foci (9). If most temporal lobe foci can be localized to mesial structures, the interpretation of electrocorticographic results must be carefully evaluated; laterally placed electrodes may show vigorous epileptic spiking that is primarily a result of projected activity.

The actual mechanism underlying the spontaneous events—how this rhythmic activity is initiated and why it is maintained—remains to be elucidated. The cells display a high threshold calcium conductance and may even generate calcium spikes (Fig. 2B); some cells may be capable of generating intrinsic paroxysmal depolarizing shift discharge such as described by Prince and Wong (5). However, most cells display only synaptic events which result from a mixture of excitatory and inhibitory input. The importance of maintaining an intact minimal circuitry for generating these rhythmic events is underscored by the absence of such activity in thin (500-μm) slices. Thicker slices preserve more circuitry, which consequently may give rise to spontaneous rhythmic postsynaptic potentials. The pacemaking mechanism for these events is unclear, but the synaptic drive certainly seems capable of initiating the cell burst firing patterns previously found in in vivo extracellular recordings from human epileptic foci (2).

Interpretation of the activity seen in our slice studies as "epileptic" must, of course, be conditional, since normal tissue from mesial temporal cortex was not available for study as a control. It is possible that cells in normal mesial tissue produce spontaneous rhythmic activity. Nevertheless, the dissociation of mesial and lateral temporal regions with respect to this activity suggests that the spontaneous activity is not simply a normal discharge pattern. Further, the likelihood of observing these potentials seems loosely correlated with the abnormality of the tissue (Table 1). Thus, these spontaneous, rhythmic PSP's may reflect an underlying pathology of epileptic tissue.

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References and Notes

1. H. Jasper *et al.*, Eds., *Basic Mechanisms of the Epilepsies* (Little, Brown, Boston, 1969); D. Purpura *et al.*, Eds., *Experimental Models of Epilepsy* (Raven, New York, 1972).
2. T. Babb, E. Carr, P. Crandall, *Electroencephalogr. Clin. Neurophysiol.* **34**, 247 (1973); W. Calvin, G. Ojemann, A. Ward, Jr., *ibid.*, p. 337.
3. G. Kerkut and H. Wheal, Eds., *Electrophysiology of Isolated Mammalian CNS Preparations* (Academic Press, London, 1981).
4. P. Schwartzkroin and D. Prince, *Brain Res.* **115**, 497 (1976).
5. D. Prince and R. Wong, *ibid.* **210**, 323 (1981).
6. P. Schwartzkroin, D. Turner, W. D. Knowles, A. Wyler, *Ann. Neurol.* **13**, 249 (1983).
7. In all cases, informed consent was obtained. All electrophysiological recording was carried out on tissue that was removed for strictly clinical reasons (G. Ojemann, A. Ward, Jr., and A. Wyler, neurosurgeons). Surgery was done under local anesthesia; all patients had previously been maintained on a variety of anticonvulsant drugs, the effects of which could not be ruled out in the in vitro studies.
8. P. Schwartzkroin, in (3), pp. 15–50.
9. A. Wyler and N. Bolender, *Ann. Neurol.* **13**, 59 (1983).
10. Supported by NINCDS grants NS 00413 and NS 17111 and by NSF grant BNS 7915115.
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Ribose Intervention in the Cardiac Pentose Phosphate Pathway Is Not Species-Specific

Abstract. *Ribose is cardioprotective in the rat in a variety of pathophysiological conditions. The metabolic basis for this effect is the low capacity of the oxidative pentose phosphate pathway in the myocardium. Ribose bypasses this pathway, elevates the available pool of 5-phosphoribosyl-1-pyrophosphate, and thus stimulates the biosynthesis of adenine nucleotides. In the study reported here the activity of glucose-6-phosphate dehydrogenase, the first and rate-limiting enzyme of the oxidative pentose phosphate shunt, was very low in the human heart and was of the same order of magnitude in the myocardium of various animal species. Furthermore, ribose had a similar stimulating effect on myocardial adenine nucleotide biosynthesis in the guinea pig, in which hemodynamic parameters are different from those in the rat. It is concluded that the metabolic basis for the effectiveness of ribose is similar in all species investigated.*

A special metabolic feature of the myocardium is the low capacity of the pentose phosphate pathway (1). In the oxidative branch of this shunt ribose-5-phosphate is produced that is converted into 5-phosphoribosyl-1-pyrophosphate, an essential substrate for the synthesis of purine and pyrimidine nucleotides. As a consequence, the rates of these synthetic processes are very low in the myocardium (2). In searching for the rate-limiting step, Eggleston and Krebs (3) found that glucose-6-phosphate dehydrogenase (G6PDH), the first enzyme of the oxidative pentose phosphate pathway, exerts a tight control in the liver. In the myocardium the activity of this enzyme is lower than in the liver and in most other organs (4), and the available pool of 5-phosphoribosyl-1-pyrophosphate is smaller than in the liver and kidney (5). Hence, the rate of adenine nucleotide biosynthesis is minute in the heart compared with other organs (2).

The limited pool of 5-phosphoribosyl-1-pyrophosphate and the low rate of adenine nucleotide biosynthesis can be overcome by ribose in the heart, both in the control state (5) and in various pathophysiological conditions. Ribose enhances cardiac adenine nucleotide biosynthesis in catecholamine-treated rats (6) and in animals recovering from oxygen deficiency (7) and experimental

Table 1. Activities of the first two enzymes of the oxidative pentose phosphate pathway in the myocardium of different species. Heart tissue was homogenized in ice-cold 0.15*M* KCl containing 8 ml of 0.02*M* $KHCO_3$ per liter. Centrifugation, dialysis of the supernatant, and measurements of enzyme activities at 25°C were done in accordance with the methods of Glock and McLean (4), and protein concentration in the dialyzate was determined with the biuret reaction. The human papillary muscles, which were kept in the ice-cold solution, were homogenized within 2 hours after the samples were obtained during cardiac surgery. When rat hearts were maintained in the same solution for this period of time after excision, enzyme activities were not altered. Values are means ± standard errors.

Species	*N**	Enzyme (units per gram of protein)		*P*†
		G6PDH	6-Phosphogluconate dehydrogenase	
Guinea pig	8	6.0 ± 0.46	11.6 ± 0.36	< 0.0005
Rat	28	4.3 ± 0.15	11.2 ± 0.25	< 0.0005
Rabbit	4	3.4 ± 0.77	12.2 ± 1.21	< 0.0005
Dog	6	1.5 ± 0.19	8.8 ± 0.43	< 0.0005
Calf	4	1.6 ± 0.35	9.3 ± 0.50	< 0.0005
Monkey	5	2.4 ± 0.59	5.0 ± 0.56	< 0.0250
Human	16	3.4 ± 0.15	5.9 ± 0.27	< 0.0005

*Number of hearts or heart samples. †Determined with Student's *t*-test for unpaired data.

myocardial infarction (*8*). In the isoproterenol-stimulated heart, the enhancement of adenine nucleotide biosynthesis was such that the decline in the level of adenosine triphosphate (ATP) was prevented and the incidence of focal myocardial cell lesions reduced (*6*). Recently, depressed heart function was normalized by preventing the reduction in ATP content with ribose (*9*). From these studies it appears that ribose qualifies as a cardioprotective substrate.

To determine whether the capacity of the oxidative segment of the pentose phosphate pathway is low in the myocardium of animals other than the rat, we measured activities of the first two enzymes of this shunt, G6PDH and 6-phosphogluconate dehydrogenase (*4*), in the hearts of seven different species (Table 1). In all hearts the activity of G6PDH, the rate-limiting enzyme, was significantly lower than that of the second enzyme in the pathway. The activity of G6PDH ranged from 1.5 U per gram of protein in the dog heart to 6 U/g in the guinea pig heart, with the human enzyme activity being in between (3.4 U/g).

We next measured heart function and the influence of ribose on myocardial adenine nucleotide biosynthesis in guinea pigs and rats. Heart function was measured in closed-chest females (240 to 260 g) by catheterizing the left ventricle with a new ultraminiature catheter pressure transducer (*9, 10*). The two species differed considerably in all hemodynamic parameters (Table 2). In the guinea pig, heart rate, left ventricular systolic pressure (LVSP), and the maximum rate of increase in left ventricular pressure (LV dP/dt_{max}) were all markedly lower than in the rat. Despite these pronounced differences, the concentrations of adenine nucleotides and the rates of biosynthesis of these high-energy phosphates were surprisingly similar in both species. Ribose, given as a single intravenous injection, enhanced adenine nucleotide synthesis in the guinea pig myocardium somewhat more than in the rat myocardium. Thus, the metabolic effect of ribose was similar in the two species although the functional parameters were different.

The results suggest a strong correlation between the activity of cardiac G6PDH and the extent of adenine nucleotide biosynthesis. This correlation holds not only for the rat and guinea pig (Table 2) but also for the dog, in which cardiac adenine nucleotide biosynthesis was recently measured at 1.0 nmole/g per hour (*11*), in accordance with the low activity of G6PDH (Table 1). In rats and guinea pigs ribose had a similar stimulating effect on myocardial adenine nucleotide biosynthesis, and in the dog ribose enhanced adenine nucleotide biosynthesis in the myocardium during recovery from ischemia (*11*). Thus, one may conclude that ribose will also have a pronounced influence in the other species, in which the oxidative branch of the pentose phosphate pathway in the myocardium is less developed (Table 1). From our data it is not possible, however, to assess the possible contribution of the nonoxidative segment of the pentose phosphate pathway (*12*) to the formation of 5-phosphoribosyl-1-pyrophosphate and to adenine nucleotide biosynthesis in the hearts of the different species. If this nonoxidative pathway plays a role, its significance appears to be limited, at least in the myocardium of the three species in which ribose had a stimulating effect.

Table 2. Heart function and myocardial adenine nucleotide metabolism in rats and guinea pigs. To measure heart function we anesthetized the animals intraperitoneally with sodium thiobutabarbital (80 mg/kg; Inactin Byk), performed tracheotomies, and placed a catheter in the trachea to keep the airway open. Heart rate, LVSP, and LV dP/dt_{max} were obtained with an ultraminiature catheter pressure transducer (model PR-249, Millar Instruments) that was advanced into the left ventricle through the right carotid artery (*9, 10*). For continuous display a multichannel Beckman RM recorder or a Gould Brush 2600 recorder was used. To determine the rate of synthesis of myocardial adenine nucleotides, we injected the tail veins of the animals with [1-^{14}C]glycine (250 μCi/kg; specific activity, 54.2 mCi/mmole; Amersham International) and killed them 60 minutes later under ether anesthesia. The hearts were rapidly excised and immediately immersed in liquid nitrogen. Radioactivity of the adenine nucleotides was related to the mean specific activity of the tissue glycine precursor pool (*2, 5*). D-Ribose (100 mg/kg; Sigma) was injected into the tail vein at the same time as the [1-^{14}C]glycine. Values are means ± standard errors for the number of experiments shown in parentheses.

Variable	Rat	Guinea pig
Heart rate (beats per minute)	409 ± 7 (19)	271 ± 11 (9)
LVSP (mmHg)	142 ± 4 (19)	94 ± 6 (9)
LV dP/dt_{max} (mmHg/sec)	6073 ± 187 (19)	3248 ± 295 (9)
ATP (μmole/g)	4.4 ± 0.1 (30)	4.1 ± 0.1 (6)
ATP + ADP + AMP (μmole/g)	5.8 ± 0.1 (30)	5.1 ± 0.1 (6)
Adenine nucleotide synthesis (nmole/g-hour)	6.0 ± 0.7 (25)	7.1 ± 4.4 (4)
Adenine nucleotide synthesis + ribose (nmole/g-hour)	26.7 ± 5.0 (7)	41.4 ± 7.2 (3)

All the human papillary muscles in which the enzyme activities were determined were obtained from hearts that had undergone surgery for aortic or mitral valve replacement. The hearts had been subjected to an increased load, as evidenced by an elevated left ventricular end-diastolic pressure, and had been treated with cardiac glycosides and diuretics or vasodilators. However, G6PDH activity in these tissues did not exceed that in the other species. Thus, conventional cardiac therapy does not seem to affect the pentose phosphate pathway.

Animals rarely develop myocardial ischemia and infarction subsequent to coronary atherosclerosis (*13, 14*), so they have no need for increased production of 5-phosphoribosyl-1-pyrophosphate through the oxidative pentose phosphate pathway for the synthesis of purine nucleotides. Man, who is exposed to cardiovascular risk factors (*15*) leading to hypertension and coronary atherosclerosis, would certainly benefit from a higher capacity of the oxidative branch of the pentose phosphate pathway in situations involving impaired oxidative phosphorylation of adenosine diphosphate (ADP) or during recovery from an ischemic insult to the heart. Perhaps man's exposure to the stress of civilization has not lasted long enough to trigger adaptation of the oxidative pentose phosphate pathway. Since an increased synthesis of cardiac G6PDH is hardly imminent, we must rely on the therapeutic approaches available. In addition, intravenous infusion of ribose would appear to be an appropriate adjunct in the medical and surgical management of certain heart diseases, since the enzymatic basis for the efficacy of ribose in the human heart seems even better than in the rat or guinea pig myocardium.

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References and Notes

1. R. L. Jolley, V. H. Cheldelin, R. W. Newburgh, *J. Biol. Chem.* **233**, 1289 (1958); L. E. Crevasse, J. C. Shipp, H. K. Delcher, *Biochim. Biophys. Acta* **86**, 402 (1964); A. H. Burns and W. J. Reddy, *Am. J. Physiol.* **232**, E570 (1977).
2. D. A. Goldthwait, *J. Clin. Invest.* **36**, 1572 (1957); H.-G. Zimmer, C. Trendelenburg, H.

Kammermeier, E. Gerlach, *Circ. Res.* **32**, 635 (1973); J. Olivares and A. Rossi, *J. Physiol. (Paris)* **78**, 175 (1982).
3. L. V. Eggleston and H. A. Krebs, *Biochem. J.* **138**, 425 (1974).
4. G. E. Glock and P. McLean, *ibid.* **55**, 400 (1953); *ibid.* **56**, 171 (1954).
5. H.-G. Zimmer and E. Gerlach, *Pfluegers Arch.* **376**, 223 (1978).
6. H.-G. Zimmer, H. Ibel, G. Steinkopff, G. Korb, *Science* **207**, 319 (1980).
7. H.-G. Zimmer, *J. Physiol. (Paris)* **76**, 769 (1980); M. K. Pasque *et al.*, *J. Thorac. Cardiovasc. Surg.* **83**, 390 (1982).
8. H.-G. Zimmer, *Prog. Clin. Pharmacol.* **3**, 13 (1982).
9. ———, *Science* **220**, 81 (1983).
10. ———, *Basic Res. Cardiol.* **78**, 77 (1983).
11. M. Mauser, H. M. Hoffmeister, C. Nienaber, W. Schaper, *Circulation* **68**, 389 (1983).
12. J. F. Williams and P. F. Blackmore, *Int. J. Biochem.* **15**, 797 (1983).
13. T. B. Clarkson and N. J. Alexander, *J. Clin. Invest.* **65**, 15 (1980).
14. H. Shimokawa *et al.*, *Science* **221**, 560 (1983).
15. R. I. Levy and J. Moskowitz, *ibid.* **217**, 121 (1982).
16. Supported by the Deutsche Forschungsgemeinschaft (Zi 199/4-3). The excellent technical assistance of G. Steinkoff and M. Sagstetter is gratefully acknowledged. We thank Dr. U. Mittmann for the supply of monkey hearts.

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Susceptibility of Skeletal Muscle to Coxsackie A_2 Virus Infection: Effects of Botulinum Toxin and Denervation

Abstract. *Coxsackie A viruses can infect denervated but not innervated mature skeletal muscles. The role of synaptic transmission in preventing susceptibility to Coxsackievirus infection was studied by surgically denervating leg muscles of mice or injecting the muscles with botulinum toxin to block quantal release of acetylcholine. Control muscles were injected with heat-inactivated toxin. Subsequent injection of Coxsackie A_2 virus resulted in extensive virus replication and tissue destruction in the denervated and botulinum toxin–treated muscles, while the control muscles showed only minimal changes. This suggests that the susceptibility of skeletal muscle to Coxsackievirus infection is regulated by synaptic transmission.*

Coxsackie A viruses are capable of infecting skeletal muscles, but only under certain circumstances. Immature (*1*) or denervated (*2*) muscles are susceptible to infection, while mature innervated muscles are relatively resistant. At present, neither the factors that determine susceptibility to Coxsackievirus infection nor the role of innervation in regulating muscle resistance to infection are understood. Certain other properties of skeletal muscles are regulated by acetylcholine (ACh) released from nerve terminals (*3*). We therefore wondered whether muscle cell resistance to Coxsackie A infection might also be regulated by synaptic transmission. To investigate this possibility, we used botulinum toxin to block the quantal release of ACh from motor nerve terminals in mouse muscles and then determined their susceptiblity to infection with Coxsackie A_2 virus.

A total of 132 adult (30 to 40 g) female Swiss mice (Buckberg) were surgically denervated or injected with botulinum toxin or given control injections of heat-inactivated botulinum toxin. Unilateral denervation of the lower limb was performed in 44 mice by excising a 5- to 6-mm length of the proximal sciatic nerve from the mid-thigh. In 48 mice the gastrocnemius muscles were surgically exposed and 40 μl of type A crystalline botulinum toxin freshly diluted in sterile Ringer solution (4.5×10^{-6} mg/ml) was injected into each muscle through a 30-gauge needle. (Surgical exposure was found to facilitate accuracy of injection in these small muscles and did not produce any artifactual effects in controls.) Another 40 mice received control injections of 40 μl of heat-inactivated (100°C for 30 minutes) botulinum toxin (4.5×10^{-6} mg/ml) into their gastrocnemius muscles. One week later all the muscles were directly injected with $2.6 \times \log_{10}$ $TCID_{50}$ (50 percent tissue culture infective dose) of Coxsackie A_2 virus suspended in 40 μl of Ringer solution (*4*). At 1.5, 3.0, 4.5, and 6.0 days after the virus injections, groups of rats were killed and their gastrocnemius muscles were excised. One hundred five muscles were assayed for the content of infectious virus (*5*) and 27 muscles were quick-frozen and prepared for histological examination (*6*).

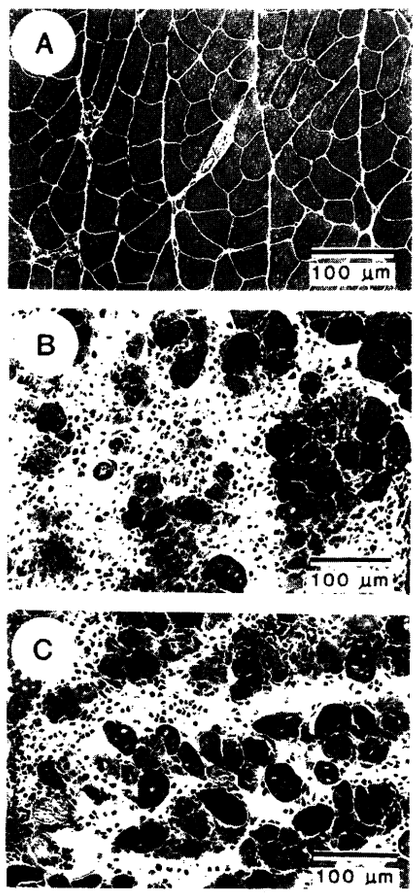

Fig. 1. Effects of various treatments on susceptibility of skeletal muscles to Coxsackie A_2 virus infection. Light photomicrographs show transverse sections of gastrocnemius muscles 4.5 days after inoculation. The muscles had been treated 7 days previously with (A) heat-inactivated botulinum toxin, (B) surgical denervation, or (C) botulinum toxin (0.18×10^{-9} g). Some artifact due to freezing is seen in the fibers in (C) and does not represent any effect of toxin or virus on the muscle. The control muscle cells (A) show only minimal changes while the denervated and botulinum-treated muscles show cellular infiltration and fiber necrosis.

In general, both denervated and botulinum toxin–treated muscles developed severe infection, while control muscles showed only mild changes. The progression of infection was slightly but significantly more rapid in denervated muscles than in botulinum-treated muscles (Table 1). At 1.5 days after inoculation, muscles from denervated and botulinum-treated animals had between 10 and 100 times more infectious virus than control muscles. At 3 days the virus concentration in denervated muscles reached its maximum, 1000 times greater than that of the original viral inoculum. By 4.5 days the concentration had decreased slightly. The concentration of virus in botulinum-treated muscles continued to rise through day 4.5, reaching levels nearly 1000 times greater than that of the original inoculum. Control muscles showed less than a tenfold increase in virus throughout the experimental period. Virus titers in all three experimental groups declined after day 4.5.

The histological changes in infected skeletal muscles followed a similar pattern, though lagging somewhat behind the changes in virus titer (Figs. 1 and 2). At 1.5 days denervated and botulinum-treated muscles showed a few focal areas of infiltration, with occasional degenerating fibers, while control muscles had only rare foci of minimal cellular infiltration. At 3 days denervated muscles showed widespread diffuse or multifocal cellular infiltration and fairly extensive