# Relation Between Work and Phosphate Metabolite in the in Vivo Paced Mammalian Heart

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**Nuclear magnetic resonance (NMR) spectroscopy was used to monitor, on a beat-to-beat basis, the concentration of creatine phosphate and adenosine triphosphate during alterations in the work output of canine hearts in vivo. Over a wide range of rate-pressure products (5,000 to 25,000 mmHg/min), the relative amounts of creatine phosphate and adenosine triphosphate within the heart remained constant. The relative concentration of free adenosine diphosphate was calculated under the reasonable assumption that the creatine kinase–catalyzed reaction is near equilibrium in this tissue. The free concentration of adenosine diphosphate also did not change over this range of rate-pressure products. The data demonstrate that the concentration of these compounds is highly regulated in vivo and suggest that factors other than their concentration may be involved in the modulation of steady-state myocardial work output with oxygen consumption.**

THE ORCHESTRATION OF OXIDATIVE metabolism, or energy conversion, and work output in the mammalian heart has been an area of active research in cellular physiology (*1*). In this regulatory process, adenosine triphosphate (ATP), creatine phosphate (CrP), adenosine diphosphate (ADP), and inorganic phosphate ($P_i$) are thought to be instrumental in the cell's ability to balance the rate of energy conversion and work output (*1–4*). This relation has long been believed to be the result of the control of oxidative metabolism by the cytoplasmic levels of these compounds, whether it involves simply their concentration (*2, 3*) or the thermodynamic relation of the phosphate potential (*4*). Briefly, the work output, or ATP hydrolysis at the myofibrils, is proposed to control the rate of oxidative metabolism occurring in the mitochondria by regulating the rate of delivery of ADP and $P_i$ to, or the amount of ADP and $P_i$ at, the mitochondria. The delivery of ADP and $P_i$ to the mitochondria is believed to occur by simple diffusion (*5*) or by means of a creatine-CrP shuttle (*6*).

In isolated heart mitochondria the rate of respiration can be controlled by the delivery or concentration of ADP alone (*3*). For an ADP concentration ([ADP]) near the value of its Michaelis constant, the relation between [ADP] and respiration is linear, with a slope of approximately unity. That is, a doubling of [ADP], in the 10- to 30-$\mu M$ range, results in a doubling of the rate of oxidative metabolism in isolated mitochondria. If [ADP] is also the regulatory agent between oxygen consumption and work output in vivo, then a similar relation between work output and [ADP] should be observed in vivo.

In these studies we used $^{31}P$ NMR to test this hypothesis and to establish the sensitivity of [CrP] and [ATP] to alterations in work output of the in vivo dog heart throughout the cardiac cycle by monitoring the relative concentrations of ATP, CrP, and, indirectly, ADP. $^{31}P$ NMR is now well established as a technique for noninvasively monitoring the concentration and turnover of these metabolites in intact tissue (*7*). The application of $^{31}P$ NMR to the study of the human heart and other organs has now begun (*8*). Therefore, our investigation should also provide information on the potential clinical usefulness of $^{31}P$ NMR in detecting alterations in myocardial metabolism in vivo.

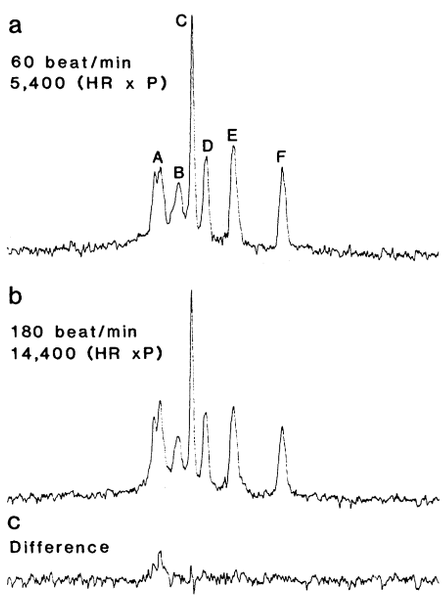


Fig. 1. Effects of increased paced heart rate and rate pressure product on $^{31}P$ NMR spectra from the dog heart in vivo. (a) This spectrum is the average of 800 scans collected with a 30-μsec pulse duration every 2 seconds. A baseline correction and 5 Hz of line broadening were applied. The paced heart rate was 60 beat/min with a mean arterial blood pressure of 90 mmHg (130/70). The peak assignments are (A) 2,3-DPG and inorganic phosphate; (B) phosphodiesters; (C) CrP; (D) gamma phosphate of ATP; (E) alpha phosphate of ATP and NAD; and (F) beta phosphate of ATP. (b) This spectrum, from the same heart as in (a) was collected under the same conditions except that the paced heart rate was increased to 180 beat/min, with a mean arterial pressure of 80 mmHg (90/50). (c) Difference spectrum: (b) minus (a).

ATP and CrP, which are present in millimolar concentrations, are directly detected in a $^{31}P$ NMR measurement of heart (Fig. 1). The free concentration of ADP in vivo (10 to 30 $\mu M$) is too low to be directly detected by $^{31}P$ NMR, but it can be estimated by assuming that the creatine kinase reaction is near equilibrium and calculating [ADP] from an equilibrium equation (*9, 10*).

Because the NMR technique is nondestructive, multiple measurements could be made on each dog, and each animal could serve as its own control. The preparation and techniques have been described in previous reports on the $^{31}P$ NMR catheter probe developed in our laboratories (*11–13*). The heart rate was altered by atrial cardiac pacing alone to minimize inotropic effects. Each anesthetized animal was allowed to stabilize in the large-bore magnet, and a control spectrum (Fig. 1a) was collected at low heart rates, either ungated or gated to the cardiac cycle. Under these control conditions the average heart rate was 65 beat/min, with average systolic and diastolic pressures of 120 and 70 mmHg, respectively. The ratio of CrP to ATP averaged 1.46 under these partial saturation collection conditions (*14*). No significant deviation from this value was observed within the cardiac cycle, in agreement with studies on unpaced control dogs (*13, 16*).

After the control period, the heart rate was doubled or tripled with cardiac pacing and allowed to stabilize for 10 to 15 minutes before ungated and gated $^{31}P$ NMR spectra were collected. An example of an ungated spectrum obtained with the heart paced at 180 beat/min is shown in Fig. 1b. A spectrum depicting the difference between spectra collected under control conditions and spectra collected when the heart rate was increased shows no significant change in the ATP and CrP peak intensities (Fig. 1c). In some cases a slight change in the total signal intensity or in the 2,3-diphosphoglycerate (2,3-DPG) region was observed when the heart rate was increased. This could be interpreted as a change in the average position of the coil with respect to the myocardi-

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Table 1. CrP/ATP areas, with partial saturation (*14*), as a function of cardiac cycle phase. Mean rate-pressure products were 15,800 ± 2,600 ($n$ = 5) and 16,500 ± 2,300 ($n$ = 6). All errors are expressed as ±SEM for $n$ animals.

| Cycle phase | $n$ | CrP/ATP |
|---|---|---|
| Systole | 6 | 1.4 ± 0.06 |
| 1/4 | 5 | 1.5 ± 0.08 |
| 1/2 | 5 | 1.5 ± 0.07 |
| 3/4 | 6 | 1.5 ± 0.08 |

um. Since the inorganic phosphate is very low in the normal heart, intracellular inorganic phosphate could not be resolved from blood inorganic phosphate and 2,3-DPG at this magnetic field. Therefore, we did not attempt to quantitate intracellular inorganic phosphate or estimate intracellular *p*H. However, in no instance was a significant change in the relative concentrations of CrP and ATP detected (Fig. 2a). In addition, the ratio of CrP to ATP was essentially invariant throughout the cardiac cycle at high heart rates (Table 1); this invariance of CrP/ATP was earlier observed at lower heart rates (*13*). In gated-acquisition experiments, no significant changes occurred in CrP/ATP as a function of the rate-pressure product for any of the four individual positions of the cardiac cycle we analyzed (*16*). A plot of CrP/ATP at systole as a function of the rate-pressure product is shown in Fig. 2b.

The present data demonstrate that even when the rate-pressure product of the dog heart in vivo is varied over a wide range (5,000 to 25,000 mmHg/min) the value of CrP/ATP does not change significantly. Because of the near-equilibrium condition of the creatine kinase reaction, the ratio of CrP to ATP is rather sensitive to alterations in the free concentration of ADP (*9, 10*). Therefore, the lack of change in the value of CrP/ATP is consistent with a constant free ADP concentration.

These data on the heart in vivo are in contrast to studies on saline-perfused hearts in vitro where, in general, changes in the work output of the heart are correlated with alterations in CrP/ATP (*10, 17*). Indeed, even cyclic changes in the CrP and ATP content have been reported in the isolated perfused rat heart with glucose as the sole substrate (*18, 19*), while in the rat heart in vivo, no beat-to-beat alterations were reported (*20*). Two major differences between hearts in vivo and perfused in vitro are the oxygen and substrate delivery to these preparations. The in vivo heart may have a more adequate delivery of oxygen and preferred substrates to the tissue than does the saline-perfused heart. In fact, many of the alterations in the value of CrP/ATP observed in the heart in vitro are greatly diminished if substrates other than glucose (for example, pyruvate or acetate) are also included in the medium (*10, 19*).

Katz and Feinberg (*21*) demonstrated that the rate-pressure product is a good index of the oxygen consumption in the dog heart. This observation, with various modifications, has been reproduced by several laboratories (*22*). Calculations made with this relation showed that the three- to fivefold increase in the rate-pressure product of the hearts used in these studies was accompanied by a two- to threefold increase in oxygen consumption (*23*). However, this increase in oxygen consumption was not associated with an increase in [ADP], as suggested by the ratio of CrP to ATP. One explanation for this observation is that the stimulation of oxygen consumption by ADP in vivo is much more sensitive than detected in isolated mitochondria. That is, in vivo, a smaller or transient change in [ADP] could produce a large change in respiration due to yet undescribed cooperativity effects. Another possibility is that mitochondrial respiration is not controlled directly by [ADP] and [$P_i$] alone, but rather by the delivery of substrates and subsequent changes in the NADH-NAD redox state (*24*). As mentioned earlier, many of the discrepancies between the in vitro and in vivo models disappear if the perfusate contains, in addition to glucose, more easily utilized substrates such as pyruvate. This might suggest that the redox state of mitochondrial NADH is more important in the control of mitochondrial respiration in vivo than was previously recognized.

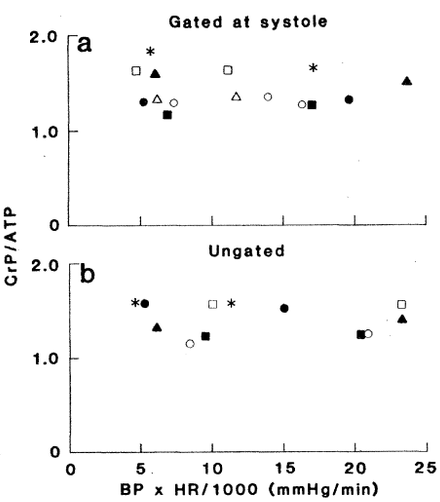


Fig. 2. Effect of variation of the rate-pressure product on the value of CrP/ATP of the heart in vivo. Data acquisition conditions were identical to those in Fig. 1. Each symbol represents one animal. (a) Ungated data acquisitions. No significant difference was detected in nine experiments ($P > 0.28$). (b) Gated acquisitions with data collected during systole [see (*16*)].

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$$K = \frac{[CrP][ADP][H^+]}{[ATP][Cr]}$$

If the total creatine pool and intracellular *p*H are assumed to remain constant, then the value of CrP/ATP should reflect alterations in [ADP].
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12. Male beagles weighing 10 to 12 kg were used in these studies. Each dog was initially anesthetized with intravenously administered sodium pentobarbital (30 mg/kg). An intravenous infusion of 0.9% saline was begun, and the femoral artery was cannulated in order to monitor the arterial blood pressure. A superficial cutdown was performed over the external jugular vein and the coil was guided into the apex of the right ventricle with the aid of x-ray fluoroscopy. The femoral vein was exposed and a nonmagnetic pacing lead was inserted into the right atrium, again under fluoroscopic monitoring. The dogs were wrapped in a foil blanket (space blanket, Boy Scouts of America, North Brunswick, NJ) and placed in a 1.89-tesla magnet (Oxford Instruments) with a 26-cm clear-bore diameter. A water-circulating heating pad was used to maintain the animal's temperature. Ventilation and anesthesia were accomplished with 0.5% to 1% halothane in 50/50 nitrous oxide and $O_2$. The ventilatory volume and rate were adjusted to maintain normal values of arterial blood gases and *p*H. The magnet was interfaced to a computer (Nicolet 1280) and pulse programmer (293C) with an amplifier (Amplifier Research). The coil was tuned to 32.5 MHz and matched to 50 ohms with a balanced matching capacitor configuration. Observation of the $^1H$ signal at 80.29 MHz was used to gauge proper positioning of the animal within the magnet and to optimize the room temperature shims to obtain the best signal intensity and line shape (*11*). Generally, $^1H$ line widths less than 25 Hz were obtained. For $^{31}P$ NMR (at 32.5 MHz) a pulse width of 20 to 30 μsec was applied to remove the 2,3-DPG signal from blood near the coil (*13*). Gating was performed with a custom-designed system that provided a precise, adjustable trigger on the rising edge of the pressure wave (*13*). Signals were acquired at four different periods evenly spaced throughout the cardiac cycle.
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14. These data were collected with only a 2-second delay time between each radiofrequency pulse in order to maximize the signal-to-noise ratio over a reasonable experimental time frame. Therefore, the signals ob-

tained are differentially saturated and do not represent the total amounts of metabolites present. However, these signals can be corrected by using the known spin-lattice relaxation times ($T_1$'s) of the phosphate compounds and the nutation angles used (*13*). We have determined these parameters previously (*13*) and calculated the correction factors for CrP ($T_1$ = 4.4 seconds) and the beta phosphate of ATP ($T_1$ = 1.63 seconds) to be 1.94 and 1.27, respectively. With these values the actual CrP to ATP ratio is 2.2, in good agreement with our previous data (*11, 13*).
15. The signal-to-noise (S/N) ratios of peaks in these spectra were calculated using Nicolet 1280 software. As a criterion for retaining a data set, the S/N of the beta phosphate of ATP had to exceed 10 to 1, with 15 Hz line broadening applied. Since the ATP beta phosphate peak, an unresolved triplet, is several times broader than 15 Hz, this can be considered as a lower limit of the S/N value. This S/N allows changes of <10% in the phosphate resonance intensities to be observed. The values of CrP/ATP were calculated by using the Nicolet NMRCAP routine to simulate experimental spectra as seven lorentzian lines (three for ATP, one for CrP, one for phosphodiesters, and two for the unresolved peaks of $P_i$, monophosphoesters, and 2,3-DPG), whose positions, heights, and widths were varied to minimize the root-mean-square deviation between experimental and simulated spectra.
16. The CrP/ATP values at low versus high heart rates were compared for each of the four points in the cardiac cycle by use of a paired one-tailed *t* test. The results gave $P > 0.15$ ($n$ = 6 dogs) for each of the four phases. Only experiments with more than a 2.2-fold increase in the rate-pressure product with increased pacing rate were used in this analysis.
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23. In previous studies of dog hearts (*21, 22*) under various conditions, including anesthesia regimes similar to that used in the present study, the relationship of oxygen consumption (moles per minute per gram) and the rate-pressure product (mmHg per minute) has been shown to be linear with a slope of 0.4 to 0.6. Therefore the 5,000 to 25,000 range in the rate-pressure product in these studies indicates that the oxygen consumption of the heart was changing two- to threefold with the pacing jump.
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# AIDS Retrovirus Induced Cytopathology: Giant Cell Formation and Involvement of CD4 Antigen

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**The formation of multinucleated giant cells with progression to cell death is a characteristic manifestation of the cytopathology induced by the AIDS retrovirus in infected T lymphoid cells. The mechanism of giant cell formation was studied in the CD4 (T4/Leu 3) positive T cell lines JM (Jurkat) and VB and in variants of these lines that are negative for cell surface CD4 antigen. By means of a two-color fluorescent labeling technique, multinucleated giant cells in infected cultures were shown to form through cell fusion. Antibody to CD4 specifically inhibited fusion, and uninfected CD4 negative cells, in contrast to uninfected CD4 positive cells, did not undergo fusion with infected cells, suggesting a direct role for the CD4 antigen in the process of syncytium formation. These results suggest that, in vivo, cell fusion involving the CD4 molecule may represent a mechanism whereby uninfected cells can be incorporated into AIDS virus infected syncytia. Because the giant cells die soon after they are formed, this process may contribute to the depletion of helper/inducer T cells characteristically observed in AIDS.**

THE T LYMPHOTROPIC RETROVIRUS known variously as LAV, HTLV-III, and ARV has been etiologically associated with the acquired immune deficiency syndrome (AIDS) and a spectrum of related disorders (*1*). Serum antibodies to viral antigens are found in most patients with these conditions (*1, 2*), and the virus is readily isolated from these individuals (*2*). In patients infected with the virus, the helper/inducer T lymphocyte subset, which bears the CD4 (T4/Leu 3) cell surface antigen, is characteristically depleted (*3*). In vitro, the virus is tropic and cytopathic for T cells and transformed cell lines expressing CD4, and monoclonal antibodies to CD4 block the infectivity of the virus in vitro (*1, 2, 4–6*). Thus CD4 may function as a specific cellular receptor for the AIDS virus (*6*).

The mechanism through which the AIDS virus induces cytopathology in susceptible cells is not well understood. In vitro, infected cells characteristically form multinucleated giant cells that produce large amounts of virus and then die over a period of a few days (*1, 2*). Multinucleated giant cells and cells expressing viral antigens have also been observed in histologic sections from infected individuals (*7*). Such cells may result from fusion of infected cells, or from uncoupling of the normal mechanisms that synchronize nuclear replication and cell division. We now present evidence that the CD4 molecule is involved in the fusion of infected cells and that $CD4^+$ cells do not need to be productively infected themselves to undergo fusion with an already infected cell.

To study the cytopathic effects of the AIDS retrovirus, we used two $CD4^+$ leukemic T cell lines, JM (Jurkat) and VB, that respond to AIDS virus infection in a manner which approximates infection in nontransformed human T helper/inducer cells. Infection of these lines resulted in a burst of virus production [assessed by measuring cell-free, particle-associated reverse transcriptase (RT) activity] that was accompanied by a pronounced cytopathic effect. Virus production then declined over a period of days to weeks, and RT activity ultimately ceased altogether. Cell surface expression of the CD4 antigen decreased after infection, so that by the time detectable virus production had ceased, all the viable cells were essentially $CD4^-$ (Fig. 1). Surface expression of other antigens, including CD3, CD5, CD7, and nonpolymorphic HLA class I determinants did not vary significantly between the initial $CD4^+$ population and the $CD4^-$ postinfection outgrowth. The JM $CD4^-$ outgrowth was completely refractory to infection with the virus. To determine whether the $CD4^-$ outgrowth was persistently but nonproductively infected, we used Southern blot analysis to test for the presence of the AIDS virus genome. No viral gene sequences were detected when the HTLV-III

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