed in soya oil under each of the perfusion conditions indicated.

was equilibrated with 95% O_2 while the heart was perfused with 20% O_2, the venous oxygen content consistently exceeded the arterial oxygen content (7.64 ± 0.15 µl/ml), thereby yielding the negative value of VO_2^M shown in Fig. 6 *A*. Table I again presents the true rate of oxygen consumption deduced via the model under each condition as well as the ambient PO_2-dependent rate of diffusive exchange. Note that the effects of both ambient and arterial oxygen tension agree remarkably well, both in amplitude and direction, among the three conditions of chamber fluid: if there is an unstirred layer on the epicardial surface, then it must be of comparable

magnitude in all three cases. Note too the extreme errors of measurement that could arise through use of the classical method, especially when the true rate of oxygen consumption of the heart is low. In fact, without a model of transepicardial exchange it would be necessary to conclude that the heart is *producing* oxygen whenever the error is <100%.

Effect of Saran. In metabolic studies of exposed skeletal muscle in situ, it is common to wrap the preparation in a film of Saran because it is reputed to be an O_2 barrier. To examine the efficacy of this putative O_2 barrier (and thereby test hypothesis 3), hearts were carefully enclosed in a film (i.e., a single layer) of ordinary, supermarket-grade Saran Gladwrap™.

Considerable caution was taken to ensure an effective seal. A minute hole was made in the centre of a square of Saran. This hole (which prevented cardiac tamponade) was placed under the apex of the heart and the Saran film folded up over the base where it was tied separately around the aortic and pulmonary arterial catheters. The result was a small hole in the Saran wrap at the apex of the heart, a very snug fit over the ventricles, and a 2–3–mm region of imperfect seal at the base of the heart between the aorta and the pulmonary artery.

Five hearts (weighing 1.28 ± 0.075 g; fractional water content, 0.784 ± 0.003) were subjected to five chamber values of FO_2. The Saran wrap was then removed and the measurements repeated, in the same order, on the same heart. That is, a repeated-measures design was adopted in which the "with-Saran" treatment always preceded the "sans-Saran" treatment. The chamber was air-filled and hearts were perfused at 5°C with 20% O_2. These conditions were chosen since they should maximize the effectiveness of any putative oxygen barrier by preventing negative values of $\dot{V}O_2^M$ at high values of chamber FO_2 (cf. Figs. 5 and 6).

The results are presented in Fig. 7. The sheet of Saran (Gladwrap™) clearly diminished the PO_2 dependency of $\dot{V}O_2^M$ but, just as clearly, it failed to prevent the phenomenon. As a result, the apparent rate of oxygen consumption still became negative when the gas-filled chamber was aerated with 95% O_2. However, the relative error in estimating $\dot{V}O_2^H$ from any given value of $\dot{V}O_2^M$ was reduced to about one-third by the presence of Saran (compare rows 2 and 7 of Table I).

Effect of the pericardium. Hypothesis 3 was reexamined with the pericardium as the putative oxygen barrier. Four hearts (1.15 ± 0.15 g, fractional water content 0.788 ± 0.002) were studied at 37°C in the usual repeated measures design but with a fixed order of presentation of treatment conditions: (*a*) pericardium intact, heart beating, (*b*) pericardium intact, heart arrested, (*c*) pericardium removed, heart arrested, and (*d*) pericardium removed, heart beating. During the beating phase the hearts were paced at 4.5 Hz. Perfusion pressure was maintained constant (at 60 torr) during the first half of the experiment (pericardium intact). In the second half of the experiment (pericardium absent) the perfusion pressure was adjusted so as to maintain the coronary flow identical to the value achieved in the corresponding state (beating or arrest) in the first half of the experiment. This maneuver avoided any possible dependence of cardiac oxygen consumption upon coronary flow rate. Because of the duration of this experiment, only two values of chamber FO_2 were employed: 0 and 95%.

The results are presented in Fig. 8. Whether the hearts were beating or arrested,

the difference in VO_2^M with 0 and 95% O_2 in the heart chamber was unaffected by the presence of the pericardium. This could not have been due to any mechanical distortion of coronary flow, subsequent to removal of the pericardium, because flow was maintained constant.

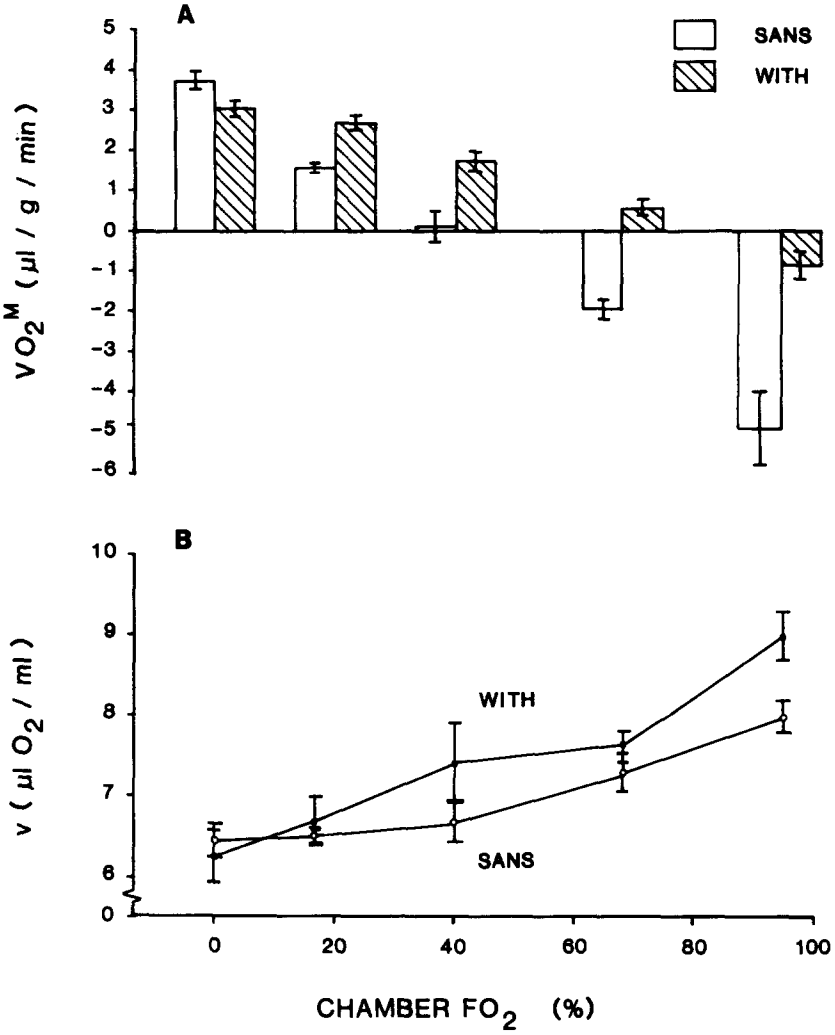

FIGURE 7. Effect of varying ambient oxygen fraction (FO_2) on measured oxygen consumption (*A*) and venous oxygen content (*B*) of five guinea pig hearts. Hearts perfused with 20% O_2 at 5°C initially enveloped in Saran (*WITH*) and then the Saran removed (*SANS*).

This experiment also provided a test of hypothesis 4. The rate of oxygen consumption during arrest fell to ~20% of the value recorded when hearts were paced at 4.5 Hz. The absolute value of the discrepancy in VO_2^M between the 0 and 95% chamber values remained comparable, however, despite the nearly fivefold differ-

ence in metabolic rate (Fig. 8, *top*). But as a consequence the relative error increased from ~15% during pacing to ~60% with arrest. Note that the rate of oxygen consumption during the final (or "recovery") period (i.e., heart beating, pericardium absent) was 93% of that measured during the initial period of activity (heart beating, pericardium present).

Effect of heart size within species. To test hypothesis 5, experiments were performed using hearts from four large (body weight, 1,080 ± 63 g) and four small (body weight, 316 ± 6 g) guinea pigs. The corresponding wet heart weights were 1.88 ± 0.12 and 0.65 ± 0.001 g. The fractional water content of the hearts was 0.782 ± 0.004 and 0.791 ± 0.005, respectively. Each heart was subjected to all four

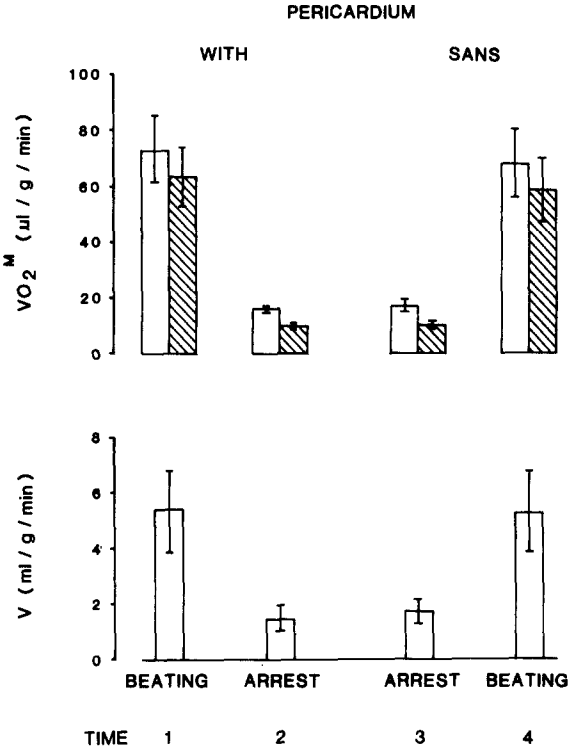

FIGURE 8. Effect on measured rate of oxygen consumption, $\dot{V}O_2^M$ (*top*), of altering chamber FO_2: 0% O_2 (*open bars*), 95% O_2 (*hatched bars*) in four guinea pig hearts. During periods 1 and 2, pericardium intact (*WITH*); during periods 3 and 4, pericardium absent (*SANS*). Perfusion pressure adjusted during periods 3 and 4 to achieve same coronary flow (*V*, *bottom*) as during periods 1 and 2, respectively. No effect of pericardium in either beating or arrested state.

combinations of 20 or 95% arterial O_2 and 0 or 95% gaseous O_2 in the chamber during 80 min of KCl arrest.

The results are presented in Fig. 9. As in all previous figures, $\dot{V}O_2^M$ with 0% O_2 in the chamber in every case exceeded the value of $\dot{V}O_2^M$ with 95% O_2 in the chamber. For the large hearts perfused with 95% O_2, the mean difference was 6.7 µl $O_2 \cdot g^{-1} \cdot min^{-1}$, a value similar to those seen in Figs. 2, 5, 6, and 8, above, under comparable perfusion conditions. The difference when the large hearts were perfused with 20% O_2 was similar (7.6 µl $O_2 \cdot g^{-1} \cdot min^{-1}$) although the absolute magnitudes of both $\dot{V}O_2^M$ values were reduced.

The effect on the small hearts was dramatic. Whether perfused with 20 or 95% O_2, the measured value of oxygen consumption, $\dot{V}O_2^M$, with 0% O_2 in the chamber

always greatly exceeded that measured with 95% oxygen in the chamber. When the arterial perfusate of small hearts contained 20% O_2, than an ambient environment of 95% O_2 reduced their apparent rate of oxygen consumption to the vicinity of zero (leftmost hatched bar of Fig. 9). In fact, for one of the small hearts VO_2^M under these circumstances was repeatedly calculated to be negative. That is, the value of the venous content of oxygen consistently exceeded that of the arterial value. This behavior had previously been seen, in larger hearts, only at very low temperature (Figs. 5–7).

The average discrepancy between the pairs of VO_2^M values for the small hearts was about double that for large hearts: 13.9 and 17.0 μl $O_2 \cdot g^{-1} \cdot min^{-1}$ for 20 and 95% arterial FO_2, respectively, vs. 7.6 and 6.7 μl $O_2 \cdot g^{-1} \cdot min^{-1}$ for large hearts (see above). The greater effect of chamber FO_2 in the smaller hearts can be attributed purely to the difference in heart size because it occurred despite a somewhat

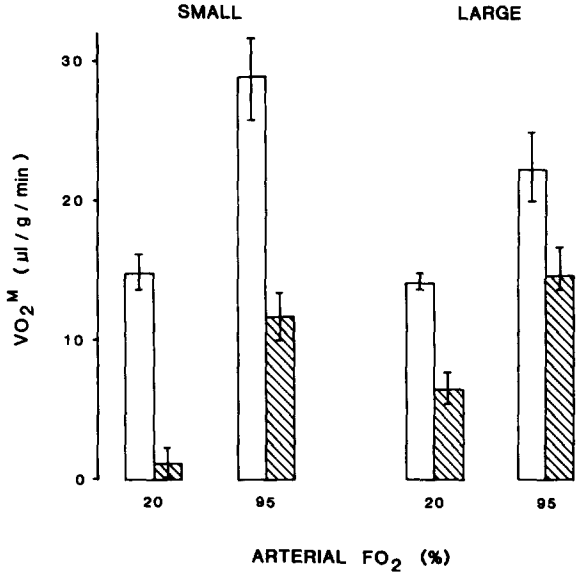

FIGURE 9. Effect on measured rate of oxygen consumption (VO_2^M) of heart size when air-filled heart chamber aerated with 0% O_2 (*open bars*) or 95% O_2 (*hatched bars*). Hearts from four small and four large guinea pigs perfused with both 20 and 95% O_2.

reduced rate of coronary flow: 4.93 ± 0.56 $ml \cdot g^{-1} \cdot min^{-1}$ vs. 6.25 ± 1.10 $ml \cdot g^{-1} \cdot min^{-1}$ in the large hearts. The corresponding values of g_H, the diffusive myocardial oxygen conductance, with 20 and 95% arterial FO_2, respectively, were 15.6 ± 1.7 and 18.7 ± 1.6 μl $O_2 \cdot g^{-1} \cdot min^{-1} \cdot atm^{-1}$ for small hearts and 6.6 ± 1.9 and 6.7 ± 1.6 μl $O_2 \cdot g^{-1} \cdot min^{-1} \cdot atm^{-1}$ for large hearts. As predicted by the model, g_H was insensitive to arterial PO_2 but varied inversely with heart size.

Effect of heart size across species. Hypothesis 5 was further tested by exploiting the natural dependence of heart size on species size. Hearts of 24 rats and eight rabbits were perfused in the air-filled chamber equilibrated with either 0 or 95% O_2. The diffusive oxygen conductance of the heart, g_H, was computed from the model. The results, combined with those from 90 guinea pig hearts, are shown in Fig. 10. There was a statistically significant inverse (hyperbolic) dependence of g_H on heart weight where the latter varied by roughly an order of magnitude. There is thus no suggestion that the guinea pig heart, with its extensive collateral circulation

(Schaper et al., 1986), has an inherently greater diffusive oxygen conductance than either the rat or the rabbit.

DISCUSSION

A voluminous literature detailing numerous, often ingenious, methods of measuring oxygen consumption of the heart has arisen since the initial report by Yeo (1885). Yeo discussed a major source of measurement error, namely the considerable loss of oxygen through the walls of rubber tubing: a lesson that was sometimes forgotten, even by his immediate successors (Barcroft and Dixon, 1906). Early workers, using volumetric techniques and heart-lung preparations, considered the loss of car-

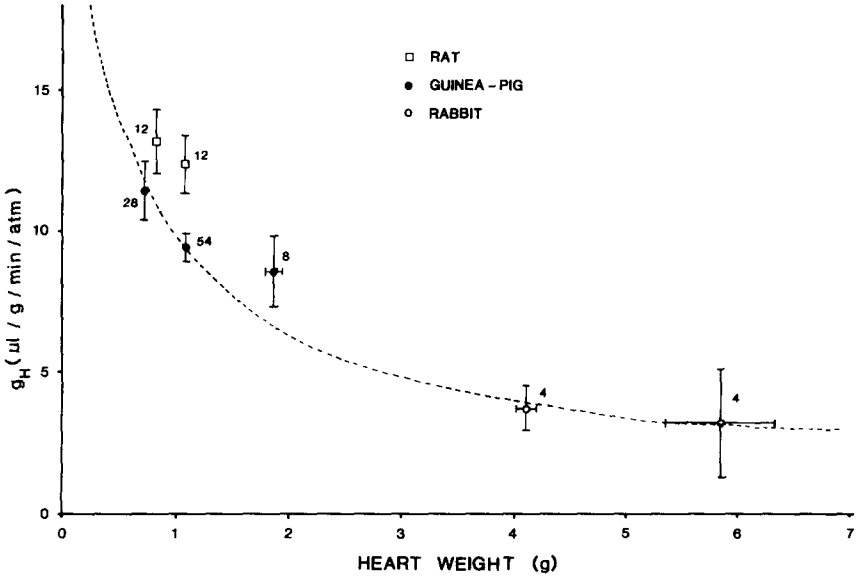

FIGURE 10. Variation of diffusive oxygen conductance of the heart, g_H, with wet heart weight, across species. Arbitrary division into bins according to body weight; for rats at 400 g, for guinea pigs at 400 and 800 g, for rabbits at 3 kg. Number of observations per bin appears beside standard error bars (latter sometimes smaller than width of symbol). (*Dashed curve*) Hyperbolic regression, $g_H = 0.97 + 14.4/(0.60 + \text{heart weight})$, $r = 0.92$.

bon dioxide from the surface of the lung (Evans, 1912) and often carefully evaluated the rate of oxygen consumption of both lung tissue (Evans, 1912; Evans and Starling, 1913) and the circulating blood (Evans and Matsuoka, 1915). But none reported any exchange of oxygen across the heart surface. Many early workers clearly recognized this possible source of error and immersed the heart in saline or oil in an attempt to minimize gaseous exchange, despite the fact that Yeo (1885) could detect no effect upon the oxygen consumption of the heart subsequent to its immersion in olive oil.

In 1971 Follert clearly showed that the diffusive loss of oxygen across the surface of the isolated guinea pig heart could lead to a severe overestimation of its rate of oxygen usage. This paper seems to have languished, however. It is not until very

recently that this source of error has been explicitly readdressed (Loock and Schmidt, 1986) and, in one case at least, fully taken into account in accurate determination of the rate of oxygen consumption of isolated rat hearts (Wilkman-Coffelt et al., 1983). However, no full description of the magnitude of this oxygen exchange phenomenon nor analysis of its determining factors has been located in the literature. These are provided in the current paper together with a simple mathematical model that quantifies the extent of exchange and provides a method of calculating the true rate of myocardial oxygen consumption from measurements such as those reported above. But independent of any mechanism proposed to explain the phenomenon, the above data make it clear that the rate of oxygen consumption of the isolated heart, as classically measured using the Fick Principle, will generally be in error.

The rate of oxygen consumption of the heart, measured according to the Fick Principle (Eq. 1), is expected to be independent of ambient PO_2. The results presented in Fig. 2 *A*, for the Langendorff-perfused guinea pig heart isolated in an air-filled glass chamber, show that this is not the case. When the perfusate has been equilibrated with 95% O_2, the apparent (i.e., measured) rate of myocardial oxygen consumption, VO_2^M, decreases linearly with increasing ambient PO_2 (see also row 1 of Table I). The simplest explanation for this observation is that oxygen freely diffuses across the epicardial surface of the heart. The model of myocardial oxygen exchange proffered in the current paper (Eqs. 6 or 10) simply adds a term to the usual Fick model (Eq. 1) to quantitate this transepicardial flux of oxygen. The added term is comprised of the product of a purely physical factor (the transepicardial gradient of oxygen partial pressure) with a physiological factor unique to each heart (its diffusive oxygen conductance, g_H).

Now the diffusion of gaseous solutes is driven solely by their gradients of partial pressure. Hence it was predicted that the magnitude of the effect seen in Fig. 2 *A* would be independent of the nature of the fluid filling the heart chamber. This expectation was fulfilled. Whether the heart was suspended in air (Fig. 2 *A*), saline (Fig. 5 *A*), or oil (Fig. 6 *A*), its measured rate of oxygen consumption underwent the same (linear) decline as ambient PO_2 increased.

A second expectation, arising from the hypothesis of transepicardial oxygen diffusion, is that the net direction of diffusive exchange can be made positive, negative, or zero by suitable choice of experimental conditions. This expectation was also fulfilled. When the oxygen fraction of the perfusate was lowered to 20%, then the measured oxygen consumption fell from a positive value at low ambient PO_2, through zero, to become negative at high ambient PO_2. In the latter case the oxygen content of the coronary venous outflow exceeded that of the arterial inflow (Figs. 5–7).

These results clearly established the phenomenon of an ambient PO_2 dependence of the measured rate of oxygen consumption of the isolated heart and gave confidence that the model was essentially correct. But the model gained further credibility when additional predictions were confirmed experimentally. Thus the diffusive oxygen conductance (or, equivalently, the rate of transepicardial oxygen exchange for any given PO_2 gradient) was shown to be independent of metabolic rate (Fig. 8), reduced (though not eliminated) by the use of Saran wrap (Fig. 7), and greater in small than in large hearts both within (Fig. 9) and across (Fig. 10) species.

Fig. 10 emphasizes that the phenomenon of diffusive oxygen exchange is not specific to the heart of any particular species. This result contradicts a recent report by Loock and Schmidt (1986) that the effect does not appear in rat hearts. Also contradicted is their assertion that varying the external O_2 environment from 0 to 95% causes only a 12% reduction in the apparent rate of oxygen consumption of KCl-arrested guinea pig hearts. As can be seen in Fig. 2 *A* and Table I, the effect is rather some three or four times larger.

Implications of the Phenomenon

To what extent does the phenomenon of diffuse oxygen exchange across the heart wall matter, in practice, to the accurate measurement of cardiac oxygen consumption? The above results are unambiguous. Unless it is arranged that the environment has a PO_2 identical to that of the myocardium, then the resulting calculated rate of oxygen consumption will be in error. The absolute magnitude of the error is roughly independent of the metabolic rate (Fig. 8) so measurements made during cardiac arrest are at much greater relative risk. Immersion of the heart in either saline (Fig. 5) or oil (Fig. 6) confers no advantage. (A qualification is required here. If the heart is immersed in saline within an air-tight chamber which functions to collect the coronary venous effluent, after the manner of Wilkman-Coffelt et al. [1983], then complete accuracy in the steady-state can be achieved by sampling this saline pool for venous oxygen content. But transient changes in v [and hence VO_2^H] will be highly damped because the pool of saline will act as an oxygen "capacitor." That is, the frequency response of the measurement system will be compromised: a result which may be of little consequence in some applications.)

The error is still present when the FO_2 of the perfusate is lowered to 20% (Figs. 5–7 and 9). Hence it is expected to occur even in blood-perfused preparations. In such cases the error would be less, however, due to the reduced gradient of PO_2 from myocardium to surrounds (room air). The error is likely present even in open-chest preparations in situ because the pericardium appears to present no barrier to oxygen diffusion (Fig. 8). Covering the exposed surface with Saran would diminish but not prevent the problem (Fig. 7).

The most insidious error, however, may arise in experiments in which the heart is housed in an air-tight glass chamber specifically designed to avoid such problems. For even under conditions of perfect metabolic steady state, the PO_2 of the chamber would rise or fall (depending on its initial condition) to reach a plateau corresponding to mean myocardial PO_2. That is, even an air-tight heart chamber adiabatically isolated from room air will nevertheless act as an "oxygen capacitor" as oxygen diffuses into it from the heart. During the charging of this "capacitor" the oxygen consumption of the heart, calculated via the Fick Principle, will show an entirely spurious time-dependent decline. Because the maximum possible diffusive loss is of the order of 10 $\mu l \cdot g^{-1} \cdot min^{-1}$ (see Table I) it is clear that even a very small heart chamber can have an enormous oxygen capacitance. If the heart is merely suspended in room air that is subjected to variable stirring, then the error is more difficult to assess.

Because of this diffusive exchange phenomenon, many reported measurements of myocardial oxygen consumption may be in error. This is especially true of experi-

ments where the metabolic rate of the heart is low, the heart is small, or the perfusate has been equilibrated to a high partial pressure of oxygen. Such experimental conditions typically prevail in studies of the basal rate of cardiac metabolism.

I wish to thank Dr. P. McN. Hill for pointing out the parallel between the proposed model of diffusive oxygen exchange in the heart and the well-characterized diffusing capacity of the lung. It is a pleasure to thank Dr. Poul Nielsen for assistance in developing the nonlinear regression solution. Miss Sally Lark provided invaluable technical assistance in performing the experiments.

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REFERENCES

Armiger, L. C., R. N. Seelye, M. A. Morrison, and D. G. Holliss. 1984. Comparative biochemistry and fine structure of atrial and ventricular myocardium during autolysis in vitro. *Basic Research in Cardiology.* 79:218–229.

Armitage, W. G., and D. E. Pegg. 1977. An evaluation of colloidal solutions for normothermic perfusion of rabbit hearts: an improved perfusate containing Haemaccel. *Cryobiology.* 14:428–434.

Barcroft, J., and W. E. Dixon. 1906. The gaseous metabolism of the mammalian heart. Part I. *Journal of Physiology.* 35:182–204.

Dejours, P. 1975. Principles of Comparative Respiratory Physiology. North-Holland Publishing Co., Amsterdam.

Evans, C. L. 1912. The gaseous metabolism of the heart and lungs. *Journal of Physiology.* 44:213–234.

Evans, C. L., and Y. Matsuoka. 1915. The effect of various mechanical conditions on the gaseous metabolism and efficiency of the mammalian heart. *Journal of Physiology.* 49:378–405.

Evans, C. L., and E. H. Starling. 1913. The part played by the lungs in the oxidative processes of the body. *Journal of Physiology.* 46:413–434.

Follert, E. K. 1971. Significance of diffusion loss of oxygen in determining respiration of isolated, perfused organs. *Pflügers Archiv European Journal of Physiology.* 323:80–85.

Hill, A. V. 1964. Trails and Trials in Physiology. Edward Arnold Publishers Ltd., London.

Loiselle, D. S. 1985. The effect of temperature on the basal metabolism of cardiac muscle. *Pflügers Archiv European Journal of Physiology.* 405:163–169.

Loock, W., and H. D. Schmidt. 1986. Relevance of oxygen diffusion loss through the surface in isolated hemoglobin free perfused hearts. *Pflügers Archiv European Journal of Physiology.* 406(Suppl. 1):R32. (Abstr.)

Lorber, V. 1953. Energy metabolism of the completely isolated mammalian heart in failure. *Circulation Research.* 1:298–311.

Mahler, M., C. Louy, E. Homsher, and A. Peskoff. 1985. Reappraisal of diffusion, solubility, and consumption of oxygen in frog skeletal muscle, with applications to muscle energy balance. *Journal of General Physiology.* 86:105–134.

Ray, A. A., editor. 1982. SAS (Statistical Analysis System) User's Guide. SAS Institute Inc., Cary, NC.

Robinson, L. A., M. V. Braimbridge, and D. J. Hearse. 1983. Myocardial damage induced by particulate contamination of cardioplegic solutions. *Journal of Molecular and Cellular Cardiology.* 15(Suppl.1):305. (Abstr.)

Rodger, R. S. 1975. The number of non-zero, post hoc contrasts from ANOVA and error-rate I. *British Journal of Mathematics and Statistics in Psychology.* 28:71–78.

Schaper, W., J. Schaper, and B. Winkler. 1986. The collateral circulation of the heart. *Journal of Molecular and Cellular Cardiology.* 18(Suppl. 1):60. (Abstr.)

Wilkman-Coffelt, J., R. Sievers, R. J. Coffelt, and W. W. Parmley. 1983. The cardiac cycle: regulation and energy oscillations. *American Journal of Physiology.* 245:H354–H362.

Yeo, G. F. 1885. An attempt to estimate the gaseous interchange of the frog's heart by means of the spectroscope. *Journal of Physiology.* 6:93–121.