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An Improved Isolated Working Rabbit Heart Preparation Using Red Cell Enhanced Perfusate

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The performance of isolated working rabbit hearts perfused with Krebs-Henseleit (KH) buffer was compared with those in which the buffer was supplemented with washed human red blood cells (KH + RBC) at a hematocrit of 15 percent. When perfused with KH alone at 70 cm H_2O afterload and paced at 240 beats/minute, coronary flow was more than double, whereas aortic flow was 40–60 percent of that in hearts perfused with KH + RBC, regardless of left atrial filling pressures (LAFP). Peak systolic pressure reached a plateau at 120 mm Hg in KH + RBC, but at 95 mm Hg in the KH group. Stroke work, however, was similar in the two groups. Despite the high coronary flow, oxygen uptake by hearts perfused with KH was substantially less and did not respond to increases in LAFP as in those perfused with KH + RBC. There was a 20 percent drop in ATP and glycogen content after 90 minutes' perfusion. In contrast, isolated hearts perfused with RBC-enriched buffer remained stable for at least 150 minutes. Irrespective of the perfusate, triacylglycerol content of the muscle remained at similar levels throughout the course of study. Increasing RBC in the perfusate from 15 percent to 25 percent had no additional effect on cardiac performance or oxygen consumption. Our findings demonstrate that in the isolated working rabbit heart inclusion of RBC in the perfusate improves mechanical and metabolic stability by providing an adequate oxygen supply.

INTRODUCTION

The isolated perfused working rat heart preparation developed by Neely et al. [1] has been used extensively in studying myocardial performance and metabolism. Metabolic activities such as oxygen consumption and substrate utilization can be measured directly and related to mechanical work over a range of controlled physiological workloads. An additional advantage is that isolated hearts are not subjected to autonomic or circulating factors such as occur in intact animals. Working heart preparations using guinea pigs [2], rabbits [3–5], and newborn pigs [6] have also been developed. Generally, these preparations are perfused with Krebs-Henseleit (KH) bicarbonate buffer which has a much lower oxygen-carrying capacity than whole blood. Although adequate oxygen supply is maintained in isolated rat and guinea pig hearts [1,2,7], recent findings by Paradise et al. [8] have shown that perfusing with KH alone does not provide sufficient oxygen for the retrogradely perfused isovolumic (non-working) rabbit hearts subjected to lower levels of work than working hearts. These preparations had been shown to perform better when perfused with red cell-enriched buffer [9].

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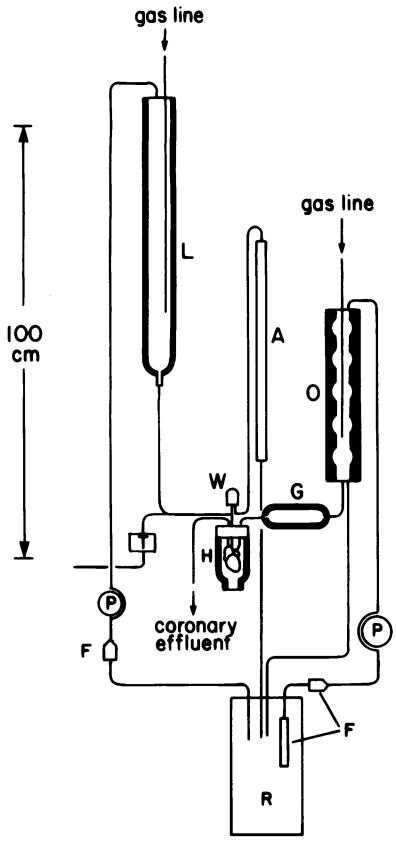

FIG 1. Schematic diagram of isolated perfused working rabbit heart apparatus. **A**, afterload column; **F**, filters; **G**, Graham condenser; **H**, heart chamber; **L**, Langendorff preperfusion column; **O**, perfusate oxygenator; **P**, drive pumps; **R**, perfusate reservoir; **T**, pressure transducer; **W**, Windkessel chamber.

The purpose of this report is to describe functional and metabolic characteristics of an improved isolated working rabbit heart preparation in which KH is supplemented with red cells (KH + RBC). Comparison is made with similar preparations perfused with KH alone. The findings indicate that left ventricular performance and responses to pressure loads in the KH + RBC group are comparable to those found *in vivo*. Moreover, the myocardium remains functionally and metabolically stable for at least 2½ hours.

MATERIALS AND METHODS

Isolated Working Heart Apparatus

The perfusion apparatus was a modification of that developed for rat hearts [1,7]. The elements included a heart chamber, a perfusate oxygenator, a cannula assembly connected to a modified Graham condenser (with water jacket-type inlet and outlet), an afterload column, and a Langendorff preperfusion column (Fig. 1). An Allihn condenser 60 cm long served as the oxygenator to ensure the buffer was adequately gased. A Graham condenser was placed before the cannula assembly and functioned as a second heat exchanger to maintain temperature of the perfusate entering the heart at 37°C. Initial experience indicated that, without it, temperature of the perfusate dropped by as much as 2–3°C (depending on room temperature) before entering the hearts and thus created a temperature gradient across the muscle. This condition often

led to arrhythmia, notably pulsus alternans. Buffer in the Langendorff column was kept at a height of 100 cm to generate a pressure of approximately 75 mm Hg at the tip of the aortic cannula. Different sizes of various cannulas were tested for this study because the flow of perfusate is in part dependent on the bores, hence resistance imposed by the cannulas. Those being used approximated the sizes of the vessels cannulated and allowed cardiac work to increase linearly over a range of left atrial filling pressures. Only Tygon special or food-grade tubing was used to transport the perfusate because cellular fragments do not readily adhere to its surface. The bores of all stopcocks were enlarged to facilitate buffer flow. The enlargement was especially crucial in preventing hemolysis. All condensers were siliconized before use.

The buffer was filtered through a fritted disc filter before reaching the heart. Without filtration the heart preparations were found to deteriorate rapidly. The filter was replaced after every 30-minute perfusion to avoid hemolysis. The top of the oxygenator was partially closed to ensure that the entire column was saturated with 95 percent O_2–5 percent CO_2. To prevent foaming, the perfusate was not gased directly, but aerated as it flowed in a thin film down the inner surface of the condenser. The pO_2 and pCO_2 of the perfusate were maintained at 450–500 and 35–40 mm Hg throughout the course of perfusion. The average oxygen-carrying capacity of KH, KH + RBC_{15}, and KH + RBC_{25} were 1.4, 6, and 11 ml oxygen/100 ml buffer, respectively.

Preparation of Perfusates

All hearts were perfused with a recirculating volume of 1 liter of modified KH bicarbonate buffer (pH 7.4), without or with supplementation of washed human RBC. The medium consisted of: 118 mM NaCl, 4.7 mM KCl, 2.4 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 5.5 mM glucose, 1.5 mM lactate, 0.45 mM free fatty acids (FFA), 100 μU/ml insulin, and 0.5 mM bovine serum albumin (BSA). BSA used contained 0.21–0.32 mM FFA. Palmitate (0.13–0.24 mM) was added to make a final concentration of 0.45 mM. The concentrations of insulin and substrates in the perfusate were comparable to those found *in vivo*. In hearts perfused with RBC-enriched buffer, washed red cells were added to provide a hematocrit of 15 (KH + RBC_{15}) or 25 (KH + RBC_{25}) percent. Packed human RBC were obtained from the hospital blood bank. Human RBC were used because of their availability and comparable size to rabbit RBC. Similar performance between hearts perfused with human or rabbit RBC was observed in preliminary studies. They were washed twice with saline and twice with KH buffer at 4°C with a hematocrit of 25 percent just before use. The saline and KH were supplemented with 0.15 mM and 0.5 mM BSA. Preliminary studies showed that the BSA is important in maintaining RBC stability. A minimum of four washes was required to remove non-red cell elements (e.g., hormones and proteins). The washed cells were filtered through surgical gauze to remove microaggregates before mixing with KH buffer. Hematocrit of the buffer was maintained using a magnetic stirrer. A floating stir bar was used to avoid hemolyzing the RBC.

Isolation Techniques and Perfusion Protocols

Male New Zealand white rabbits weighing 2.5–3.0 kg were used in this study. The animals were anesthetized with pentobarbital sodium (30 mg/kg, intravenously). A bolus of heparin was administered (1,000 U/kg, intravenously) 20 minutes before the animals were tracheotomized and connected to a precalibrated respirator. After

another five minutes to allow stabilization, the chest cavity was opened and the pericardium incised. The heart was excised and immediately transferred to ice-cold KH buffer and rinsed twice to remove most of the blood. Special care was taken not to allow bubbles to enter the heart. The aorta was then cannulated and the heart perfused retrogradely with non-circulating KH to wash out residual blood. The left atrial appendage was incised, gathered around the left atrial cannula, and tied in place. The pulmonary veins were ligated. A small incision was made at the bifurcation of pulmonary arteries. The pulmonary artery cannula was inserted and tied in place. The vena cavae and azygous vein were ligated. Thus, all coronary effluent was collected by the pulmonary artery cannula. The Langendorff column was shut off and simultaneously antegrade perfusion with KH, KH + RBC_{15} or KH + RBC_{25} via left atrium was begun. The time elapsed between excision of the heart to cannulation of the aorta was one to two minutes; and that to commencement of antegrade perfusion was seven to ten minutes.

The heart was electrically paced at 240 beats/minute and allowed to stabilize for 30 minutes at 10 cm H_2O left atrial filling pressure (LAFP). Cardiac work was varied by altering LAFP in steps from 5 to 10, 15, 20, 25, 30, and finally back to 10 cm H_2O. Each level of LAFP was maintained for five minutes and the following measurements were made: peak systolic (PSP) and diastolic (DP) pressure, and coronary and aortic flow. Oxygen consumption was estimated from A-V differences in oxygen content determined by the Lexington oxygen content analyzer (Lex-O_2-Con). Mechanical performance was assessed from the relationships of PSP and stroke work to LAFP.

To evaluate stability, the hearts were freeze-clamped at liquid nitrogen temperature after 90 or 150 minutes of perfusion. A portion of the frozen heart was deproteinized with perchloric acid. Myocardial ATP and glycogen concentrations were determined, using the neutralized (with KOH) perchlorate extracts [10]. Muscle triacylglycerols were extracted with chloroform and measured according to Froberg [11]. The remaining portion of the frozen heart was dried at 90°C for 72 hours for estimation of tissue water content. For comparison, unperfused rabbit hearts were also freeze-clamped *in situ* and ATP, glycogen, and triacylglycerol content determined.

Statistical Analysis

Data are expressed as means ± SE/g dry weight. Results from KH and KH + RBC perfused rabbit hearts were analyzed by one-way ANOVA followed by Newman-Kuel's test. Performance of individual hearts at different LAFP was analyzed with dependent Student's *t*-test. Significance was set at $p < 0.05$.

RESULTS

Intrinsic heart rates averaged 184 ± 5 beats/minute ($n = 33$). Without pacing, hearts perfused with KH were unable to maintain an afterload of 70 cm H_2O. This was also observed in paced hearts perfused at 5 cm H_2O LAFP. However, with red cells added to the perfusate, they maintained afterloads as high as 120 cm H_2O. For comparative purposes, all hearts were perfused at 70 cm H_2O afterload.

Irrespective of the preloads, coronary flow in hearts perfused with KH was greatly elevated (Fig. 2). At 20–30 cm H_2O LAFP, the flow rates were approximately 2½ times those of the KH + RBC group. This also resulted in a higher total cardiac output even when aortic flow was significantly less than in hearts perfused with KH + RBC (Fig. 2, left middle and lower panels). Moreover, maximum coronary and aortic flow

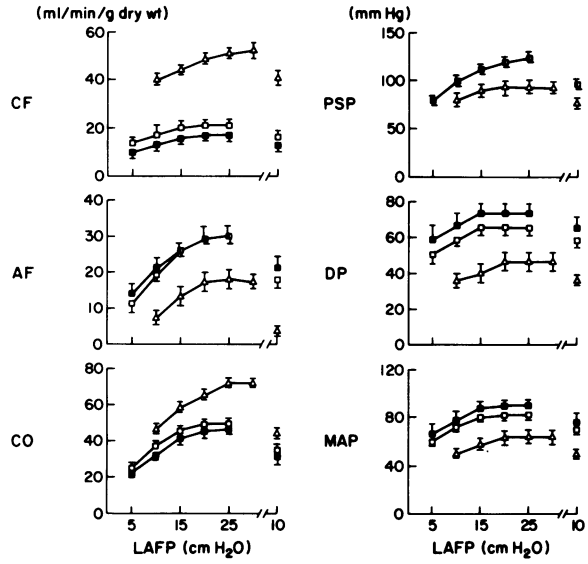

FIG. 2. Flow rates and left ventricular mechanical function of isolated working rabbit hearts perfused with KH buffer alone *(open triangles)* or supplemented with RBC at a hematocrit of 15 percent *(open squares)* or 25 percent *(solid squares)*. Values are means ± SE of four to six hearts. Flow rates and pressures of KH-perfused hearts are significantly different from those perfused with KH + RBC ($p < 0.05$). Changes in hematocrit were without effect. **CF,** coronary flow; **AF,** aortic flow; **CO,** cardiac output; **PSP,** peak systolic pressure; **DP,** diastolic pressure; **MAP,** mean arterial pressure; **LAFP,** left anterior filling pressure.

values in KH-perfused hearts were attained at 25 cm H_2O LAFP, whereas in KH + RBC perfused hearts, the flow rates reached a plateau at 15 cm H_2O LAFP. To evaluate mechanical stability, following each run, LAFP was reduced gradually from 25/30 down to 10 cm H_2O. Coronary and aortic flow in the KH + RBC groups were comparable to values observed previously at that LAFP; however, aortic flow in hearts perfused with KH alone was 50 percent lower ($p < 0.001$).

Left ventricular pressure generation was substantially smaller in hearts perfused with KH alone at the various preloads (Fig. 2). When the filling pressure was raised from 10 to 25 cm H_2O, PSP increased only 18 percent. In hearts perfused with KH + RBC_{15} or KH + RBC_{25} PSP increased 50 percent when LAFP was raised from 5 to 25 cm H_2O. Increases in diastolic and mean aortic pressures showed similar patterns (Fig. 2, right middle and lower panels).

Hearts perfused with KH consumed less oxygen than those perfused with KH + RBC (Fig. 3, upper panel). Moreover, they were unable to increase oxygen consumption when subjected to increasing LAFP. This contrasts with the RBC-enhanced groups which showed progressive increases in O_2 consumption. A significant relationship was found between myocardial oxygen consumption and coronary flow in hearts perfused with KH + RBC_{15} or KH + RBC_{25}. Linear regression analysis showed correlation coefficients of 0.87 and 0.92 ($p < 0.001$), respectively.

Myocardial work was assessed from the relationship of double product (CO × PSP) or stroke work (CO × PSP/HR) to LAFP. The responses were similar between hearts perfused with KH and KH + RBC. Both the double product (data not shown) and stroke work (Fig. 3, lower panel) increased with increases in LAFP, and reached a plateau at 20 cm H_2O.

Since mechanical functions and oxygen consumption between hearts perfused with KH + RBC_{15} and KH + RBC_{25} were not significantly different, data from the two groups were pooled for subsequent comparisons.

There was a direct correlation between oxygen consumption and double product in hearts perfused with KH containing RBC (Fig. 4), but not with KH alone (data not

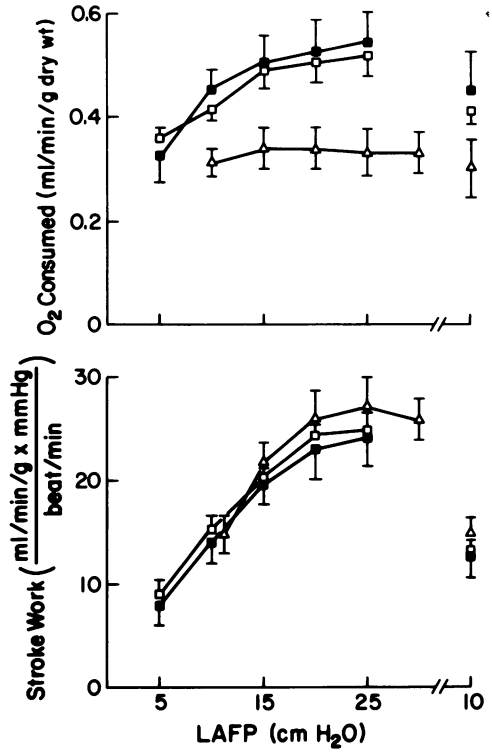

FIG. 3. Oxygen consumption and stroke work of isolated working rabbit hearts perfused with KH buffer alone *(open triangles)* or supplemented with RBC at a hematocrit of 15 percent *(open squares)* or 25 percent *(solid squares)*. Values are mean ± SE of five to six hearts. Oxygen consumption is significantly different between hearts perfused with KH and KH + RBC ($p < 0.05$). Stroke work curves are identical.

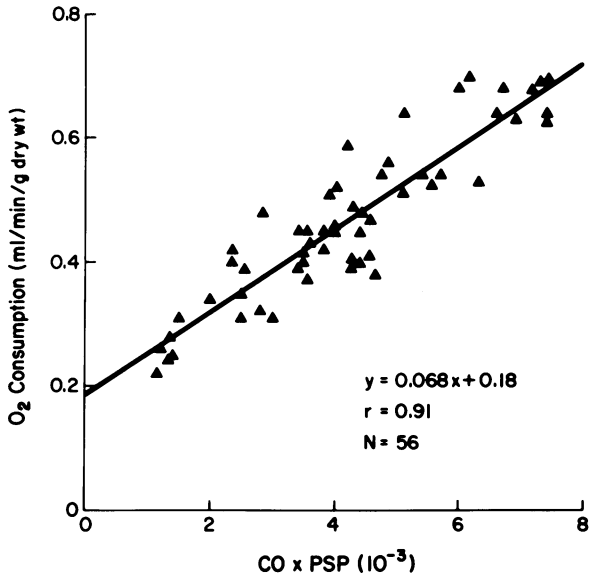

FIG. 4. The relationship of oxygen consumption to the double product of cardiac output (**CO**) × peak systolic pressure (**PSP**) in isolated rabbit hearts perfused with KH containing 15 or 25 percent RBC. Correlation coefficient is significant at $p < 0.001$.

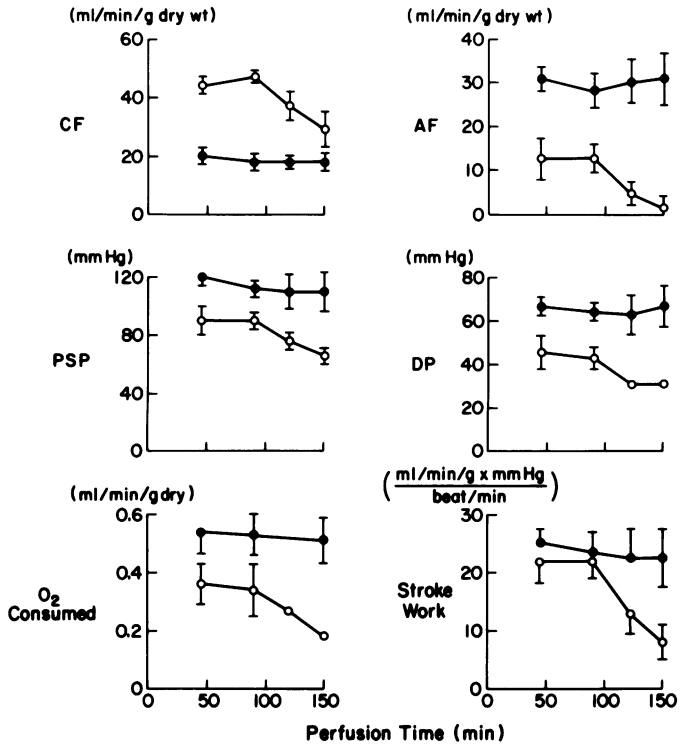

FIG. 5. Time course study on the performance of isolated working rabbit hearts perfused with KH buffer with *(solid circles)* or without *(open circles)* added RBC. The hearts were maintained at 20 cm H_2O left atrial filling pressure, 70 cm H_2O afterload, and paced at 240 beats/minute. Values are means ± SE of three to five hearts. Left ventricular mechanical function of the buffer-perfused hearts deteriorated significantly ($p < 0.05$) after 120 minutes' perfusion. Those with added RBC were stable for 150 minutes.

shown). The correlation coefficients were 0.90 ($p < 0.001$) and 0.29 ($p > 0.05$), respectively.

Mechanical stability of the isolated working hearts was also examined over a period of 150 minutes. All were perfused at a constant preload of 20 cm H_2O. As shown in Fig. 5, the performance of both groups remained unchanged for 90 minutes. Thereafter, in those perfused with KH mechanical function and oxygen usage began to deteriorate. This is in contrast to hearts perfused with RBC at either 15 or 25 percent hematocrit where all parameters remained stable for the full 150-minute period.

Myocardial ATP and glycogen values are shown in Table 1. In the KH group these were significantly decreased (−23 and −25 percent) after 90 minutes and declined further (−42 and −31 percent) after 150-minute perfusion. Tissue water content was also greater after 90-minute perfusion. In contrast, ATP and glycogen concentrations, as well as water content, did not change between 90 and 150 minutes in hearts perfused with KH + RBC_{15} or KH + RBC_{25}. Moreover, the values were comparable to those (24.4 ± 1.8 and 74.6 ± 3.2 μmol/g and 78 ± 0.5 percent, respectively) obtained from the *in situ* hearts ($n = 4$). Despite a reduction in glycogen store, triacylglycerol concentration in hearts perfused with KH remained the same during the course of study (Table 1). The value was similar to that obtained from hearts perfused with red cell-enriched buffer or freeze-clamped *in situ* (28.4 ± 1.6 μmol/g).

DISCUSSION

This study describes an isolated working rabbit heart preparation in which the heart is perfused with RBC-enriched KH bicarbonate buffer. The preparation has met the

TABLE 1
Myocardial ATP, Glycogen, and Tissue Water Content

	Perfusate	Perfusion Time (minutes) 90	150
ATP (μmol/g)	KH	18.0 ± 0.8	14.3 ± 0.9
	KH + RBC	23.3 ± 2.3[a]	24.3 ± 2.1[a]
Glycogen (μmol/g)	KH	63.1 ± 2.6	50.6 ± 3.2
	KH + RBC	76.1 ± 3.8[a]	78.1 ± 4.0[a]
Triacylglycerol (μmol/g)	KH	28.8 ± 2.7	26.7 ± 4.4
	KH + RBC	30.5 ± 4.9	31.8 ± 5.7
Tissue water content	KH	83.1 ± 0.5	83.8 ± 0.6
(% wet weight)	KH + RBC	78.7 ± 0.6[a]	79.7 ± 0.5[a]

Values are means ± SE of three to five hearts. KH + RBC has 15 percent or 25 percent hematocrit.
[a] $p < 0.05$ from KH-perfused hearts

two most important criteria for an *in vitro* heart preparation: the ability to respond to controlled physiological workloads and the potential to remain stable for a prolonged time period. Both coronary flow (3.1–3.7 ml/minute/g wet) and mean aortic pressure (83–90 mm Hg) at high filling pressures are comparable to those found in intact rabbits; these average 2.7 ml/minute/g wet and 85–105 mm Hg, respectively [12]. Moreover, ATP, glycogen, and triacylglycerol content, even after 150-minute perfusion, are similar to those of hearts freeze-clamped *in situ*. The working rabbit heart preparation possesses significant advantages in that it does not require expensive custom-fabricated components and it can be easily modified to suit specific experimental requirements.

The present findings reiterate the need for an additional oxygen-carrying vehicle in maintaining functional and metabolic integrities of the KH-perfused rabbit hearts. Adequate oxygen supply to the myocardium is maintained as indicated by a highly significant relationship between oxygen consumption and mechanical work (Fig. 5). When perfused with KH supplemented with RBC at 15 percent hematocrit and a preload of 5 cm H_2O, oxygen consumed by each heart averaged 0.5 ml/minute and coronary flow was 20 ml/minute. Because of the much lower oxygen-carrying capacity of KH (1.4 ml oxygen/100 ml buffer at 37°C) the amount of dissolved oxygen passing through the coronary vasculature was only 0.28 ml/minute. It thus appears that even at the lowest preload, KH alone is inadequate to meet the oxygen demands of the perfused rabbit heart.

The importance of red cell augmentation has also been demonstrated in the retrogradely perfused isovolumically beating rabbit hearts in which oxygen requirements are considerably less than in working hearts perfused at high filling pressures [9]. Both pressure developments and oxygen consumption were markedly elevated after sheep RBC were added to the perfusate. The increases were more substantial when the hematocrit was increased from 25 to 40 percent. Raising hematocrit in the perfusate, however, had no additional effect on the mechanical function or oxygen consumption by the working rabbit hearts (Figs. 2 and 3). The unaltered oxygen consumption, despite increases in hematocrit, has also been demonstrated in the isolated working rat hearts [14]. The discrepancy is partly attributable to differences in the perfusion conditions of the two heart preparations, especially in the regulation of

coronary flow. Coronary flow in the working heart is dependent on the filling pressures. In the isovolumic heart, the flow rate was maintained by 60 mm Hg perfusion pressure [9]. It is conceivable that the perfusion pressure was insufficient to sustain optimal coronary flow and the myocardium had become oxygen-deficient when perfused with RBC at 25 percent hematocrit.

Our findings also confirm the importance of filtering media containing cell elements for the perfusion of isolated organs. This point has been emphasized previously [9,13,14]. Different blood transfusion filters were tested and found to be unsatisfactory mainly because they were unable to handle the high volume of filtration required for this study. As a result, hemolysis and functional deterioration appeared rapidly. The fritted disc filters employed satisfactorily removed microaggregates of RBC and other debris from the perfusate. By replacing the filters regularly, hemolysis as indicated by hemoglobin released from ruptured cells is negligible after 2½-hour perfusion. Besides appropriate filters, maintaining RBC stability during the washing procedure and assuring non-restricted flow of buffer through the perfusion apparatus, especially through stopcocks, are equally important in reducing red cell trauma.

The use of vehicles besides RBC to increase oxygen-carrying capacity of KH has been explored. Notable is perfusion with the perfluorochemical emulsion FC-43 which has an oxygen-carrying capacity [15] approximating that of KH + RBC_{15} (5.6 versus 6.0 volume percent) employed in the present study. Chemnitius et al. [5] have recently demonstrated that the performance of isolated working rabbit hearts perfused with FC-43 is significantly improved and oxygen consumption is increased when compared with those perfused with KH. The emulsion has also been shown to preserve mechanical function of isolated rat hearts [16]. The ameliorating effect is not due to increased oxygen supply, however, as indicated by similar oxygen consumption rate between hearts perfused with FC-43 and KH. Deterioration in the performance of rat hearts perfused with KH is probably attributable to the very high coronary flow and absence of BSA in the buffer. Albumin is known to play an important role in maintaining stability of the isolated heart preparations [14,15]. It is also important to mention that BSA is essential in studies of myocardial fatty acid metabolism, where it functions as a carrier for the FFA.

Despite the greatly enhanced oxygen-carrying capacity, FC-43 perfused hearts exhibit a major limitation in that they become edematous. The 83 percent tissue water content [5,16] is actually identical to that in hearts perfused with KH alone (Table 1). The deleterious effect of interstitial edema on ventricular function is, in fact, evident in the isolated rat hearts perfused with FC-43 [16].

In addition to adequate oxygen supply the availability of exogenous substrates is also crucial in maintaining metabolic stability of isolated hearts. In this study, the perfusate was supplemented with a mixture of substrates instead of glucose alone. It is well recognized that glycolytic flux of glucose in the myocardium is rate-limiting and glucose oxidation is not sufficient to support its energy demand even when the heart is perfused against moderately high workloads [17–21]. Consequently, endogenous lipids are used to maintain energy balance. In contrast, working rabbit hearts perfused with KH + RBC rely primarily on exogenous substrates as energy source, as indicated by the unaltered endogenous substrate contents (Table 1).

No correlation was found between oxygen consumption and mechanical performance in the KH-perfused rabbit hearts (Fig. 3) in contrast to rat and guinea pig hearts [1,2,7]. This may be a function of diffusion limitations in the rabbit hearts.

Differences in capillary-to-myocyte ratios and diffusion distances, if present, may be contributory. Nevertheless, this pattern is consistent with the suggestion that saline-perfused rabbit hearts are hypoxic [8,22]. Consequently, the myocardium is likely to be more dependent on glycolysis for energy production. This fact probably accounts for the observed gradual decline in glycogen, but not triacylglycerol stores (Table 1). The amount of energy generated, however, remained insufficient to sustain cardiac work and the hearts rapidly deteriorated (Fig. 5). It is noteworthy that decreased ATP concentrations precede manifestations of impaired cardiac function. Thus these preparations are capable of maintaining substantial mechanical function in the face of acute energy deficiency.

The observation that oxygen consumption by KH-perfused hearts did not change with increasing workloads is consistent with the notion that oxygen extraction by the myocardium at 10 cm H_2O LAFP is already at or near maximum. In most systems the magnitude of coronary flow is largely dependent on myocardial metabolic demand. Its regulation is thought to involve primarily the generation of local metabolites. During hypoxia or ischemia, coronary flow in the isolated rabbit or guinea pig hearts is markedly elevated and this state is accompanied by massive release of adenosine, a potent vasodilator [23–24]. This condition presumably reflects accelerated breakdown of energy-rich phosphates and may account for the more than twofold greater coronary flow in hearts perfused with KH alone (Fig. 2). To a small extent, lower viscosity of the buffer may also be contributory.

Changes in coronary flow, PSP, oxygen consumption, and stroke work with increases in filling pressure in rabbit hearts perfused with RBC-enriched buffer are comparable to those reported in KH-perfused rat and guinea pig hearts [1,2]. The highly significant correlation between oxygen consumption and coronary flow is also in accord with unchanged oxygen extraction at different preloads. This relationship was unaltered when the RBC concentration of the perfusate was raised from 15 to 25 percent. Hence, oxygen-carrying capacity of KH + RBC_{15} is fully adequate to maintain myocardial performance under these experimental conditions.

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