Hemodynamic and Metabolic Responses of the Working Heart in Relation to the Oxygen Carrying Capacity of the Perfusion Medium

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Abstract. Hemodynamic and metabolic adaptations of isolated working heart perfused alternatively with normal or low oxygen carrying capacity medium were studied in an experimental model. A step change in arterial oxygen content (1.75 to 15.3 ml O_2/100 ml) was followed by a decrease in coronary flow, an increase in aortic flow, external work, myocardial oxygen consumption and efficiency, respectively. Metabolic investigations (steady state values) showed the activities of both glycolysis and the Krebs cycle to increase with the oxygen carrying capacity of the perfusion medium. Within the limits of these aerobic conditions, most of the cardiac changes were reversible. The use of reconstituted blood provides physiological conditions of oxygenation, allows a dynamic equilibrium between oxygen supply and oxygen requirements and maintains a near physiological regulation between cardiac dynamic and metabolic functions. These conclusions stress the importance of optimal O_2 carrying capacity of perfusion medium in metabolic studies on isolated working heart.

Key words: Oxygen capacity — Isolated working heart — Reconstituted blood

Introduction

Isolated heart preparations have been widely used for a variety of experimental studies. The effects of workload, ischemia or hypoxia on hemodynamic and metabolic functions of cardiac muscle have been studied by Neely et al. (1967 a, b; 1973; 1976), Rovetto et al. (1973), Penney and Cascarano (1970), Kao et al. (1976), Nishiki et al. (1978), Reibel and Rovetto (1978). Several models of perfused hearts, based on a modified technique of Langendorff (1895) or Neely et al. (1967a) and using balanced electrolyte solution as perfusion medium, have been developed (Bunger et al. 1975, 1979; Taegtmeyer et al. 1980).

The use of blood or reconstituted blood as perfusion medium was developed in several studies (Duvelleroy et al. 1976; Bergman et al. 1979; Gauduel et al. 1982).

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The present work was performed to elucidate the hemodynamic and metabolic adaptations of the isolated working rat heart perfused alternatively with a medium containing erythrocytes (physiological oxygen carrying capacity) and a red cell free perfusate (low oxygen carrying capacity).

Also, our aim was to define the conditions of an aerobic limit of the working heart, and to establish relations between this limit and the activity of the cardiac muscle.

Materials and Methods

Technical procedures

Male Wistar rats (200 to 300 g body weight) maintained on standard laboratory diet ad libitum, were anesthetized with ether. A thoracotomy was then performed and the heart rapidly excised and immersed in iced saline containing heparin. The heart was mounted on an aortic canula, while immersed in saline, in order to prevent any entry of air into the coronary arteries. The heart and canula were then transferred to a perfusion apparatus. Initially, the heart was perfused using the Langendorff method (1895), with blood, until a second canula was inserted into the left atrium and a small catheter was placed into the pulmonary artery.

Modifications of Neely's perfusion apparatus (1967a) consisted principally in an artificial peripherical circulation system, in which preload and afterload were regulated by servo-controlled pumps (Martin 1979; Duruble et al. 1985). This system, in which two circuits were set up in parallel, provided following advantages: absence of interface between gas phase and blood, the possibility of a sudden change of one perfusate to another. This change was made via electropneumatically activated guillotine type valves and controlled by a computer HP 21 MX. Moreover, this system allows the measurements of dynamic responses to an acute change in perfusate. Aortic and coronary flows could be recorded continuously in real time by the computer. The perfusate was not recirculated and the entire perfusion device was enclosed in a thermostatic chamber at 37 °C. The hearts were spontaneously beating and preloads and afterloads were imposed at 9 and 67 mm Hg, respectively. Aortic pressure was monitored by a transducer located just before the damping chamber.

Perfusion media

The blood was reconstituted by mixing 450 ml washed porcine red cells with 550 ml of an electrolyte solution. Final ionic concentrations were: NaCl (120 mmol . l^{-1}), KCl (5.9 mmol . l^{-1}), free calcium 2.5 mmol . l^{-1}), $MgSO_4$ (0.5 mmol . l^{-1}), NaH_2PO_4 (1.2 mmol . l^{-1}), $NaHCO_3$ (28 mmol . l^{-1}). Bovine albumin fraction V, glucose, pyruvate and lactate were present to a final concentration in blood of 1 %, 11 mmol . l^{-1}, 1.2 mmol . l^{-1} and 0.9 mmol . l^{-1}, respectively. The arterial blood concentration of glucose, lactate and pyruvate was kept stable throughout the experiments. With the reconstituted blood, the hematocrit was 35.8 %. Prior to the experiments, the blood was carefully filtered through a Swank filter (Swank filter IL200, pore size 10 microns, Extra corporeal medical specialities Inc.).

The red-cell free electrolyte solution was a modified Krebs Henseleit bicarbonate buffer (hematocrit 0 %, NaCl 118 mmol . l^{-1}, KCl 5.9 mmol . l^{-1}, free calcium 2.5 mmol . l^{-1}, $MgSO_4$ 0.5 mmol . l^{-1}, NaH_2PO_4 1.17 mmol . l^{-1}, $NaHCO_3$ 28 mmol . l^{-1}, glucose 11 mmol . l^{-1}, lactate 0.9 mmol . l^{-1}, pyruvate 1.2 mmol . l^{-1}, and bovine albumin 1 %).

The reconstituted blood was oxygenated using a membrane oxygenator Travenol (5MO321), and a gas mixture containing 20 % O_2, 6 % CO_2 and 74 % N_2. The electrolyte solution was exposed to a gas mixture consisting of O_2 (94 %) and CO_2 (6 %). The oxygen content was determined with a galvanic cell (Lex-O-Con), (Lexington Instruments Corp.), and PO_2, PCO_2 and pH were measured with

Table 1. Hematocrit and blood gas analysis of perfusates.

	Reconstituted blood (Groups B1 and B2)		Krebs Henseleit Buffer (Group K)	
Hematocrit (%)	35.8 ± 0.4	(8)	0	(8)
Arterial PO_2 (mm Hg)	135 ± 3	(8)	550 ± 15	(8)
Arterial O_2 content (ml/100 ml)	15.3 ± 0.2	(8)	1.75 ± 0.05	(8)
Arterial PCO_2 (mm Hg)	37 ± 2	(8)	35 ± 1.5	(8)
Arterial pH	7.4 ± 0.01	(8)	7.43 ± 0.02	(8)
Coronary sinus PO_2 (mm Hg)	44 ± 1	(8)	77 ± 4	(8)

standard electrode techniques (Radiometer PHM 72). A comparison of gas analyses of the perfusates is shown in Table 1.

Perfusion protocols

Three groups of hearts were used. Group B_1: the hearts were perfused for 60 minutes with reconstituted blood, hematocrit 35 %. Group K: the hearts were perfused with blood for 45 minutes and subsequently with red-cell free solution for 15 minutes. Group B_2: perfusion with blood for 45 minutes followed by a perfusion with electrolyte solution for 15 minutes, and then with blood for 10 minutes.

Preparation of tissues and biochemical analytical methods

After each perfusion (groups B_1, K, B_2), the hearts were frozen by clamping them between Wollenberger tongs precooled in liquid nitrogen. A frozen aliquot of ventricular tissue was kept under liquid nitrogen until the time of analysis. The frozen tissue was powdered in a mortar chilled with liquid nitrogen, and 0.5 g of the frozen powder was homogenized in 5 ml of cold perchloric acid (0.6 N). The homogenate was centrifuged for 25 minutes at $13{,}500 \times g$ in a RC5 Superspeed refrigerated centrifuge (Sorvall). The supernatant fraction was decanted and neutralized with K_2CO_3 ($3 \text{ mol} \cdot l^{-1}$) or KOH ($1.5 \text{ mol} \cdot l^{-1}$) at respective pH according to the compound to be assayed. Analyses were performed with enzymatic techniques using an automatic dual beam spectrophotometer (Perkin—Elmer 551). Tissue glycogen was determined according to the method of Good et al. (1933) and expressed in terms of micromoles of glucose equivalent per gram of dry weight. Lactate and pyruvate were determined with lactate dehydrogenase (Hohorst 1974). Other enzymatic procedures used were: glucose-1-phosphate (Bergmeyer and Michal 1974), glucose-6-phosphate (Lang and Michal 1975), fructose-1-6-diphosphate and dihydroxyacetone phosphate (Michal and Beutler 1974), acetyl-coenzyme A (Decker 1974). Alpha-ketoglutarate was determined by means of glutamate dehydrogenase, using an ammonia-free enzyme in glycerine (Bergmeyer and Bernt 1974). Adenosine triphosphate was assayed according to the method of Lamprecht and Trautshold (1974). In blood perfused hearts, the cellular metabolites were corrected for those present in red blood cells. First grade enzymes and chemical reagents were used (Boehringer Mannheim). The protein concentration was assayed in the pellets according to the Biuret method. All tissue concentrations were expressed in terms nano or picomoles per mg of protein.

Calculations

Oxygen contents of arterial and venous samples were evaluated using a Lex-O_2-Con oxygen analyser. Myocardial oxygen consumption, expressed in $\mu L \cdot min^{-1} \cdot g^{-1}$ wet weight, were calculated from coronary flow and differences in arterial and venous oxygen content.

External left ventricular work was computed as the product of the pressure gradient with the

Table 2. Hemodynamic performance of the blood perfused working rat heart during several hours of perfusion at constant left arterial pressure. Each value is the mean ± SEM of ten experiments.

Parameters	Duration of blood perfusion		
	60 min	120 min	180 min
Aortic flow (ml/min/g)	91 ± 3	88 ± 3	85 ± 7
Coronary flow (ml/min/g)	6.5 ± 0.7	6.8 ± 0.5	6.2 ± 0.4
External work (mJ/min/g)	0.84 ± 0.04	0.80 ± 0.08	0.78 ± 0.05
Oxygen consumption (ml O_2/min/g)	0.35 ± 0.03	0.32 ± 0.06	0.30 ± 0.07

cardiac output and a coefficient ($1.33\ 10^{-4}$) which defined work in Joules minute^{-1}. The pressure gradie[illegible]calculated as the difference between the mean aortic pressure and the mean left atrial filling pressure. Efficiency was defined as the ratio external left ventricular work/ energy equivalent of oxygen consumption.

Statistical evaluation

All results were expressed as mean ± SEM (number of experiments). P values were calculated by the Student t-test. $P < 0.05$ was taken as the limit of significance.

Results

Cardiac performance

The system of perfusion was evaluated for stability in a series of ten experiments. The results are summarized in Table 2. When hearts were perfused with reconstituted blood, a steady hemodynamic state was reached after 45 minutes of perfusion. Aortic and coronary flows, external work and myocardial oxygen consumption did not significantly change during three hours of perfusion. The transient responses of the coronary and aortic flows to a sudden change in the oxygen carrying capacity of the perfusate is shown in Figure 1. The coronary flow which was 6 ml . min^{-1} . g^{-1} after one hour of blood perfusion increased, in less than fifteen seconds, to 15.5 ml . min^{-1} . g^{-1} upon a change to red cell free perfusate. Simultaneously, the aortic flow decreased from 90 ml . min^{-1} . g^{-1} to 24 ml . min^{-1} . g^{-1}. The results obtained with group B_2 suggested that the recovery was nearly complete for the coronary flow and less for the aortic flow after the return to perfusion with blood.

The steady state responses for the different groups of hearts (B_1, K, B_2) are summarized in Figure 2 and Table 3. When hearts were perfused with reconsti-

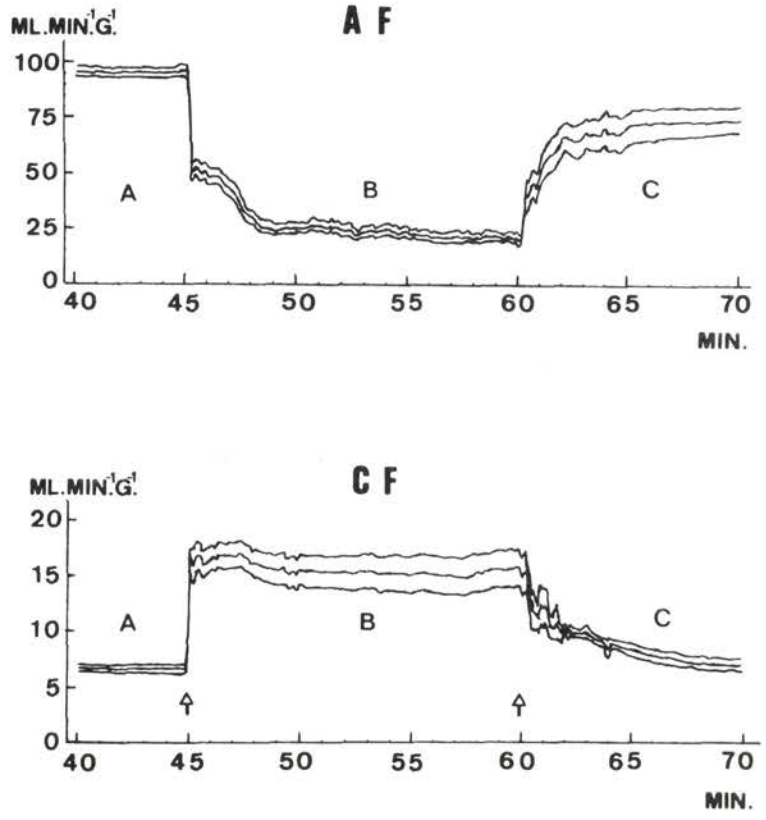

Fig. 1. Dynamic responses of the cardiac muscle to a step change in the arterial oxygen content (CaO_2 in ml O_2/100 ml) of the perfusate. The values of aortic flow (AF) and coronary flow (CF) are mean ± SEM values of 8 experiments. The hearts were perfused with blood for 45 minutes (*A*), $CaO_2 = 15.3$; 15 minutes with electrolyte solution (*B*), $CaO_2 = 1.75$ and 10 minutes with blood again (*C*), $CaO_2 = 15.3$. This perfusion protocol was used for the group B_2.

tuted blood (hematocrit 35 %), the cardiac performances were better than with an electrolyte solution (hematocrit 0 %). The cardiac output/tissue oxygenation (coronary flow) ratio was inversely related to the oxygen carrying capacity of the perfusate. The effects of low oxygen carrying capacity during Krebs Henseleit perfusion included: reduced myocardial oxygen consumption, and the reduced external work, stroke volume, systolic aortic pressure and efficiency. The recovery was not complete after a return to blood perfusion (group B_2). Nevertheless, coronary flow, oxygen utilization and arterial pressure reached values near those of control hearts (group B_1). Heart rate was not influenced by the oxygen carrying capacity of the perfusion medium.

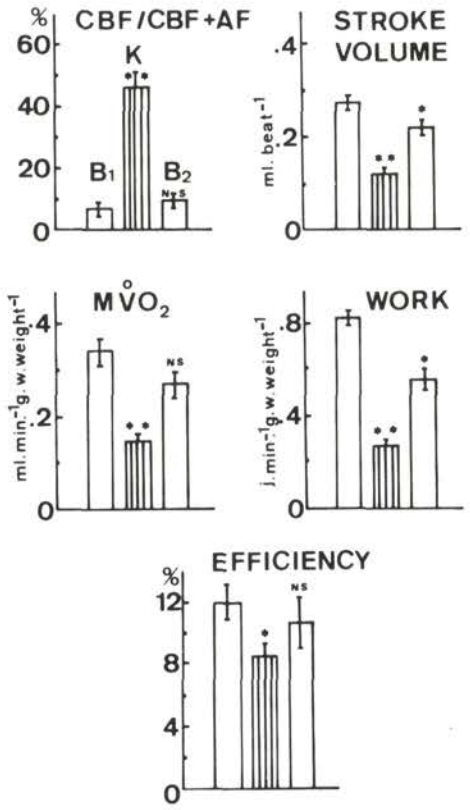

Fig. 2. Various indices of cardiac performance shown for the three experimental groups. Group B_1: values obtained after 60 minutes of perfusion with blood ($n = 8$); group K: perfusion with blood for 45 minutes and subsequently with electrolyte for 15 minutes ($n = 8$); group B_2: hearts alternatively perfused with blood (45 minutes), electrolyte solution (15 minutes) and blood again (10 minutes) ($n = 8$). K vs B_1 and B_2 vs B_1. **: p 0.01; *: $p < 0.05$; ns: not significant ($p > 0.05$).

**Metabolic responses*

The results of metabolic studies are summarized in Table 4. The glycogen stores reflected the quality of myocardial oxygenation. Their cellular levels were in direct proportion to the oxygen carrying capacity of the perfusate. Low arterial O_2 contents of the perfusion medium (group K), were associated with a reduction of the tissue glycogen content, an increase in glucose-1-phosphate concentration and a decrease in the glucose-6-phosphate stores. Perfusion with reconstituted blood resulted in an increased cellular concentration of glucose-6-phosphate.

Table 3. Hemodynamic parameters of isolated working hearts, perfused with different oxygen carrying capacities.

	Blood perfusion 60 min -B1-		Blood perfusion: 45 min KHB perfusion: 15 min -K-		Blood perfusion: 45 min KHB perfusion: 15 min Blood perfusion: 10 min -B2-	
Aortic flow ($ml\ min^{-1}\ g^{-1}$ w.wt.)	91 ± 3	(8)	21.5 ± 2	(8)**	75 ± 6	(8)*
Coronary flow ($ml\ min^{-1}\ g^{-1}$ w.wt.)	6.4 ± 0.4	(8)	15.1 ± 0.7	(8)**	7.2 ± 0.7	(8) ns
Heart rate (beats min^{-1})	356 ± 8	(8)	330 ± 11	(8) ns	361 ± 10	(8) ns
Systolic pressure (mm Hg)	113 ± 3	(8)	77 ± 2	(8)*	107 ± 5	(8) ns
Diastolic pressure (mm Hg)	28 ± 1	(8)	33 ± 1	(8)*	30 ± 1	(8) ns

Each value is the mean ± SEM of the numbers of experiments shown in parentheses.
K vs B1 and B2 vs B1: $*p < 0.05$ $**p < 0.01$ ns: not significant ($p > 0.05$)

Table 4. Tissue metabolite levels, during perfusion with different oxygen carrying capacities, of isolated, working rat heart.

		Perfusion -Group B1- Blood: 60 min		-Group K- Blood: 45 min KHB: +15 min		-Group B2- Blood: 45 min KHB: +15 min Blood: +10 min
* GLYCOGEN μmol. Gluc. Eq. w.wt.	(6)	29.9 ± 2.9	(6)	11.5 ± 1.6*	(6)	17.9 ± 1.5*
* GLUCOSE-1P pmol . mg^{-1} protein	(6)	56.7 ± 5.6	(6)	89.0 ± 16*	(4)	77.1 ± 3.9*
* GLUCOSE-6P nmol . mg^{-1} protein	(6)	1.9 ± 0.15	(6)	1.2 ± 0.1*	(4)	2.3 ± 0.17 ns
* FRUCTOSE-1-6diP nmol . mg^{-1} protein	(6)	0.108 ± 0.02	(6)	0.222 ± 0.04*	(4)	0.080 ± 0.01 ns
* DIHYDROXYACETONE-P nmol . mg^{-1} protein	(6)	0.106 ± 0.01	(6)	0.149 ± 0.03 ns	(4)	0.137 ± 0.08 ns
* PYRUVATE nmol . mg^{-1} protein	(6)	1.6 ± 0.2	(6)	3.1 ± 0.3**	(4)	2.7 ± 0.5 ns
* LACTATE nmol . mg^{-1} protein	(6)	21.8 ± 2.2	(6)	49.8 ± 3.6**	(4)	24.1 ± 3.4 ns
* LACTATE/PYRUVATE	(6)	10.7 ± 1.7	(6)	17.5 ± 3.1*	(4)	9.0 ± 1.9 ns
* ACETYL coA nmol . mg^{-1} protein	(6)	4.5 ± 0.5	(6)	1.5 ± 0.2**	(4)	2.8 ± 0.45*
* αKETOGLUTARATE nmol . mg^{-1} protein	(6)	2.2 ± 0.2	(6)	1.3 ± 0.1**	(4)	2.14 ± 0.45 ns

Values are means ± SEM of the numbers of experiments shown in parentheses.
(K) vs (B1); (B2) vs (B1) * $p < 0.05$ ** $p < 0.01$ ns: not significant

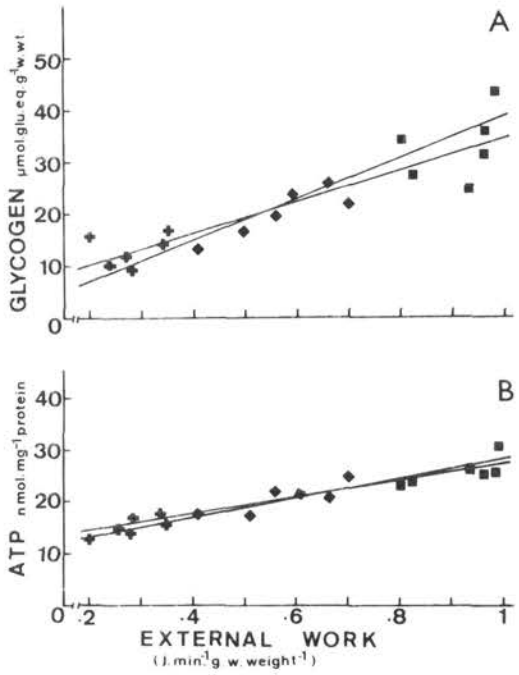

Fig. 3. Second order linear regression between external work and tissue glycogen concentration (*A*) and between external work and cellular ATP level (*B*). (*A*): $X = 0.59 + 0.025\ (Y - 22)$ and $Y = 22 + 30.3\ (X - 0.59)$; $r = 0.88$ (*B*): $X = 0.59 + 0.052\ (Y - 20.7)$ and $Y = 20.7 + 18.02\ (X - 0.59)$; $r = 0.96$. ■ group B_1, + group K, ◆ group B_2.

The endogenous pool of dihydroxyacetone phosphate remained unchanged despite changes in oxygenation of the cardiac muscle (groups B_1 and K). However, the level of tissue oxygenation influenced the lactate/pyruvate ratio and the intermediates of the Krebs cycle, acetyl coenzyme A and alpha-ketoglutarate. During perfusion with blood, high concentrations of acetyl-coenzyme A and alpha-ketoglutarate were accompanied by a low lactate/pyruvate ratio. This metabolic situation was reversed when the hearts were perfused with red cell free electrolyte solution. A recovery of metabolic activity during the period of reperfusion with blood (group B_2) was suggested by the presence of glycogen synthesis. This was shown by the return of the glycogen pool to near control values and by a reduction of the glucose-1-phosphate concentration. The tissue concentrations of glucose-6-phosphate, fructose-1-6-diphosphate, dihydroxyacetone phosphate, and the lactate/pyruvate ratio returned to values observed before perfusion with the Krebs Henseleit solution. Alpha-ketoglutarate concentration reached its control value in ten minutes, acetyl coenzyme A concentration increased more slowly.

The relationship between the external work and the cardiac levels of adenosine triphosphate (ATP) and glycogen are shown in Figure 3. In all conditions of myocardial oxygenation, with normal or low arterial oxygen content, cardiac performance was directly related to ATP level and tissue glycogen content.

Discussion

**Hemodynamic adaptations during aerobic perfusion*

A modified working heart preparation (each heart acting as its own control) was used to study the hemodynamic and metabolic adaptations during sudden variations in hematocrit of the perfusion medium. The perfusion system used in the present study included an extracorporeal circuit for control of the work performed by the heart. It enabled rapid and controlled changes of perfusates and continuous recording of hemodynamic parameters. The isolated blood perfused heart was capable of a constant hemodynamic performance and could be adapted for pharmacological studies.

A high left atrial filling pressure (LAP) was not adequate for the blood perfused heart. Physiological LAP (2 mm Hg) was high enough to result in a substantial aortic flow (50 ml . min^{-1}) but it was ineffective during perfusion with Krebs Henseleit buffer. For this reason, 9 mm Hg preload were chosen as an optimal compromise with the different hematocrits used in this study.

When the oxygen carrying capacity of the perfusion medium was modified by the presence or absence of hemoglobin, extensive cardiac hemodynamic adaptations were apparent. During perfusion with red cell free electrolyte solution, the mean cardiac output value remained low in comparison with other studies in which Krebs Henseleit buffer was used (Neely et al. 1967a; Hearse et al. 1978; Kannengiesser et al. 1979). This discrepancy may be due to the fact that in the present study performance measurements were made after one hour of perfusion. Nevertheless, for a left atrial filling pressure of 9 mm Hg, the cardiac outputs obtained were comparable with values obtained by de Leiris et al. (1978) and Bunger et al. (1979). The stroke volume was similar to that reported by Neely et al. (1967a) and Bunger et al. (1979) who used conditions of enhanced heart perfusion. The cardiac output fraction used for myocardial oxygenation (coronary flow) was less than physiological. The almost instantaneous coronary flow response observed during a step change in perfusates could mainly be explained by variations in viscosity (Chien 1972; Messmer et al. 1972) while the role of a change in vessel tone was difficult to assess quantitatively.

Cardiac perfusion with reconstituted blood was characterized by significant changes in hemodynamic parameters. After one hour of perfusion, mean cardiac output, which was the best indicator of cardiac activity, reached values similar to those reported by Dowell et al. (1975) for the rat heart "in situ". The coronary

flow/cardiac output ratio and stroke volume were similar to those observed during physiological cardiac activity. The external work was also increased as compared to hearts perfused with red cell free solution. The spontaneous heart rate was similar to that observed in the intact animal. The oxygen carrying capacity of the perfusion medium had no effect on this parameter. The absence of an interface between perfusate and gas was probably essential to maintain the integrity of red cells and plasma proteins, thus supporting the stability of the preparation. Hence, the quality of myocardial oxygenation was dependent on the mode of oxygen transport to the tissue, and the working capacity of the myocardium was largely dependent on the presence of oxygen bound to hemoglobin.

**Hematocrit and myocardial oxygen consumption*

In the myocardium, oxygen availability is determined by the coronary flow, the contact time at the site of capillary exchange and the capillary density (Martini and Honig 1969; Duvelleroy et al. 1980). In the present study, as already pointed out by Bassingthwaighte (1974) and Wittenberg (1970), oxygen availability to the perfused heart was also determined by the oxygen carrying capacity of the perfusate and the gradient in oxygen tension along the diffusion path of the capillaries (Duvelleroy et al. 1981).

In the red cell free electrolyte, oxygen is in solution and the arterial oxygen content will remain low even if arterial oxygen pressure is high. Although the presence of a high venous pO_2 is generally assumed to reflect adequate myocardial oxygenation, there was no evidence to support the validity of this assumption because myocardial oxygen uptake and venous oxygen pressure changed in opposite directions (Duvelleroy et al. 1976). The autoregulated adjustment of the coronary flow, which can be influenced by microcirculatory adaptations (Lombardo et al. 1953; Trenouth et al. 1976), was not sufficient to maintain physiological tissue oxygenation when the arterial oxygen content was low due to the absence of erythrocytes. During the change from blood to Krebs Henseleit buffer, oxygen utilization and cardiac activity were limited due to a reduction in oxygen availability as a result of the absence of oxyhemoglobin. When hearts were perfused with reconstituted blood, myocardial oxygen consumption values (4.8—5.4 mmoles $h^{-1}\,g^{-1}$ dry weight) were higher than those reported by authors using isolated working hearts at near physiological workload (4.4—4.6 mmoles $h^{-1}\,g^{-1}$ dry weight) (Rovetto et al. 1973; Neely et al. 1972a, b) or those using an improved perfusion system (4.2—5.1 mmoles $h^{-1}\,g^{-1}$ dry weight) (Bunger et al. 1979; Taegtmeyer et al. 1980). The rate of oxygen utilization obtained in the present study was associated with physiological coronary flows and venous oxygen pressures.

Hematocrit and metabolic activity

This study demonstrated that in different conditions of aerobic perfusion (hematocrit 35 % or 0 %), the cardiac performance was related to the cellular glycogen levels. Hearts perfused with solutions with hematocrits of 35 % showed physiological performance and the endogenous pool of glycogen remained high. This level of glycogen reserves indicated a constant state with the glycogenolysis and glycogenesis being in equilibrium. By contrast, when the oxygen carrying capacity of the perfusate was low (hematocrit 0 %), the limitation in cardiac external work may have been ascribed to a decrease in the cellular glycogen level, since glycogen stores are decisive in the cardiac response to oxygen deprivation (Scheuer et al. 1970). Our results were in accordance with those reported in previous studies (Williamson et al. 1976; Bing et al. 1976) in which glycogen has been shown to preserve the mechanical capacity of perfused hearts.

In hearts perfused with electrolyte solution, the cellular glycogen depletion could indicate (Marshall et al. 1981) that anaerobic glycogenolysis and glycolysis were accelerated by the supply-demand oxygen imbalance. The depletion in glucose-6-phosphate and ATP concentrations, associated with a significant increase in cellular inorganic phosphates and AMP (Gauduel et al. 1982), probably activated glycogen phosphorylase "b" (Opie et al. 1971b; Morgan and Parmeggiani 1964; Neely et al. 1970); this could explain the reduction in the cellular glycogen stores during the Krebs Henseleit perfusion period. Simultaneously, the increase in fructose-1-6-diphosphate concentration was associated with a strong activity of phosphofructokinase (Mansour 1963). This state appeared reversible when the heart was subsequently perfused with blood (group B_2). When the arterial oxygen content was reduced, a rise in cellular lactate, pyruvate concentrations and the lactate/pyruvate ratio indicated the development of an imbalance between oxygen requirements and oxygen supply (Scheuer 1967; Rovetto et al. 1973; Marshall et al. 1981). During the perfusion with zero hematocrit solution a high concentration of lactate was essential to allow regeneration of NAD^+, to maintain the cytoplasmic redox potential, and to ensure the glycolytic production of ATP in the cardiac cells. The poor energy levels in hearts alternatively perfused with blood and Krebs appeared to result from a lack of substrate (acetyl coA) for the citric acid cycle. In blood perfused hearts (groups B_1 and B_2), high cellular acetyl coA levels corresponded to the optimum cellular oxygenation (Messmer et al. 1972).

Cellular ATP level, the first energy compartment necessary for contractile activity, was related to myocardial oxygen uptake. The same relation has also been defined by Pelosi et al. (1969) in intact rats. Thus, the hematocrit value modulated oxygen uptake and secondarily cardiac energy metabolism (Gauduel et al. 1982). In accordance with results of Reibel et al. (1978), we have observed that in different conditions of aerobic perfusion, ventricular performance was directly

related to ATP concentration. This observation suggested that the cellular ATP level was an indicator of the ability of the heart to maintain mechanical activity.

All the hemodynamic and metabolic adaptations observed during changes of hematocrit were due to changes in oxygen availability, since this was the only parameter modified in our experimental conditions. According to Weber et al. (1980), the myocardial oxygen availability determines the aerobic limit for cardiac activity. This limit was reached when the oxygen demand/oxygen supply ratio became inadequate. The presence of oxygen bound to hemoglobin increased the arterial oxygen delivery and enhanced oxygen transport to the tissue. Under these conditions, physiological cardiac activity, defined as a dynamic equilibrium between hemodynamic and metabolic functions, could be only maintained when the oxygen demand/oxygen supply ratio was granted by a perfusion medium with a sufficient oxygen carrying capacity.

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