are protected from enzyme activity in an acidic environment, then their mode of life would place a premium on their capacity to produce and excrete organic acids. Selection would favor anaerobiosis independent of environmental PO_2.

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Normalization of Depressed Heart Function in Rats by Ribose

Abstract. *Severe constriction of the abdominal aorta and simultaneous injection of isoproterenol in rats induced depression in heart function and reductions in cardiac adenosine triphosphate and total adenine nucleotides. When ribose was continuously infused for 24 hours, biosynthesis of cardiac adenine nucleotides was stimulated to such an extent that the reductions in adenosine triphosphate and total adenine nucleotides were prevented and left ventricular hemodynamic parameters were normal. These results support the hypothesis that adenosine triphosphate is primarily responsible for depression in myocardial contractility and that ribose is cardioprotective through its pronounced effects on adenine nucleotide metabolism in heart muscle.*

Various interventions have been proposed to improve myocardial adenine nucleotide metabolism in situations in which there is a discrepancy between energy supply and demand. In such a pathophysiological situation, the degradation of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and adenosine monophosphate (AMP) further proceeds to adenosine, inosine, and hypoxanthine (*1*), which are released (*2*) and thus lost from the myocardial cell for restitution of adenine nucleotides through the "salvage pathways." These pathways include the phosphorylation of adenosine to AMP through adenosine kinase and the conversion of hypoxanthine to inosine monophosphate, a reaction catalyzed by hypoxanthine-guanine phosphoribosyltransferase. In this reaction, 5-phosphoribosyl-1-pyrophosphate, which is also an important precursor substrate for the biosynthesis of adenine nucleotides (*3*), is consumed.

One procedure for studying myocardial adenine nucleotide metabolism is designed to stop the total breakdown of adenine nucleotides and bases. This may be accomplished by applying allopurinol, an inhibitor of xanthine oxidase (*4*). Such an inhibition may have been responsible for the improvement of function and metabolism in dogs with experimental myocardial infarction (*5*) and hemorrhagic shock (*6*). Another approach is to inhibit adenosine transport within or efflux from the myocardial cell by administering dipyridamole (*7*), which also has pronounced dilatory effects on the coronary vascular system (*8*).

Apart from affecting degradation and transport of adenine nucleotides and their breakdown products, there are basically two ways to stimulate the synthesis of myocardial adenine nucleotides. The first involves the administration of adenosine (*9*), inosine (*10*), and adenine (*11*), all of which can be used to restore cardiac adenine nucleotides through the salvage pathways, which depend on the availability of 5-phosphoribosyl-1-pyrophosphate as regards inosine and adenine. The second approach is aimed at enhancing the biosynthesis of adenine nucleotides with ribose (*12*). It is based on the fact that the rate of biosynthesis is very low (6 nmole/g per hour) compared with the total content of adenine nucleotides, and that stimulation with ribose is possible not only under control conditions (*12*) but also in such situations as recovery from lack of oxygen (*13*), cardiac hypertrophy (*14*), and stimulation with catecholamines (*15*). The pronounced effect of ribose can be attributed primarily to the increased availability of 5-phosphoribosyl-1-pyrophosphate, a substrate that appears to be the major limiting factor for biosynthesis of myocardial adenine nucleotides (*3*, *12*). In the experimental conditions mentioned, the stimulation of adenine nucleotide biosynthesis is so considerable that the decline in adenine nucleotides is attenuated or even prevented. Although the effects of ribose on adenine and uridine nucleotide metabolism (*16*) and on the morphology of the myocardium have been documented (*15*), it has not been known whether the impairment of heart function associated with ATP depletion can be prevented by ribose.

To examine this, a reduction of myocardial adenine nucleotides was induced in rats (240 to 260 g) experiencing impaired heart function. This combined effect was elicited by constricting the abdominal aorta to a final diameter of 0.65 mm (*17*) and by simultaneously adminis-

Table 1. Effect of aortic constriction (AC) and simultaneous injection of isoproterenol (ISO; 25 mg/kg, subcutaneously) on the myocardial content of ATP and total adenine nucleotides, left ventricular systolic pressure (LVSP), maximum rate of increase in left ventricular pressure, and the product of LVSP and heart rate (HR) in rats that had received a continuous intravenous infusion of 0.9 percent NaCl or ribose (200 mg/kg per hour) for 24 hours. Values are means ± standard errors for the number of experiments given in parentheses.

Treatment	ATP (μmole/g)	ATP, ADP, and AMP (μmole/g)	LVSP (mmHg)	Maximum rate of increase in LVSP (mmHg/sec)	LVSP × HR (mmHg/min)
Control	4.4 ± 0.07 (30)	5.8 ± 0.10 (30)	142 ± 4 (19)	6,073 ± 187 (19)	58,342 ± 1,897 (19)
AC + ISO + NaCl	3.4 ± 0.10 (14)*	4.7 ± 0.16 (14)*	111 ± 5 (11)*	4,699 ± 338 (11)*	34,764 ± 4,600 (11)*
AC + ISO + ribose	4.2 ± 0.10 (18)	5.7 ± 0.12 (18)	133 ± 6 (12)	6,231 ± 308 (12)	52,569 ± 2,637 (12)†

*Significantly different from corresponding control value ($P < .0005$, unpaired t-test). †$P < .05$.

tering isoproterenol (25 mg/kg, subcutaneously). Twenty-four hours later, the animals were anesthetized with sodium thiobutabarbital (Inactin, Buyk; 80 mg/kg, intraperitoneally) and an ultraminiature pressure transducer (model PR-249, Millar Instruments) was advanced into the left ventricle through the right carotid artery (*18*). Heart rate, left ventricular pressure, and maximum rate of increase in left ventricular pressure were continuously recorded on a multichannel Beckman RM Dynograph or a Gould Brush 2600 recorder. All functional parameters and the levels of ATP and adenine nucleotides were depressed (Table 1).

To determine whether ribose can stimulate cardiac adenine nucleotide biosynthesis in this pathophysiological condition, ribose or saline was infused in unanesthetized, unrestrained rats (*19*). When saline was infused, adenine nucleotide biosynthesis was enhanced, but only by about 100 percent (Table 2). But when ribose was administered, the increase in biosynthesis was comparable to that observed in other pathophysiological situations (*12–15*). As a result, ATP and total adenine nucleotides were essentially normal (Table 1). Likewise, the hemodynamic parameters were close to the control values, except for the product of left ventricular systolic pressure and heart rate (*20*), which was some 10 percent below control, since heart rate was still slightly reduced. Since ribose itself had no effect on cardiac hemodynamics (*18*), it is likely that the improvement in heart function resulted from the normalization of cardiac adenine nucleotides brought about by ribose. The exact site of ATP action remains unknown. It may be that ATP controls cardiac contractility primarily through its modulatory effects on calcium entry into the myocardial cell (*21*) or on calcium efflux from the sarcoplasmic reticulum (*22*).

The procedure used in this study is essentially a modification of a model in which cardiomyopathy is induced by catecholamines in open-chest rabbits (*23*). That catecholamine administration and constriction of the abdominal aorta significantly reduced all hemodynamic parameters in the closed-chest rat is not surprising, since catecholamine infusion alone impairs cardiac pump function (*23*). Of greater interest is the observation that the depression of heart function was prevented by ribose. Thus ribose qualifies as a cardioprotective agent. Its effect is primarily the stimulation of cardiac adenine nucleotide biosynthesis to such a degree that ATP and total adenine nucleotides are kept at normal levels.

Table 2. Rate of myocardial adenine nucleotide biosynthesis under control conditions and 24 hours after aortic constricton (AC) and subcutaneous injection of isoproterenol (ISO; 25 mg/kg) in rats intravenously infused with 0.9 percent NaCl or ribose (200 mg/kg per hour). Infusion rate: 5 ml/kg per hour. Measurements were done after exposing the rats to [1-^{14}C]glycine (250 μCi/kg, intravenously; specific activity, 54.2 mCi/mmole; Amersham) for 60 minutes in vivo (*25*). Values are means ± standard errors for the number of experiments given in parentheses.

Treatment	Adenine nucleotide biosynthesis (nmole/g per hour)
Control	6.6 ± 0.6 (31)
AC + ISO + NaCl	14.0 ± 1.3 (3)
AC + ISO + ribose	76.7 ± 0.3 (3)

These results support the hypothesis that ATP deficiency is primarily responsible for the impairment of cardiac contractility (*24*), since such impairment can be reversed when the decline in myocardial ATP is prevented. This approach may be an appropriate intervention in clinical states of depressed heart function due to energy deficiency. The advantage of ribose over other metabolic interventions is that it does not affect the hemodynamics of the heart with an ultimate change in oxygen demand and that it has no vasoactive properties which may result in afterload alterations.

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Adenylate Cyclase Activation Shifts the Phase of a Circadian Pacemaker

Abstract. *Forskolin, a highly specific activator of adenylate cyclase, produced both delay and advance phase shifts of the circadian rhythm recorded from the isolated eye of the marine mollusk* Aplysia. *The phase dependence of the response to forskolin was identical to that with serotonin, which also stimulates adenylate cyclase in the eye. The ability of agents to activate adenylate cyclase in homogenates was correlated with their ability to shift the phase of the circadian oscillator. These results along with earlier findings show that adenosine 3′,5′-monophosphate mediates the effect of serotonin on the rhythm and regulates the phase of the circadian pacemaker in the eye of* Aplysia.

In the last decade significant progress has been made in understanding the physiology of circadian systems (*1, 2*). Although circadian pacemakers have been localized in the nervous systems of a number of multicellular organisms, the cellular and biochemical mechanisms that regulate circadian oscillators are still elusive. Serotonin, a putative neurotransmitter in the eye of *Aplysia* (*3*), causes a phase shift in the oscillator that is responsible for the circadian rhythm of spontaneous nerve impulses from the isolated eye of this marine mollusk (*4*). We report that both serotonin and forskolin activate adenylate cyclase in the eye, and that both shift the phase shift of the circadian rhythm in the same way. These experiments and our earlier work with serotonin show that adenosine 3′,5′-monophosphate (cyclic AMP) mediates the effect of serotonin on the