to diploid hosts. In postmetamorphic life (Fig. 2), the contributions from these ventral blood islands was evidenced as 11 to 19 percent of the total population of spleen and bone marrow cells. The small number of cells in the thymus (about 7 percent) that were derived from the ventral blood islands supports our earlier conclusion that, unlike those of birds, anuran thymic lymphocytes are derived almost exclusively from the area of the presumptive thymus gland. Furthermore, since the bone marrow and spleens of adult frogs are myeloid and erythroid as well as lymphoid, it seems most likely that the primary contribution of the ventral blood islands is to the granulocyte and nucleated erythrocyte populations of these peripheral tissues. Detailed differential cell counts, however, are needed to clarify this possibility.

Our findings provide strong and direct evidence that most cells of the frog thymus are not derived from circulating embryonic mesenchymal cells. Rather, they differentiate from elements within developing thymus itself. These cells or their progeny then migrate out and colonize the kidney, spleen, and bone marrow. Circulating cells do not make a significant contribution to the thymus of postmetamorphic frogs. In short, it appears that during frog ontogeny, the thymus gland is the stem cell source of diverse populations of lymphocytes (for example, T and B cells). These data reinforce the fact that amphibians have provided and will continue to provide a powerful model with which to study the cellular basis of immunity (*11*).

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12. Intermediate values connecting the 2*n* and 3*n* distributions in Figs. 1 and 2 signify the presence of nuclei undergoing DNA replication.
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## Oscillation of Cyclic Adenosine Monophosphate Concentration during the Myocardial Contraction Cycle

Abstract. *The concentration of adenosine 3′,5′-monophosphate (cyclic AMP) rises and falls during each myocardial contraction cycle. Peak concentrations of cyclic AMP precede peak development of systolic tension. Epinephrine alters the normal oscillation in myocardial cyclic AMP and increases both diastolic and systolic concentrations of the cyclic nucleotide. These transient changes in myocardial cyclic AMP indicate a potential role for cyclic AMP as a beat-to-beat regulator of myocardial contractility.*

Adenosine 3′,5′-monophosphate (cyclic AMP) was originally proposed to mediate the inotropic action of catecholamines because they activated particulate membrane bound adenylate cyclase in a dose-related manner similar to their in vivo inotropic potency (*1*). Catecholamines increase myocardial cyclic AMP levels, and the increase in cyclic AMP precedes the peak developed tension by several seconds (*2*). It is now rather clear that an elevation of myocardial cyclic AMP is well correlated with the inotropic action of catecholamines (*3*). The exact mechanism by which cyclic AMP mediates these inotropic events is unknown. However, it has been suggested that cyclic AMP mediates the many physiological processes it controls by activation of cyclic AMP–dependent protein phosphorylation (*4*). Calcium ion is an important regulator of myocardial contractility. Calcium is thought to directly activate the contractile proteins by dissociating the troponin-tropomyosin inhibitory complex (*5*). Cyclic AMP–dependent protein kinase mediated phosphorylation of troponin has now been demonstrated (*6*). In addition, phosphorylase kinase will also phosphorylate troponin (*7*). The physiological significance of these phosphorylations remains to be demonstrated. The sarcoplasmic reticulum is believed to be the site from which calcium ion is released to initiate contraction. Relaxation is thought to begin by resequestration of the calcium (which activated the contractile proteins) back into the sarcoplasmic reticulum. Cyclic AMP increases calcium uptake in a cardiac microsomal fraction containing fragments of sarcoplasmic reticulum (*8*) and the cyclic AMP–dependent protein kinase increases calcium uptake into fragments of cardiac sarcoplasmic reticulum (*9*).

Evidence is now presented that myocardial cyclic AMP concentrations oscillate during each myocardial contraction (*10*) and that this oscillation is altered in the presence of epinephrine. These findings suggest that cyclic AMP could regulate normal and hormone-induced changes in myocardial contractility by regulating myocardial calcium metabolism.

Frog ventricular strips from *Rana pipiens* were suspended horizontally between a strain gauge and binding post. They were electrically stimulated with a 4-volt square wave pulse of 10-msec duration (12 $min^{-1}$) and maintained at 22° to 25°C. Contraction duration was about 2 seconds, and the strips were superfused with physiological salt solution containing 1 m*M* $CaCl_2$ (*3*). The experimental data in Fig. 1 was obtained on whole ventricle strips with a 1-g resting tension. The strips in Fig. 2 had an 0.5-g resting tension, and two strips from the same ventricle were simultaneously superfused and electrically stimulated so that their contractions were out of phase with each other. Thus simultaneous freezing of the strips between spring-loaded blocks cooled in liquid nitrogen stopped one strip in systole and the other in diastole. The contractions were monitored on a memory oscilloscope to confirm the exact time of tissue fixation during the contrac-

tion cycle (*11*). The frozen tissue was extracted into 5 percent trichloroacetic acid (TCA) with a Polytron PT-10 for 10 seconds at full speed; the TCA was removed by extraction with ether. Protein in the TCA pellet was determined by the method of Lowry *et al.* (*12*). Cyclic AMP was determined (*13*) on tissue extracts diluted 200 times or more (Fig. 1). The data in Fig. 2 was obtained from samples purified on AG-1 X-2 anion-exchange columns (*3*), and charcoal was used to terminate the binding assay described by Brown *et al.* (*14*) and modified as described (*15*).

Ventricle strips were initially frozen at intervals during the myocardial contraction cycle (Fig. 1). Cyclic AMP in ventricles frozen within each 10 percent of the total contractile duration were determined, and the mean and standard errors are indicated (Fig. 1). A significant rise in myocardial cyclic AMP is seen between 10 to 20 percent of the contraction cycle ($P < .025$) and between 20 to 30 percent ($P < .05$). The ventricles frozen during the first 10 percent of the contraction show an apparent elevation in cyclic AMP ($P < .10$). These comparisons were made against cyclic AMP levels determined during the last 20 percent of the contraction cycle. Cyclic AMP appears to decline after reaching a peak in the first 10 to 30 percent of the contraction cycle. Peak cyclic AMP precedes peak systolic tension development. It appears that peak cyclic AMP follows the initial phase of membrane depolarization, which occurs before any mechanical response is seen. In another series of experiments with two ventricle strips taken from the same ventricle, there was a significant rise in cyclic AMP in the respective strips frozen during peak systolic tension development (Fig. 2). In five pairs tested, the cyclic AMP was significantly ($P < .05$) higher in strips frozen in systole (*16*). Control diastolic cyclic AMP was $2.75 \pm 0.64$ pmole per milligram of protein, while systolic levels were $3.60 \pm 0.78$. Another series of five pairs of ventricle strips were perfused for 2 minutes with $1 \times 10^{-5} M$ *l*-epinephrine. Diastolic cyclic AMP rose to $4.61 \pm 0.51$ pmole per milligram of protein and systolic cyclic AMP reached $7.14 \pm 1.32$ pmole/mg (Fig. 2) ($P < .025$ by paired analysis). Thus the oscillation in myocardial cyclic AMP during the contraction cycle was maintained in the presence of epinephrine. In addition,

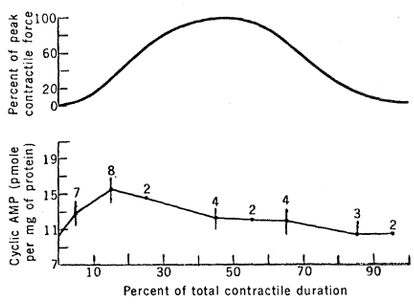


Fig. 1. Cyclic AMP levels in frog ventricle strips frozen during various phases of the contraction cycle. Vertical bars show the standard error of the mean and numbers refer to number of ventricles at each point.

significant increases in both diastolic ($P < .05$) and systolic ($P < .025$) cyclic AMP were seen with epinephrine when compared to control diastolic and systolic cyclic AMP.

These data demonstrate that during the normal contractile process oscillations in myocardial concentrations of cyclic AMP occur and the amplitude of the oscillation is increased by epinephrine. Since the myocardial cyclic AMP rises after membrane depolarization, it seems possible that each depolarization of the cell membrane could transiently activate membrane bound adenylate cyclase through a conformational change in membrane structure. Because epinephrine increases cyclic AMP by activating adenylate cyclase, it seems unlikely that the rise in cyclic AMP during the normal contraction cycle is due to the transient inhibition of cyclic nucleotide phosphodiesterase. In that the rate of membrane depolarization is increased in the presence of epinephrine and diastolic cyclic AMP is elevated in the presence of epinephrine, cyclic AMP could also regulate the events which occur during membrane depolarization. If cyclic AMP mediates its effects by activating protein kinases (*4*) then it would be necessary to envision similar transient phosphorylations and dephosphorylations during each contraction cycle.

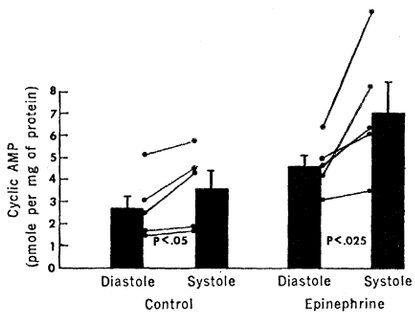


Fig. 2. Cyclic AMP in frog ventricle strip pairs frozen in diastole and during the peak mechanical response (systole). Lines between diastole and systole connect individual pairs.

Cyclic AMP reaches maximum concentrations just before or coincident with the initiation of relaxation, allowing adequate time for cyclic AMP to regulate relaxation by increasing the rate of calcium sequestration by the sarcoplasmic reticulum. The characteristic inotropic action of epinephrine is to decrease the time to peak tension development and increase the rate of relaxation. Again, higher systolic cyclic AMP in the presence of epinephrine could explain the characteristic increased rate of relaxation and decreased time to peak tension during the inotropic action of catecholamines.

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16. The use of two strips from the same ventricle (one frozen in diastole and the other in systole) enabled the detection of elevated cyclic AMP at the peak of systole, while this change could not be detected in a small number of unpaired ventricle strips. The wide variation in cyclic AMP within each experiment and the absolute differences in cyclic AMP shown in the data in Figs. 1 and 2 is not due to inaccuracies in the assay method. The only unexplained difference might be a nutritional difference in the frogs. Those used in the experiments of Fig. 1 came from Minnesota while those used in the experiments of Fig. 2 came from South Carolina.
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