HEART IN SITU

EXAMINATION OF THE HEART IN THE RANA *SP.*

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OBJECTIVES

The objectives of this laboratory experiment were to measure the isometric contraction of the heart of a leopard frog *(Rana pipian).* The rate of contraction of the heart was observed and measured. In addition to this, the effects of various drugs (adrenaline, caffeine, acetylcholine, and KCl) on the rate of contraction of the heart were examined.

INTRODUCTION

Cardiac Muscle Contraction:

The process of contraction in the vertebrate heart is basically similar to that of striated skeletal muscle. The resulting action potential of the cardiac muscle can be represented graphically, as shown: See Fig 2.1(Eckert and Randall, 1978).

The action potential of cardiac muscle possesses a plateau of roughly hundreds of milliseconds long. Also, it can be noted that the duration of the action potential is relatively long compared to the velocity of the conduction. This insures that all the cells of the ventricle are innervated at roughly the same time. This in turn causes the ventricular muscle to contract as a whole. This is important for efficient cardiac pumping action (Eckert and Randall, 1978).

The action potential of the cardiac fiber is caused by the opening of two different types of channels: the fast sodium channels and the slower calcium channels. The calcium channels, however, remain open longer than do the sodium channels. A large amount of both calcium and sodium ions flow in through both channels to the interior of the fiber. This prolongs the period of depolarization of the muscle fiber, thus causing the plateau seen in the action potential (Guyton and Hall, 1996).

The calcium ions that enter the heart help to excite the muscle contraction process of the cardiac muscle. Immediately after every action potential in the heart, there is a period of increased membrane threshold in the cardiac muscles that lasts for several hundred milliseconds. This is called the refractory period. It is the refractory period that prevents tetanic contraction, since it requires the cardiac muscle to relax, and permits the ventricle to fill with blood between action potentials. Due to this, the heart contracts adequately as a pump (Eckert and Randall, 1978).

Also immediately after every action potential in the heart, the membranes of the cardiac muscle fibers experience roughly a five-fold decrease in potassium ion permeability. This may be due to the increased influx of calcium ions into the muscle fibers. This action greatly decreases the potassium ion efflux, and in turn prevents the premature return of the action potential to resting level. Closing of the calcium channels reverses this, ending the action potential (Guyton and Hall, 1996).

Cardiac muscles cells of the frog possess a rudimentary sarcoplasmic reticulum and a system of transverse tubules. Cardiac muscle is activated by the release of calcium ions from the sarcoplasmic reticulum. This is analogous to the action of skeletal muscle. Frog cardiac muscle fibers are much smaller than skeletal muscle. The relatively high surface-to-volume ratio of the cardiac fibers lessen the need for a complex sarcoplasmic reticulum for storage, release and re-absorption of calcium ions. Much of the calcium utilized for cardiac muscle contraction enters through the cell membrane of the muscle fibers due to the increased calcium permeability of the membrane during depolarization of the muscle fibers (Eckert and Randall, 1978).

As the cardiac muscle fibers are depolarized, calcium ions diffuse into the fibers due to the increase calcium conductance across the their cell membranes. Due to the voltage dependence of the

calcium ion influx, tension develops as a function of the depolarization. As the depolarization of the muscle fibers increases, so too does the tension produced. As the concentration of extracellular calcium ions decreases, a weaker contraction for a given depolarization results. This is most probably due to the fact that fewer calcium ions enter the fiber when the concentration gradient across the cell membrane is reduced (Eckert and Randall, 1978).

When the action potential travels long the membranes of the transverse tubules in the interior of the muscle fiber, the T tubule action potentials act upon the membranes of the longitudinal sarcoplasmic tubules. This causes release of calcium ions from the sarcoplasmic reticulum into the sarcoplasm. The calcium ions then diffuse into the myofibrils and catalyze reactions promoting sliding of the actin and myosin filaments, producing muscular contraction. A large amount of extra calcium also diffuses from the T tubules into the sarcoplasm, thus increasing the force of contraction. This is know as excitation-contraction coupling (Guyton and Hall, 1996).

The force of muscular contraction depends greatly on the concentration of calcium ions in the extracellular fluids of the cardiac muscle fibers. This is due to the fact that the ends of the T tubules open directly to the outside of the cardiac muscle fiber. This allows the extracellular fluid in the intersitium to percolate across the T tubules as well (Guyton and Hall, 1996).

At the termination of the plateau in the action potential, the influx of calcium ions is cut off sharply and the calcium ions in the sarcoplasm are quickly pumped out of the sarcoplasm and into the sarcoplasmic reticulum. This ends the action potential (Guyton and Hall, 1996).

The heart functions via simultaneous contraction of both atria, followed by simultaneous contraction of both ventricles. The SA node generates the impulse, which travels to the AV node where it is slowed. Both atria are then depolarized and contract. At about the same time that the atria experience repolarization and relaxation, the ventricles will experience activation and contraction simultaneously. This entire cycle lasts approximately 300 milliseconds (F. G. de Lorenzi, 1996).

There is then a wave of depolarization through the Purkinje network of the septum and the apex, and then through the subendocardial Purkinje network. This wave of depolarization from the apex to the base of the ventricle, inducing simultaneous contraction of both ventricles (F. G. de Lorenzi, 1996). See Fig. 2.2 for the basic structures of the frog heart.

Effects of Drugs on Cardiac Muscle:

Epinephrine: Epinephrine is a catecholamine that increases the rate and force of contraction of the cardiac muscle. Its effect on the rate of contraction is mediated via the AV node pacemaker. The increased force of contraction is the result of a general effect on ally myocardial cells. The increase in heart rate is a result of changes in the conductance of the pacemaker cell. This increase is due to an increase in the conductance of sodium. This in turn increases the rate of depolarization of the pacemaker potential, and shortening the interval between action potentials. Norepinephrine increases the velocity of conduction across the atrio-ventricular node (Eckert and Randall, 1978).

Acetylcholine: The hormone acetylcholine has two effects on cardiac muscle. It decreases the rate of rhythm of the sinus node. Acetylcholine also decreases the excitability of the A-V junctional fibers between the atrial muscles and the A-V node. This slows the transmission of the action potential from the atria to the ventricles (Guyton and Hall, 1996).

Caffeine: Caffeine is believed to increase athletic performance, though this is unconfirmed. Muscle fibers will contract without any change in their membrane potentials if caffeine is introduced into the muscle. Caffeine induces contraction in the muscle fibers by causing release of calcium stored

in the sarcoplasmic reticulum via direct activation of the C elements. Caffeine also inhibits the uptake of calcium by the sarcoplasmic reticulum. Once caffeine is removed, calcium is then once again taken up by the sarcoplasmic reticulum, and the muscle relaxes. There is a delay to the response of the muscle to caffeine applied externally due to the diffusion time of caffeine to the calcium-release sites of the muscle fiber. This delay is negligible when the caffeine is injected directly (Eckert and Randall, 1978).

MATERIALS AND METHOD

Preparation - Open Chest In-Situ Heart:

A specimen of *Rana pipians* was obtained decapitated and pithed. At room temperature a V shaped incision was made into the upper thoracic cavity to expose the pericardium. The pericardium was then carefully dissected away and the myocardial muscle teased apart from neighboring tissues.

Transducer Setup:

A Harvard Apparatus LTD 50-9364 was obtained and an appropriate isometric transducer used to measure the force of ventricular contraction was connected. A suture silk package was then obtained and threaded through the apex of the ventricles and attached to the transducer. A second suture was then obtained and threaded though the atria and attached to a base. The entire preparation was then elevated so that the suture line would be on the same plane as the transducer attachment. This experiment varied slightly from the standard setup in that it employed metallic hooks, which were inserted directly into the myocardium, attached to suture line.

Chemical Substances:

Adrenaline, caffeine, acetylcholine (2mM), and KCl (15mM) were superfused via Pasteur pipettes directly onto the myocardium. After measuring the effect of the drug, the muscle was rinsed with Ringer's solution and given time to recover.

RESULTS

The following data illustrates any changes in frequency due to the drug in question. The last data set, pertaining to KCI recovery, is a measure of the force of contraction.

Table 1: FIRST DOSE OF ADRENALIN

Time (sec)	0	5	10	15	20	25	30
Frequency	2.5	2	2.5	3	0	0	0

Table 2: SECOND DOSE OF ADRENALIN

Time (sec)	0	5	10	15	20	25	30	35	40
Frequency	3	2.5	2.5	2.5	2.5	2.5	2.5	2	3

Table 3: CAFFEINE

Time (sec)	0	5	10	15	20	25	30	35	40	45	50	55
Frequency	3	3	3	3	3.5	3	3	3	2.5	2.5	3	2.5

Table 4: KCL

Time (sec)	0	5	10	15	20	25	30	35	40	45
Frequency	1.5	2	1.5	2	2	2	1.5	1.5	1.5	1.5

Table 5: KCL RECOVERY

Time (sec)	0	5	10	15	20	25	30	35	40	45	50
Frequency	1.5	1.75	2	2.25	2.5	2.5	2.75	3	3	3	3.1

DISCUSSION AND CONCLUSIONS

The first dose of adrenaline produced no significant effects. This may be attributed to the incomplete diffusion of the drug into the myocardium.

The second dose of adrenaline increased the amplitude of the wave owing to a stronger contraction of the ventricles, but produced no significant increase in the frequency of contraction.

An excessive dose of caffeine produced an a stronger contraction of the ventricles. Later effects showed a slight increase in the frequency of contraction, due to facilitated propagation of the action potential along the myocardial conducting pathway.

Acetylcholine produced the anticipated effect, with both a decrease in the amplitude and frequency of contraction.

KCI produced no initial effects that could be clearly distinguished from that of the weakened heart following application of ACh. Although KCl should have produced an unrecoverable ischemia of the myocardial tissue, the problem with diffusion of the chemical substances into the myocardium may have inhibited the desired effect. However a significant recovery of amplitude owing to a stronger contraction of the ventricles may also indicate that the drug produced an effect at least on the outer myocardium.

The primary sources of error for this experiment include (I) the relative (non numerical) measurements of the amplitude of contraction, (ii) the method of preparation in attaching both the apex of the ventricles and the atria to the transducer, (iii) the sensitivity of the machine used, and (iv) the superfusion of chemical substances producing an incomplete effect.

Data and results were approximated from a subjective interpretation of the physiograph recordings. Although the height of the wave representing the force of ventricular contraction was insufficient to produce numerical results, significant changes in the height of the wave could be visually determined and evaluated in terms of a notable response to the stimulus provided.

In lieu of using a suture setup, this setup instead used metallic hooks inserted directly into the aforementioned areas. These hooks were then attached to the electronic transducer via silk string. Unfortunately, this may have proved disadvantageous in that the metallic hooks inadvertently served to absorb a significant amount of the force of contraction exhibited by the heart. This had the effect of producing a less than optimal recording on the physiograph.

The Harvard Apparatus LTD 50-9364 did not possess sufficient sensitivity to provide an effective measure of ventricular contraction. If time had permitted, it would have been beneficial to repeat this experiment on another machine.

Although under these experimental conditions it would have been impractical to perfuse the heart with the drugs, some observable effects may have been produced. Superfusion often produced the desired effect in the epithelial myocardium, however, the inner myocardium tissue was likely unaffected. The total effect of the drug was often difficult to distinguish on the physiograph record.

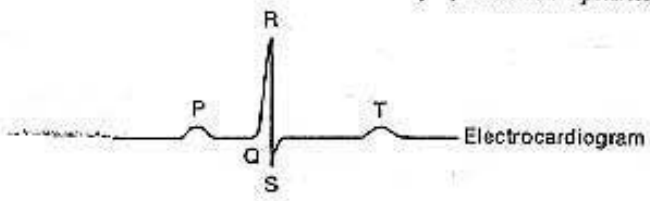
Finally, there is also the possibility that the heart was simply weak and could not respond to the stimuli. This is a logical conclusion when considering the difficulties encountered during the heart preparation.

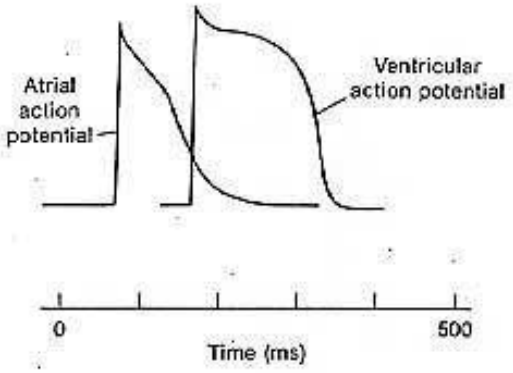
As a result of this experiment, an analysis of the change in frequency of contraction due to either facilitation or inhibition of the myocardial conduction pathway was made after the addition of each drug. A graphical analysis of significant changes have been presented as a plot of frequency

versus time in figures 2.3 to 2.5. In addition, a graphical representation of the recovery of amplitude owing to an increasing ventricular contraction has been presented. The aforementioned problems with complete diffusion of the applied drug, particularly KCI, can be indirectly examined from this data.

APPENDIX

The electrocardiogram and related potential changes in individual cardiac muscle cells. P, atrial depolarization; QRS, ventricular depolarization; T, ventricular repolarization.





The effect of vagus nerve stimulation on the pacemaker action potential. Several action potentials are shown. Stimulation of the vagus nerve produces a rise in diastolic transmembrane potential, a decrease in the rate of depolarization of the pacemaker potential, and a decrease in the duration of the action potential. [After Hecht, 1965.]

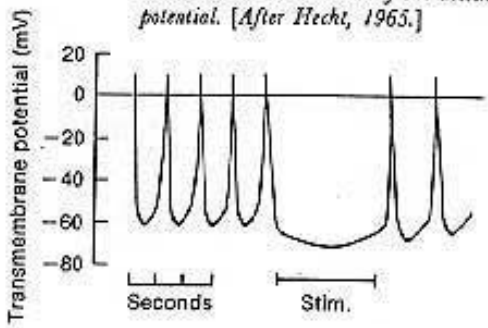


Fig 2.1: Action Potential of Cardiac Muscle (Byron and Dorothy Schottelius, 1978).

Ventral view of the internal structure of the frog heart. [After Goodrich, 1958.]

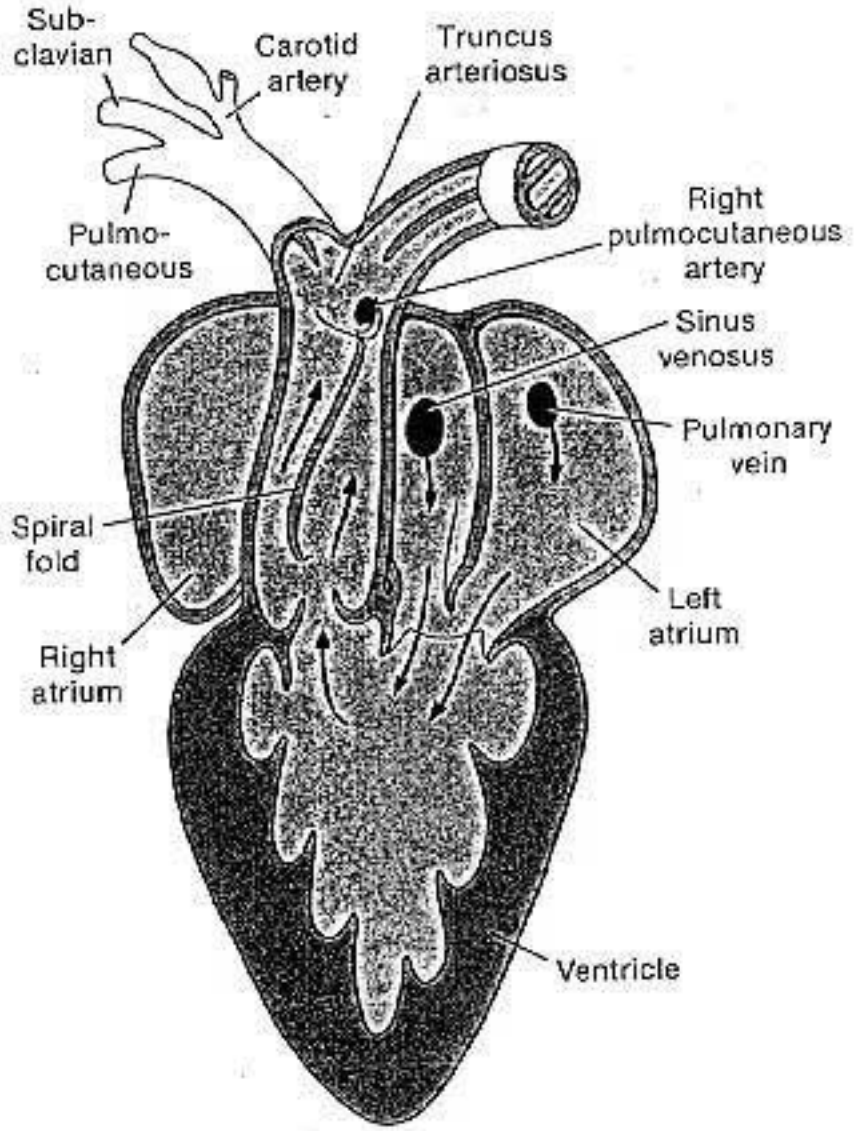


Fig. 2.2: (Roger Eckert and David Randall, 1978).

Figure 2.3: First Dose of Adrenalin

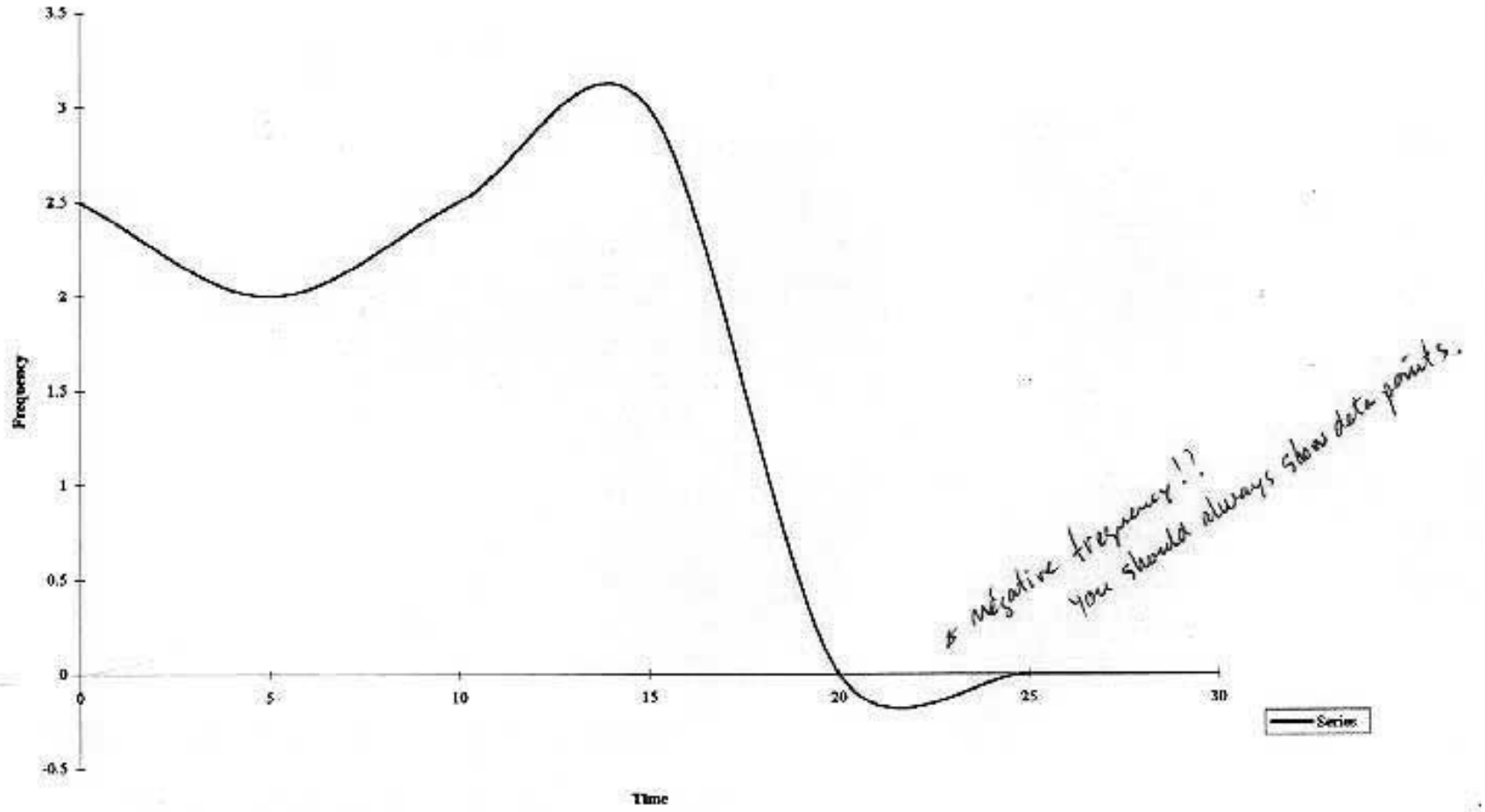


Figure 2.4: Second Dose of Adrenalin

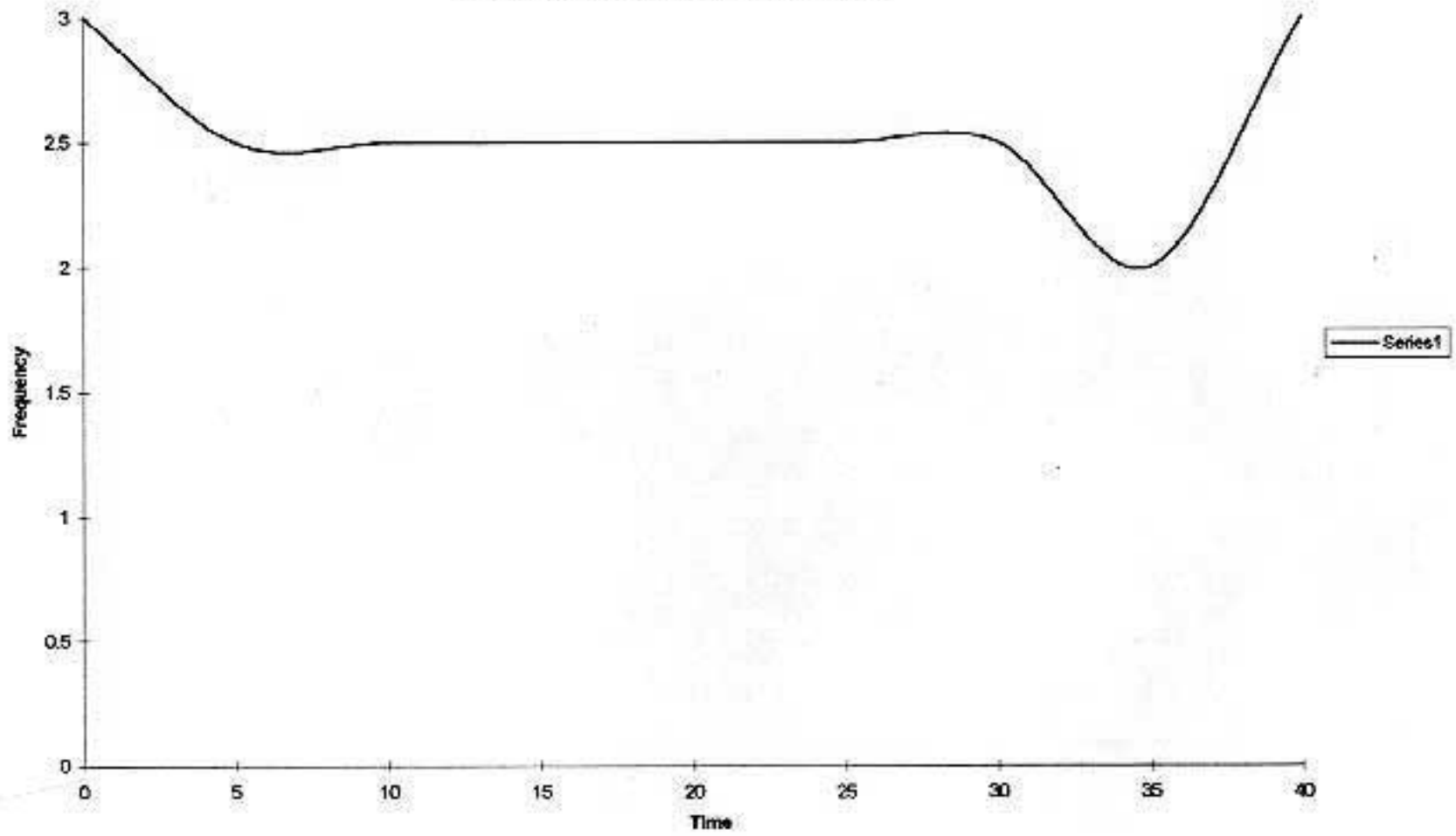


Figure 2.5: Caffeine

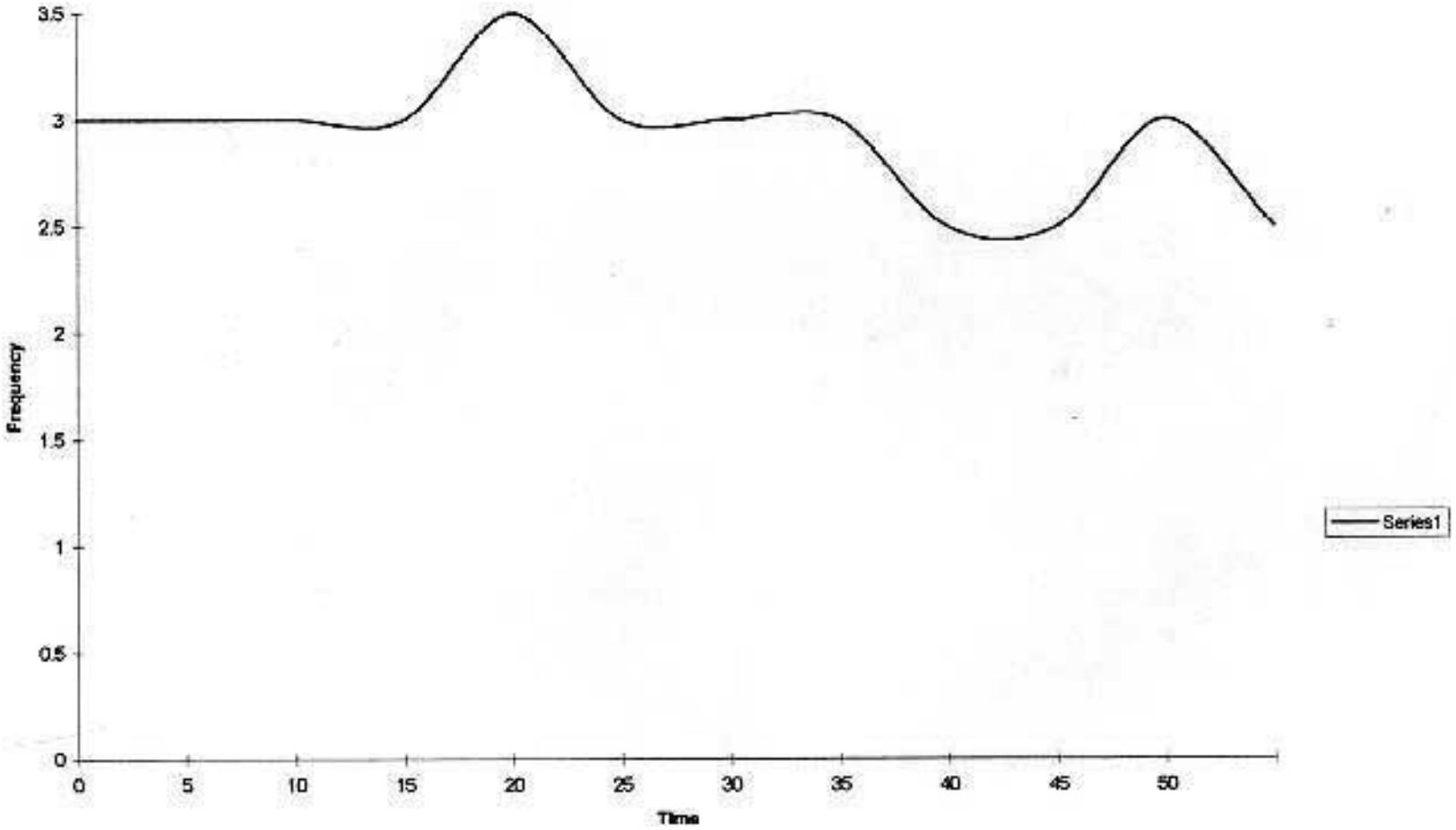


Figure 2.6: KCL

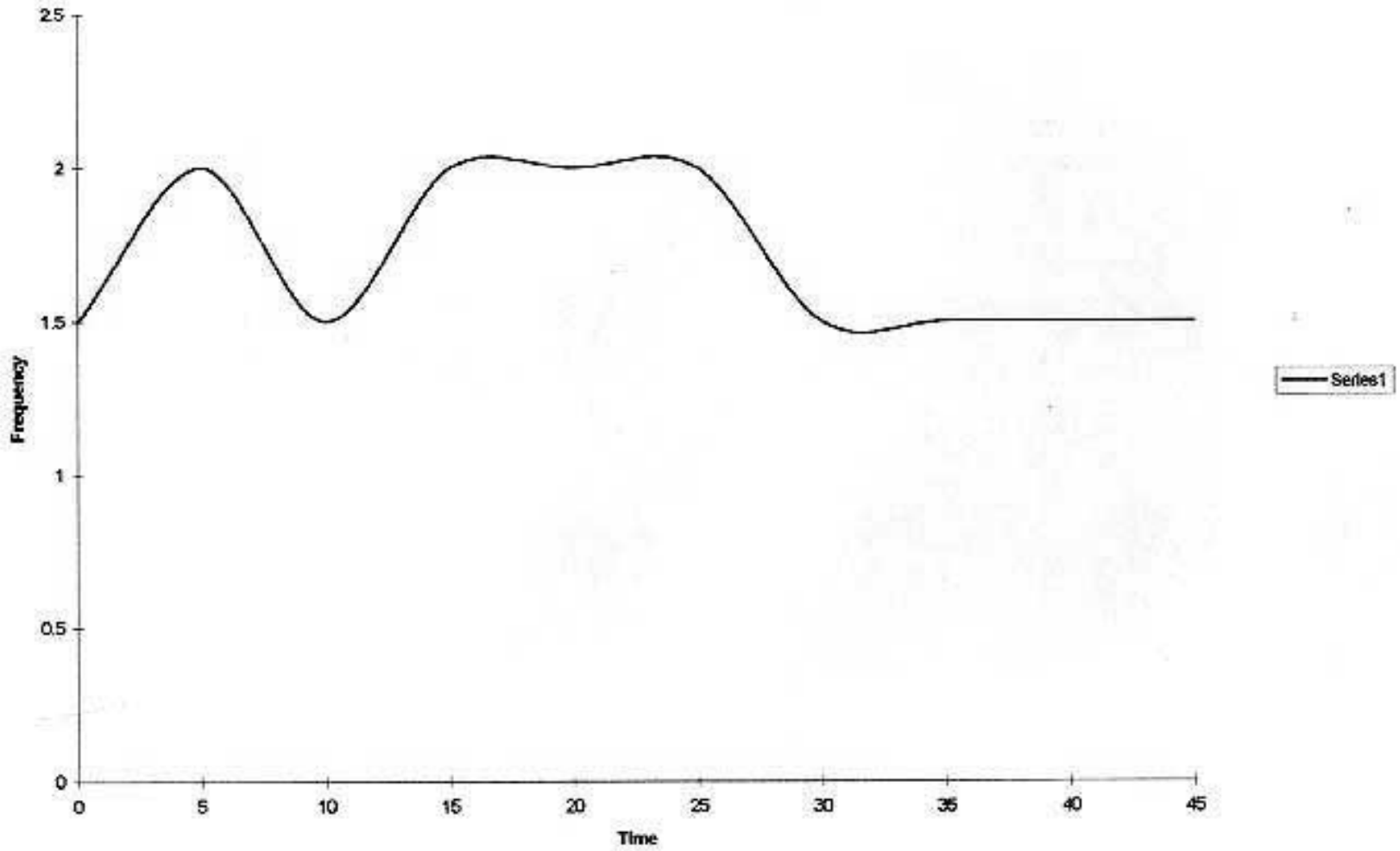
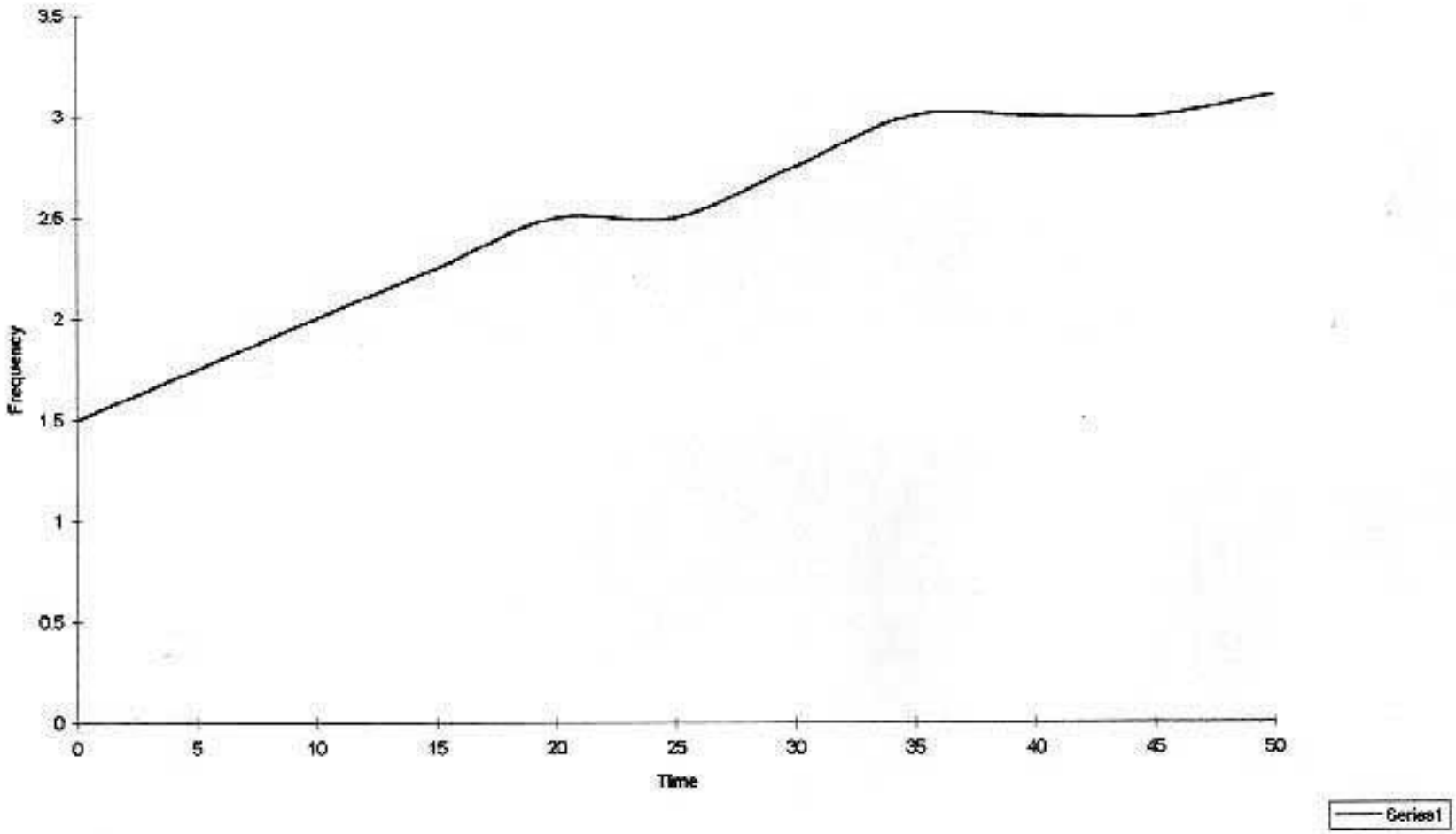


Figure 2.7: KCL Recovery



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- Guyton, Arthur C and Hall, John E., *Textbook of Medical Physiology, W* B. Saunders Company, Toronto, 1996, pp. 109-1 10, 126.