ELECTRONIC SIMULATION
OF THE
BIOLOGICAL CLOCK

by

James L. Melsa

A Thesis Submitted to the Faculty of the
DEPARTMENT OF ELECTRICAL ENGINEERING
In Partial Fulfillment of the Requirements
For the Degree of
MASTER OF SCIENCE
In the Graduate College
THE UNIVERSITY OF ARIZONA

1962

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in The University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in their judgment the proposed uses of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED:

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below.

Andrew P. Sage, Jr.
Associate Professor
Electrical Engineering

January 17, 1962
Date

ABSTRACT

The term "biological clock" has been given to the phenomenon displayed by organisms, both plants and animals, that pace their functions in a cyclic fashion related in some way to environmental stimuli. Since most of the quantitative studies of the biological clock have been made using members of the Animal Kingdom, the study has been restricted to this area.

The thesis begins with a general review of the literature on the biological aspects of light and temperature in relation to pace-making. Both periodic and random shock effects, including the effects of periods different from twenty-four hours, frequency demultiplication, variation of intensity and duration of shocks, and multiple shocks, are described.

The above data are translated into a general mathematical model for the biological clock. A hybrid (digital-analog) model for the biological clock is then proposed.

The electronic model consists basically of a sequential logic circuit, an analog response computer, and an endogenous clock unit. The sequential logic circuit is the basic decision mechanism which determines the changes to be made in activity state on the basis of past and present activity states and external stimuli. The analog portion of the model is used to determine the exact amount of change to be made; while the endogenous clock unit represents the present activity state and receives all command inputs for state changes.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. Andrew P. Sage for his interest, guidance and assistance during the preparation of this thesis. The author also wishes to thank Dr. Keith E. Justice for his advice on the biological sections and my wife, Kathy, for her understanding and assistance.

TABLE OF CONTENTS

CHAPTER 1	INTRODUCTION	Page
1.1	The Biological Clock Phenomenon	1
1.2	Electronic Simulation	1
1.3	Definition of the Problem	2
1.4	Method of Solution	4
CHAPTER 2	BIOLOGICAL DATA	
2.1	Continuous Light or Darkness	7
2.2	Periodic Light-Dark Regimes	11
2.3	Light Shocks	20
2.4	Temperature Independence of Free-Running Period	24
2.5	Temperature Entrainment in Constant Darkness	25
2.6	Conflicting Light and Temperature Signals	25
2.7	Non-Periodic Temperature Signals	28
CHAPTER 3	THE MATHEMATICAL MODEL	
3.1	Onset of Activity	30
3.2	Termination of Activity	42
3.3	Demonstration of Validity	45

CHAPTER 4 THE ELECTRONIC MODEL	Page
4.1 Oscillator Models.	48
4.2 General Philosophy of Proposed Model.	48
4.3 General Block Diagram of Electronic Model.	50
4.4 Master Clock and Trigger.	54
4.5 Sensitivity Curve Simulator.	56
4.6 Conditional Response Controller.	64
4.7 Logic or Decision Circuits.	70
4.8 Light Source.	73
4.9 Example of Operation.	76
4.10 Parameters for the Model.	81
 CHAPTER 5 CONCLUSIONS	
5.1 The Proposed Model.	84
5.2 Suggestions for Future Work.	86
 BIBLIOGRAPHY	 88
 APPENDIX A CIRCUIT DIAGRAMS	 91

LIST OF FIGURES

FIGURE	TITLE	PAGE NO.
2. 1	Free Running Periods for Nocturnal Animals in Constant Light or Constant Darkness	9
2. 2	Probability Density Function for Free Running Period in Constant Light or Constant Darkness	10
2. 3	Entrainment of Nocturnal Animals to Twenty-Four Hour Periodic Light-Dark Cycles, with Gradual Transition	13
2. 4	Entrainment of Nocturnal Animals to Twenty-Four Hour Periodic Light-Dark Cycles, with Gradual Transition	14
2. 5	Entrainment of a Nocturnal Animal to Twenty-Four Hour Periodic Light-Dark Cycle. Ratio of Light to Darkness Varied	16
2. 6	Frequency Demultiplication	18
2. 7	Entrainment to Non Twenty-Four Periodic Light-Dark Cycles	19
2. 8	Effect of Ten Minute Light Shocks of a Given Intensity on Time of Onset of Activity	21
2. 9	Sensitivity of Clock to Light Shocks	21
2.10	Effect of Light Intensity in Resetting Clock	22
2.11	Effect of Light Shock Duration Upon Phase Delay	22
2.12	Effect of Twenty-Four Hour Temperature Cycle on Activity Rhythm in Constant Darkness	26
2.13	Steady State Onset of Activity as a Function of Phase Difference Between Conflicting Light and Temperature Regimes	27
4. 1	Block Diagram of Proposed Model	49
4. 2	General Block Diagram of Proposed Electronic Model	52

LIST OF FIGURES (Continued)

FIGURE	TITLE	PAGE NO.
4. 3	Block Diagram of Master Clock and Trigger Circuit	55
4. 4	Typical Waveforms during the Operation of the Master Clock and Trigger	57
4. 5	Block Diagram of the Sensitivity Curve Simulator	58
4. 6	Typical Output of the Sensitivity Curve Simulator	61
4. 7	Typical Waveforms during the Operation of the Sensitivity Curve Simulator.	62
4. 8	Block Diagram of the Conditional Response Controller.	65
4. 9	Waveforms for Example of Operation of the Conditional Response Controller	69
4.10	Block Diagram of the Decision Circuits	71
4.11	Example of Operation of the Decision Circuits	72
4.12	Block Diagram of Light Source	74
4.13	Time Relations for Example of Operation	77

LIST OF TABLES

TABLE	TITLE	PAGE NO.
I.	Logic Symbols and Definitions	35
II.	Table of Stable States	36
III.	Primitive Flow Table	38
IV.	Merger Diagram	40
V.	Mergered Flow Table	40
VI.	Output and Excitation Matrices	41
VII.	Logical Symbols and Definitions	43
VIII.	Symbols and Notations	53

CHAPTER ONE

INTRODUCTION

1.1 The Biological Clock Phenomenon.

The term biological clock is used to designate the phenomenon, displayed by organisms, of pacing activity in a cyclic manner related to environment. Many nocturnal animals, for example, are capable of entraining the onset of their activity with the light-to-dark transition in a periodic light-dark regime in which the period or ratio of light to darkness varies widely.

1.2 Electronic Simulation.

Electronic simulation of the biological clock involves reproducing certain biological characteristics of the "clock" by use of electronic circuitry. This will necessitate simplifying assumptions or generalizations to reduce the amount of circuitry to a practical amount, since the true biological model contains large amounts of feedback and redundancy. The electronic model will run on a much reduced time scale as compared to the true biological model.

There are three basic reasons for electronic simulation of the biological clock. First, the mathematical formulation of the features of the "clock" which is necessary for the development of a model may indicate inadequacies in the biological data. Many of these "gaps in information" would not become evident except under the concise

organization needed for "model-making."

Secondly, the model will make possible the acquisition of greater knowledge of the "clock" phenomenon. By using a reduced time scale, the electronic model will develop data for several years of biological experiments within a few hours. Thus, unusual and original experiments may assist the biologist in recognizing new features of the clock to be investigated. In this manner, the model may improve or extend the basic theories of the "clock" action. Since man has been shown to display at least a "pseudo-clock"^(1,2), greater knowledge of the phenomenon may prove valuable to future space travelers.

Thirdly, previous electronic simulation of features of the "life" sciences has led to advances in the state of electronic technology or aided in the solution of similar electronic problems. It is reasonable to assume that the same may be true of this study. The relation of the biological clock to many adaptive control systems should be noted.

1.3 Definition of the Problem.

Since the majority of biological work on the "clock" has involved nocturnal animals, the model developed in this paper has been developed primarily for nocturnal animals. It is expected that simple modifications, such as changing the sign for certain variables, will allow the model to be extended to diurnal animals. The model is intended to be quantitative in nature and adjustable to allow simulation of the clock phenomenon for many different animals.

The biological clock may be studied from two points of view.

First, the endogenous rhythm or innate time-keeper of the animal may be investigated to determine its cause; or second, the adaptive nature of the clock in relation to external stimuli may be studied. This paper deals basically with the adaptive features of the clock although some discussion of the endogenous rhythm is, of necessity, included.

The only external stimuli to be considered are light and temperature. There may be other important stimuli, such as cosmic radiation or sound, but at present no biological proof of correlation has been presented in the literature.

The general biological clock phenomenon involves rhythms with periods ranging from a few seconds to a year or longer. The model, however, is limited to periods of approximately twenty-four hours or circadian periods.

Pittendrigh⁽³⁾ has shown the presence of permanent or semi-permanent alterations of endogenous period due to some forms of external stimuli. This phenomenon has been described as a "learning effect." Although this effect may be of great interest, it does not appear to be essential for the operation of the biological clock. This paper has, therefore, neglected this factor in the interest of simplicity of the initial model. It is hoped that at a later date this phenomenon can be investigated and ultimately included in the model.

Specifically then, this thesis presents the development of an electronic model for simulation of the adaptation of the endogenous circadian rhythm of nocturnal animals due to light and temperature stimuli.

1.4 Method of Solution.

There are two forms which simulation models may take. The first is the exact or "one-to-one" model in which each element of the item to be simulated is matched by an element in the model. The second type of model attempts only to reproduce certain external characteristics. In general, the model presented in this thesis will be of the latter type, although some attempt at "one-to-one" simulation will be made.

The simulation process involves three distinct phases. First, data describing the phenomenon to be simulated must be presented in a form suitable for mathematical analysis. Any deficiencies in the data must be removed either by logical inference or further experimentation. In the second phase, a model is proposed in the form of mathematical statements. Certain assumptions are made during this phase for simplicity of the model. This model must be investigated to determine its validity. In many cases, it may be necessary to make changes in the model or to develop a completely new form for the model. The assumptions may need to be re-examined and perhaps new data developed in order to assure the model's validity. The third phase involves the translation of the mathematical statements into actual electronic circuitry. This paper will follow a very similar process in the development of an electronic simulation model for the biological clock.

First, biological data describing the "clock" phenomenon will be presented. In general the data are complete, but in some cases, particularly in relation to temperature effects, there are inadequacies. This paper, however, will make no attempt to develop additional

biological information; all inadequacies will be handled by use of logical deductions. It is hoped that at some future date, complete data will become available and additional features of the model can be determined.

The next step will be the development of a mathematical model in the form of logic and algebraic equations. This model will be examined to determine its validity in reference to the available biological data.

The third step is the actual development of electronic circuits which operate in the manner described by the mathematical model.

At certain points throughout the development, previously proposed models will be discussed for their historic significance.

CHAPTER TWO

BIOLOGICAL DATA

To determine the biological effects which describe the action of the "clock" phenomenon, an extensive survey of the available literature was made. However, only the investigations which were adequately controlled and which are pertinent to the aspects of the biological clock being examined have been referenced. Emphasis was placed on the general trend and qualitative nature of the results rather than specific or quantitative factors. In some cases, contradictory results were obtained by different experimenters, who were often using different techniques. In these cases, an attempt was made to determine the most logical results on the basis of other observed effects. Until these discrepancies can be resolved, quantitative accuracy of the model can never be assured.

The biological data presented in this chapter have been divided into three sections: (1) light stimuli, (2) temperature stimuli and (3) other stimuli. In the results of the biological work, an attempt was made to eliminate all probable "latent time-givers." Animals were provided with food and water in excess of normal requirements.

I. LIGHT STIMULI

Pittendrigh⁽⁴⁾ has presented convincing arguments that light will generally be the most reliable and best perceived time-giver. This argument hinges on the assumption of the relatively precise timing and easy perception of the light to dark and dark to light transition, in contrast to the greater day to day variability of changes in temperature.

The response due to light stimuli will be shown for continuous light or dark signals, periodic light-dark regimes, and non-periodic and random light shocks. The effects of light stimuli were studied under conditions of constant temperature.

2.1 Continuous Light or Darkness.

To study the results of continuous light or darkness, animals were kept in constant darkness for a control period, then subjected to constant light and finally returned to constant darkness. Experiments of this type are the starting point for most biological studies of the "clock" and are generally used to demonstrate the endogenous nature of the "clock." The persistence of some biological rhythm such as activity or metabolic rate under constant conditions is, of course, prerequisite in these studies. Persistence is, in fact, the primary evidence of the existence of an internal pacemaker.

In constant darkness, most nocturnal animals display a period less than twenty-four hours. The period in constant darkness has been shown by DeCoursey⁽⁵⁾ to be the endogenous period for a nocturnal animal. In constant light, most nocturnal animals display a basic period greater than twenty-four hours.⁽⁶⁾ This is accomplished by

delaying the onset of activity each day. Typical results are shown in Figure 2.1a.

In contrast, some nocturnal animals display a period greater than twenty-four hours both in constant darkness and constant light.⁽⁷⁾ However, the period in constant light must always be greater than the period in constant darkness.⁽⁸⁾ Figure 2.1b shows typical results for this type of animal.

In general, each animal exhibits its own characteristic endogenous period within a range of perhaps twenty-three and one-half and twenty-four and one-half hours.^(8,9) Figure 2.2 presents a typical distribution of periods for nocturnal animals in constant light and constant darkness. The trend toward endogenous period of less than twenty-four hours should be noted. This trend has been used by Roberts⁽¹⁰⁾ as a partial rebuttal of the hypothesis that uncontrolled periodic variables such as cosmic ray intensity or the earth's rotation with a mean period of twenty-four hours are pacing the rhythm.

Clear demonstration⁽⁴⁾ has also been given that the endogenous period is an innate characteristic of the animal and not an after-effect (or memory expression) of a twenty-four hour periodicity learned early in the ontogenetic experience.

For the most part, the free-running periods in constant conditions remain at some fixed value unless a temporary disturbance occurs. There are, however, several cases when the endogenous period apparently changed spontaneously. This phenomenon was noted by both Roberts⁽⁸⁾ and DeCoursey.⁽¹¹⁾ It is maintained that these period

(a) Endogenous period
less than twenty-four hours

(b) Endogenous period:
greater than twenty-four hours

Figure 2.1. Free Running Periods for Nocturnal Animals
in Constant Light or Constant Darkness.

Figure 2:2. Probability Density Function for Free Running Period in Constant Light or Constant Darkness.

changes reflect some unknown internal change in the animal since no known disturbance of the environment occurred simultaneously.

In some cases, exposure to constant light resulted in strong inhibition of activity which made it very difficult for the time of onset or termination of activity to be determined.⁽¹²⁾ For this reason, the intensity of light in all experiments was kept low enough so that no inhibition would occur.

2.2 Periodic Light-Dark Regimes.

The experiments in the area of periodic light-dark regimes can be divided into two basic classes by the type of period considered: (1) exact twenty-four period, and (2) non twenty-four hour periods. Each class can be further subdivided into four basic experiments: (1) entrainment with gradual light-dark transitions, as in natural twilight, (2) entrainment with abrupt transitions, (3) variation of light-dark ratio and (4) frequency demultiplication. The experimental results are presented so that the variation of each factor (i.e., period, light-dark ratio) is made separately in an attempt to more explicitly demonstrate each aspect of the "clock."

2.2.1 Twenty-Four Hour Entrainment with Gradual Transitions.

To study the entrainment process in nocturnal animals for gradual light-dark transitions, animals were maintained for several days in constant darkness and then exposed to an artificial day of ten hours of light and fourteen hours of darkness. The light was turned on and off gradually and initially interrupted the active period of

the animals. The animals were returned to constant darkness following the experiment.

Figure 2.3a presents the results of this experiment for animals with an endogenous period less than twenty-four hours. The leading edge of the activity cycle was entrained with the dusk (light to dark) transition. It should be noted that the amount of daily phase delay was relatively constant throughout the approach to entrainment.⁽¹³⁾

Figure 2.3b shows the result of the same experiment for nocturnal animals with an endogenous period greater than twenty-four hours. In this case, the trailing edge of the activity cycle was entrained with the dawn (dark to light) transition.

Figure 2.4 presents the results of a similar experiment except that the light did not initially interrupt the leading edge of the activity cycle but did interrupt the trailing edge. Entrainment was the same as above in both cases.

2.2.2 Entrainment With Abrupt Transitions.

Experiments very similar to the ones described above were used to study the entrainment of nocturnal animals with abrupt transitions of light to darkness.⁽¹⁴⁾ The only change was that abrupt rather than gradual transitions were used when the light was turned on and off. The entrainment process was identical with the previous results except for a characteristic overshoot which is present in each case.⁽¹⁵⁾ Again, the amount of delay or advance per day was relatively constant throughout the entrainment process.

(a) Endogenous period
less than twenty-four hours
(dusk entrainment)

(b) Endogenous period
greater than twenty-four hours
(dawn entrainment)

Figure 2.3. Entrainment of Nocturnal Animals to Twenty-Four Hour Periodic Light-Dark Cycles with Gradual Transitions.

(a) Endogenous period
less than twenty-four hours

(b) Endogenous period
greater than twenty-four hours

Figure 2.4 Entrainment of Nocturnal Animals to Twenty-Four
Hour Periodic Light-Dark Cycles.

2.2.3 Variation of Light-Dark Ratio.

For a nocturnal animal, the length of the activity period depends basically on the length of the dark cycle and the animal's normal activity period.⁽¹⁶⁾ In constant darkness a nocturnal rodent is active for about the same number of hours each day. This may be considered its maximum duration of activity. The duration of the active state under conditions of constant light may be called the minimum duration of activity. It is possible to entrain both the onset and termination of daily activity by making the duration of the dark cycle shorter than the duration of the animal's maximum activity cycle. Figure 2.5 presents the results of an experiment used to illustrate this phenomenon.

An animal with a normal activity period of eight hours was initially placed in an artificial day of twelve hours of light and twelve hours of darkness. Entrainment was with the dusk transition. When the duration of the dark period was abruptly changed to six hours, both the onset and termination of the activity period were entrained.

The duration of the dark period was then abruptly changed to four hours. The entrainment of the onset of activity was not retained, indicating the minimum period for which the animal must be active.

2.2.4 Frequency Demultiplication.

The experiments demonstrating frequency demultiplication⁽¹⁶⁾ are a natural outgrowth of the variation of light-dark ratio experiments. In this case, animals were subjected to periodic signals which were

Figure 2.5. Entrainment of a Nocturnal Animal to Twenty-Four Hour Periodic Light-Dark Cycle. Ratio of Light to Darkness Varied.

submultiples (i.e., $1/2$, $1/3$) of twenty-four hours. Figure 2.6 shows the results of a frequency demultiplication experiment. Initially the period was twenty-four hours and entrainment was with the dusk transition. As the period was changed to twelve, eight and then six hours, entrainment was maintained. Again the animal's minimum period of five hours becomes evident. When the period was finally reduced to four hours, entrainment was no longer possible, and the animal delayed the onset of activity on each day.

It should be noted that the termination of daily activity is normally not as regular as the onset of activity, and therefore the smooth curves for termination of activity shown in Figures 2.5 and 2.6 are not an exact representation of the biological data.

2.2.5 Non Twenty-Four Hour Periods.

To study the entrainment process for non twenty-four hour periods, animals were subjected to artificial days with periods ranging from twenty-two to twenty-six hours.⁽¹⁶⁾ The results of this experiment are shown in Figure 2.7. Entrainment was possible for periods from twenty-three to twenty-five hours.

For periods greater than the animal's endogenous period, the leading edge of the activity cycle was entrained with the dusk transition, whereas for periods less than the animal's endogenous period the trailing edge of the activity cycle was entrained with the dawn transition. The inability to entrain to periods greater than twenty-five and less than twenty-three hours may indicate that there is some maximum amount of phase shift both advancing and delaying for each animal. In this case, the maximum amount appears to be approximately one hour.

Figure 2.6. Frequency Demultiplication

Figure 2.7. Entrainment to Non Twenty-Four Periodic Light-Dark Cycles.

2.3 Light Shocks.

Animals were subjected to light shocks of varying intensity and duration in order to determine the transient effects of light upon the basic clock mechanism. An exact measure of the effects of a light shock on the "clock" can be made by determining the difference between the expected and actual time of onset of activity on the night after the shock. The expected time of onset may be determined by extrapolation of the average period during the preceding period of constant darkness. Typical results from an experiment of this type are shown in Figure 2.8.

By the use of many such experiments, it is possible to develop a plot of the shift of the following day's onset of activity by a light shock versus the time at which the shock was applied relative to the onset of activity. This plot is commonly called a sensitivity curve; a typical sensitivity curve is shown in Figure 2.9. DeCoursey⁽¹⁷⁾ attempted to determine whether the zero portion of the curve was due to the animal's inactivity by awakening the animal before application of the light shock. Her results indicate that the insensitivity is not due to the animal's inactivity.

The light shock technique can also be used to study the effects of the intensity of light in causing a shift in the time for onset of activity.⁽¹⁸⁾ Figure 2.10 shows the effect of light intensity on the resetting of the "clock." There appears to be some level at which saturation occurs (i.e., further increase of intensity will have no effect). For some animals this saturation level is very low and may be the reason that some researchers have concluded that the intensity

Figure 2.8 Effect of ten minute Light Shocks of a Given Intensity.

Figure 2.9 Sensitivity of Clock to Light Stocks.

Figure 2.10. Effect of Light Intensity in Resetting Clock

Figure 2.11. Effect of Light Shock Duration Upon Phase Delay.

of light had no effect.

By varying the duration of the light shock while maintaining a constant intensity and time of application, it is possible to plot a curve of shock duration versus the shift of onset of activity: (18)

Figure 2.11 presents such a curve and clearly indicates that the duration of the shock has little effect in shifting the onset of activity for the following day.

DeCoursey⁽¹⁸⁾ has shown that when an animal is subjected to multiple short duration shocks on any given day, the total shift of onset of activity is somewhat less than the sum of the shifts for the same light shocks given on different days. However, the effect is greater than that for a single shock lasting for the total test period. The length of the dark period necessary between shocks to allow summation was not determined.

For example, if three ten minute light shocks are given at one, three and four hours after the onset of activity, one would expect from Figure 2.9 a total delay of twenty-nine plus twenty-seven plus twenty-two equals seventy-eight minutes. The actual delay may be on the order of fifty-five to seventy-five minutes. A single light shock of thirty minutes⁰ duration starting one hour after onset of activity would produce a delay of approximately thirty minutes.

When a delay shock and advancing shock are given in the same activity period, the response is equal to a delay shock alone, indicating that a delaying signal overrides the advancing signal. However, the entrainment of onset with dusk and termination with dawn

logically requires that under such conditions advancing signals override delaying signals for the control of termination.

II. TEMPERATURE STIMULI

It has been demonstrated that the light regime is generally a powerful entraining agent; however, temperature also has an effect (great or small) in most cases. The study of temperature effects has been made both with and without a periodic light regime present.

2.4 Temperature Independence of Free-running Period.

The virtual insensitivity to temperature change of the period of the clock mechanism has been noted by several authors. (4, 11, 19) This phenomenon has been explicitly recognized to be a functionally significant feature of a time measuring system and a universal feature of circadian rhythms. A temperature coefficient, Q_{10} , has been defined as:

$$Q_{10} = \left(\frac{P_2}{P_1} \right)^{\frac{10}{T_1 - T_2}}$$

where P_1 and P_2 are the mean period at temperatures T_1 and T_2 . Results from many experiments show that Q_{10} will generally be in the range $1 < Q_{10} < 1.05$ which indicates a high degree of temperature independence. A Q_{10} of approximately 2.5 is normal for most temperature-dependent chemical or biological processes.

Although the endogenous period of the animal appears to be relatively independent of temperature, the entrainment ability of temperature has been demonstrated for several cases.

2.5 Temperature Entrainment in Constant Darkness.

Subjected to a twenty-four hour temperature oscillation in constant darkness, a nocturnal animal will reset its phase and follow a steady state rhythm with a twenty-four hour period. The time of onset of activity will shift to a point coinciding with the high point of the temperature curve. (20)

Figure 2.12 shows the effect of a sinusoidal temperature variation upon the time of onset of activity. The phase of the rhythm shifts such that the onset of activity occurs at the high point of the temperature cycle.

2.6 Conflicting Light and Temperature Signals.

The two distinct entraining agents of light and temperature operate simultaneously in nature and generally give congruent or non-conflicting information. Sunset and the high point of the temperature curve are roughly coincident. Under laboratory conditions, however, it is possible to study the effects of simultaneous light and temperature cycles in which the high point of the temperature cycle occurs at any pre-established point in the light cycle.

Pittendrigh et al., (21) have shown that when animals or organisms are subjected to simultaneous light and temperature cycles in varying degrees of phase conflict, a "jump" phenomenon occurs when the temperature and light cycles are in approximately twelve hours' conflict. As illustrated in Figure 2.13, the onset of activity in the steady state follows the high point of the temperature cycle until the light and temperature are in maximum conflict at which time the

Figure 2.12. Effect of Twenty-Four Hour Temperature Cycle on Activity Rhythm in Constant Darkness.

Figure 2.13. Steady State Onset of Activity as a Function of Phase Difference Between Conflicting Light and Temperature Regimes.

phase of the clock rhythm shifts abruptly about twelve hours to the next dusk signal. This again shows the predominance of light as the "time-giver." In this case, it appears impossible to shift the phase of the clock such that the animals' onset of activity occurs during the light portion of the light-dark cycle.

2.7 Non-Periodic Temperature Signals.

Under constant light conditions the onset of activity for a nocturnal animal will generally shift to the time of the maximum temperature on the temperature cycle. Pulse type temperature signals will also produce a reset of phase. Animals which were running in constant darkness and whose activity times were scattered throughout the day were simultaneously subjected to a forty-eight hour temperature drop from twenty-five degrees centigrade to twelve degrees centigrade. The temperature was dropped to twelve degrees centigrade in about six hours, maintained at that level for thirty-nine hours and then raised in three hours to twenty-five degrees centigrade. The new phase for all animals closely correlated with the time of the rising temperature maximum. A twelve hour temperature drop was then given to determine whether it was the rising rather than the falling temperature which was acting as the phase giver. Results suggest that it is maximum temperature which acts as the phase giver.

III. OTHER STIMULI

Although stimuli other than light and temperature have generally been shown to cause little or no exogenous or adaptive effects on the "clock," it appears possible that they may have some influence on the endogenous rhythm. For example, the spontaneous change in endogenous period noted previously may be caused by either some unknown non-circadian rhythm or other external stimuli.

In order to determine such effects, it will probably be necessary to investigate the metabolism and biochemistry of the animals at the cellular level. Such an investigation is beyond the scope of this paper but presents a definite challenge for future and more basic investigations of the biological clock phenomenon. In such a manner, it may be possible to determine the mechanism by which the animal maintains his endogenous rhythm and reasons for the effects of light and temperature noted previously.

CHAPTER THREE

THE MATHEMATICAL MODEL

I. LIGHT STIMULI

Since light has been shown to be the most important time giver, a model for light stimuli only will be considered first. The problem is basically two-fold in nature. First, the time of onset of activity, and second, the time for termination of activity must be determined. Many other authors have attempted to develop a model in which both of these factors were highly interrelated,⁽¹⁹⁾ and the difficulties experienced should point out the reasons for developing a non-interacting solution to each of these problems.

3.1 Onset of Activity.

As shown in Figure 2.9, the onset of activity will follow the beginning of the sensitivity curve by some amount of time which is fixed for a given animal. The data appear to indicate that the sensitivity curve causes the onset of activity. For this reason, this paper will determine the time for the beginning of the sensitivity curve rather than the onset of activity.

The time between successive initiations of the sensitivity curve will be equal to the endogenous period plus the amount of phase shift accumulated on previous days. For the majority of animals studied, transients induced by light shocks are essentially one day in duration. Thus, the ultimate phase shift is reached on the day

after the stimulus was applied. Since the endogenous period is assumed to be an innate and constant characteristic of each animal, the determination of the time for the beginning of the sensitivity curve is basically one of determining the phase shift caused by external stimuli. The problem may then be simply stated: Should a phase shift be made; if so, an advance or delay and how much?

The first two parts of the problem can be answered in a digital manner--that is, by a simple yes or no answer, while the third question will need an analog answer--that is, one in which a solution must be generated continuously.

3.1.1 Two Historical Theories.

Several theories have been postulated for computing the amount of phase shift that should be received from some given light stimulus. The two most prominent of these have been integral action and differential action.⁽³⁾

The proponents of the integral action theory argue that the amount of phase shift is proportional to the area under the sensitivity curve during the period that light is present.

That is:

$$\text{Phase shift} \propto \int_{t_0}^t c(t) dt. \quad (3.1)$$

where:

$c(t)$ = sensitivity curve shown in Figure 2.9

The inadequacy of this theory can be demonstrated by reference to the multiple light shock data. It can be noted that the amount of phase shift for the thirty minute shock given one hour after onset would be

much greater than the three ten minute shocks according to the above theory. This is not substantiated by the experimental data. Also the lack of correlation between the amount of phase shift and the duration of the shock as shown in Figure 2.11 is unexplained by the integral action theory.

Basically, the differential action theory states that light affects the system only at its initiation and termination. This would indicate that there would be no phase shift for constant light--an obvious contradiction of experimental data. Also if the light is turned on gradually, when should the phase shift be determined?

A thorough investigation of the biological data indicated that the above theories did not provide a complete solution; and, therefore, the development of a new model was undertaken.

In general, it is easier to characterize a linear time varying system by its impulse response than by its steady state response. For this reason, light shock data will initially be used in the development of the model rather than entrainment information.

3.1.2 The Peak-Holding Theory.

A peak-holding theory for determination of the amount of phase shift will be presented here. To date, the author has found no mention of any similar theory and, therefore, some background for this decision needs to be presented.

Basically, the amount of phase shift will depend on three factors: (1) intensity of light, (2) time at which the light shock is applied and, (3) duration of the light shock. Or symbolically:

$$\text{Phase shift} = f(i, t, d) \quad (3.2)$$

where:

i = intensity of light

t = time at which the shock is applied

d = duration of the shock

From Figure 2.10 it appears that the amount of phase shift should be proportional to the effective intensity of light to some power, " K ", if all other variables are held constant. The effective light intensity may be different than the actual intensity due to saturation effects. Then:

$$\text{Phase shift} \propto i_e^K \quad (3.3)$$

where:

i_e = effective light intensity

The sensitivity curve describes the relation between the amount of phase shift and the time at which the shock is applied. Then for a very short shock (i.e., short enough such that the duration will be unimportant), phase shift will be given by:

$$\text{Phase shift} = I_e^K(t)c(t) \quad (3.4)$$

where:

I_e = normalized effective light intensity.

The relation of shock duration to the amount of phase shift was not as easily established. Figure 2.11 indicates that the amount of phase shift is practically independent of shock duration. This information does not tell which value of phase shift, given by the above equation to use if a light shock extends over a large portion of the sensitivity curve. The most logical solution was that the animal would use the maximum amount of phase shift available. Thus the peak-holding theory was formed. As noted previously there appears to be some maximum amount of phase shift that an animal can receive on any

given day. Thus, the amount of phase shift for any given day from a single light shock will be given by:

$$\text{Phase shift} = \left| I_e^K c(t) \right| \text{ max.} \quad (3.5)$$

with the constraint that:

Phase shift $\leq$ Maximum phase shift for a single light shock.

For multiple light shocks, the amount of phase shift will be the summation of the phase shift received from each shock, again subject to some constraint on the maximum amount of phase shift for any given day.

3.1.3 Digital Model.

The following logical statements have been developed from the biological data to decide when the beginning of the sensitivity curve should be advanced or delayed:

(1) The animal should receive a delaying phase shift if the light is on and the time is during the positive part of the sensitivity curve.

(2) The animal should receive an advancing phase shift if the light is on and the time is during the negative portion of the sensitivity curve and there has been no delaying signal on this day.

The above statements are simply a logical form of previous statements for the biological data and describe a problem in sequential logic which standard techniques⁽²²⁾ may be used to solve. Table I gives a list of the symbols used and a definition of their logical "1" state.

(Note: The negative portion of the sensitivity curve will be symbolized by the condition, $C = 0$ and $S = 1$.)

Symbol	Represents	Definition of Logical "1" State
S	Animal State	Animal is awake
L	Light	Light is on
C	Sensitivity Function	$c(t)$ is positive
D	Delay	Delaying phase shift should be made
A_0	Advance Onset	Advancing phase shift should be made
F	Secondary State	_____

Table I. Logic Symbols and Definitions.

The first step in the solution of a sequential logic problem is the development of a primitive flow table. The best method for developing a primitive flow table is by using a table of stable entries, which is shown in Table II.

The columns 000 and 010 represent states in which the animal is inactive and the sensitivity curve is not positive. Anytime that these conditions are present one of these states will be entered independent of the previous state. Therefore, each column has only one stable state and the output for each is 00.

Column 011 satisfies the first portion of statement (2) above and an advancing phase shift should be received if a delaying phase

c/s	000	001	011	010	110	111	101	100	A ₀	D
1									0	0
	2								0	0
		3							1	0
			4						0	0
				5					0	1
					6				0	1
						7			0	0
							8		0	0
	9								0	0
		10							0	0
						11			0	0
							12		0	0

Table II. Table of Stable States

shift has not been received on this day. This last condition necessitates two stable states in this column. The state entered will depend on the previous state. One of these states, (10), has an output of 00 and is entered if a delay has been previously received. State (3), on the other hand, has an output of 10 indicating that an advancing phase shift should be received.

Columns 110 and 111 represent the conditions of equation (1) above, and a delaying phase shift should be received whenever one of these columns is entered. The output is, therefore, 01 for states (5) and (6) which are the only stable states in these columns,

There are two stable states in each of the remaining columns. One is used if no delay has been received, while the other is occupied after a delaying signal.

From this table of stable states, it is possible to complete the primitive flow table by adding the correct unstable states in accordance with the above description of the stable states. Blanks indicate states which are impossible to enter. A completed primitive flow table is shown in Table III.

It should be noted that it is impossible to enter columns 001, 011, 111 or 101 from either of the stable states (1), or (4). This is due to the fact that the sensitivity function must become "1" previous to the onset of activity. (See Figure 2.9).

It is not possible to enter columns 110, 111, 101 or 100 from column 001 or 011 since once the sensitivity function has become "0" it cannot return to the "1" state on the same day.

cls										
000	001	011	010	110	111	101	100	A ₀	D	
(1)			4	5			12	0	0	1
1	(2)	3	4					0	0	2
1	2	(3)	4					1	0	3
1			(4)	5			12	0	0	4
1	9	10	4	(5)	6	7	8	0	1	5
1	9	10	4		(6)	7		0	1	6
1	9	10	4		6	(7)		0	0	7
1	9	10	4	5	6	7	(8)	0	0	8
1	(9)	10	4					0	0	9
1	9	(10)	4					0	0	10
1	2	3	4	5	6	(11)		0	0	11
1	2	3	4	5	6	11	(12)	0	0	12

Table III. Primitive Flow Table

Also it is impossible to move from column 111 or 101 to column 110 or 100 since the termination of activity cannot occur until the sensitivity function has become negative.

The next step is to form the merged flow table by use of a merger diagram. The merger diagram is shown in Table IV and clearly indicates two groups consisting of rows 1, 2, 3, 4, 11 and 12 and 5, 6, 7, 8, 9, and 10. Within each of these groups all pairs of rows can be merged. Therefore, it is possible to merge each group into a single row. The resulting merged flow table is shown in Table V.

It is not necessary to draw a transition diagram since the merged flow table has only two rows and therefore only one secondary state is needed. Although the secondary assignment for the output and excitation matrices may be made in two ways, for simplicity only one is shown. The assignment of all optional states was made consistent with the criteria of no false outputs and minimum networks. The output and excitation matrices are shown in Table VI.

The output and excitation functions may then be written:

$$D = lc \quad (3.6)$$

$$A_0 = slc'f' \quad (3.7)$$

and: $F = sf + lc + cf \quad (3.8)$

By use of the above logic equations and the peak-holding theory, the phase shift to be received from any light stimuli can be determined.

Table IV. Merger Diagram

<i>cls</i>							
000	001	011	010	110	111	101	100
1	2	3	4	5	6	11	12
1	9	10	4	5	6	7	8

Table V. Mergered Flow Table

		cls							
		000	001	011	010	110	111	101	100
f	0	0	0	0	0	1	1	0	0
	1	0	1	1	0	1	1	1	1

F

(a) Excitation Matrix

		cls							
		000	001	011	010	110	111	101	100
f	0	00	00	10	00	01	01	00	00
	1	00	00	00	00	01	01	00	00

A_0D

(b) Output Matrix

Table VI. Output and Excitation Matrices

3.2 Termination of Activity

In the past, little attention has been directed toward the termination of activity. For this reason, the data describing this aspect of the "clock" are not as extensive or conclusive as the data for the onset of activity. However, it appears quite likely that the termination of activity will correspond very closely with the end of the sensitivity curve. (i.e., the return of the sensitivity curve to zero after passing through the negative region.)

Since the time for the beginning of the sensitivity curve has been determined, the end of the sensitivity curve will be known if its length is determined. The problem of finding the time for termination of activity is then resolved to the problem of determining the length of the sensitivity curve.

The following rules of action are postulated for determining the length of the sensitivity curve:

(1) There will be a minimum length of the sensitivity curve for a given animal, corresponding to the length in constant light.

(2) There will be a maximum length of the curve, corresponding to the length in constant darkness.

(3) The length of the sensitivity curve on the next day will be decreased if a light shock occurs during both the positive and negative portions of the sensitivity curve. The amount of this decrease will be proportional to the advancing phase shift that would be received if

light did not occur during the positive portion of the curve.

(4) If the conditions of (3) above are not satisfied, the length of the sensitivity curve for the next day will be increased by some constant amount.

Due to the similarity of statement (3) above to statement (2) in the digital model for the onset of activity, the following logical equation can be written directly. (Symbols not defined in Table I are defined in Table VII.)

$$A_T = slc'f \quad (3.9)$$

Then note that:

$$A_T + A_O = slc' = A \quad (3.10)$$

Symbol	Represents	Definition of Logical "1" State
A_T	Decrease	The length of the sensitivity curve should be decreased.
A	Advance	An advancing signal should be computed.

Table VII. Logical Symbols and Definitions.

Therefore, an advancing phase shift will be computed independent of whether a delay has previously been received on a given day. If a delaying signal has been received, the delaying phase shift will be applied to the time for the beginning of the sensitivity curve and the advancing phase shift will be used to decrease the length of the sensitivity curve for the next day. If no delaying signal has been

received, the advancing phase shift will be applied to the time for the beginning of the sensitivity curve and the length of the sensitivity curve for the next day will be increased by some fixed amount.

The exact proportionality constant between the advancing phase shift and the decrease of sensitivity curve length will have to be determined experimentally. The same is true for the fixed amount that the length will be increased.

This completes the development of the mathematical model for light stimuli.

II. TEMPERATURE STIMULI

As noted previously, the only effect of temperature stimuli will be to change the time of onset of activity by adding some amount of phase shift to the endogenous period. The amount of this phase shift depends on (1) the time at which the maximum temperature is reached relative to the onset of activity and (2) the rate of change of light-induced phase shift.

The effect of the time at which the maximum temperature occurs can be accounted for by a sensitivity curve similar to the one for light. Since a temperature sensitivity curve has not appeared in any of the available literature, not even the general nature of the curve is known.

The relation of the rate of change of light-induced phase shift to the amount of temperature-induced phase shift to be received has not been determined. From the biological data, it appears reasonable that the amount of temperature-induced phase shift will be reduced during rapid changes in light-induced phase shift. On the other hand, when

the animal becomes entrained or is in a free-running state, the amount of possible temperature-induced phase shift will increase. This effect appears analogous to some form of automatic gain control.

Thus, there does not appear to be enough information available for the formulation of a reliable mathematical model for temperature stimuli.

3.3 Demonstration of Validity.

Before proceeding with the development of the electronic model, it is necessary to demonstrate the validity of the proposed mathematical model. If this can be done, simulation of the mathematical model will assure the validity of the electronic model.

It is not necessary to discuss the light shock data. Since this information was used in the development of the model, the validity should be inherent. The variation of intensity and duration of a light shock and the sensitivity curve have been directly incorporated in the model. In effect, the mathematical model has been developed to give the correct impulse (light shock) response; however, it is necessary to show that the steady-state (entrainment) response will be correct.

In constant darkness, the period of the model will be equal to the animal's endogenous period since no phase shift will be received. The absence of light, and thus advancing phase shifts, will allow the sensitivity curve to increase to its maximum length. Both of these results are consistent with the biological data.

In constant light, the model will delay the onset of the sensitivity curve on each day so that this free-running period will be

longer than the endogenous period. The continuous light will cause both delaying and advancing phase shifts to be computed and therefore the length of the sensitivity curve will be decreased to its minimum. Again, the model's action is consistent with the biological data.

To entrain to periodic signals, the model will align either the leading or trailing edge of the sensitivity curve with the dusk or dawn transition respectively. The type of entrainment will depend on the relative lengths of the light signal and the endogenous period. This will be done in such a manner that the amount of phase shift received when added to the endogenous period will equal the light regime period. If the period of the light regime is too long or too short, entrainment will not be possible since the amount of phase shift received on any day is limited.

When gradual transitions are used, it will be possible for the model to seek the entrainment point without overshooting since the amount of phase shift will be reduced as the transition region is entered. The abrupt transition, however, may cause an overshoot since the amount of phase shift may be large even at a point just prior to entrainment. Thus, the model may delay too much on the last day and overshoot.

The peak-holding theory also explains why the amount of phase shift is relatively constant throughout the entrainment process. As the sensitivity curve indicates, the maximum amount of phase shift is reached relatively soon after onset. Therefore until the last few days prior to entrainment, the amount of phase shift will be constant.

The variation of light-dark ratio and the frequency demultiplication experiments may be discussed simultaneously since the phenomenon displayed is very similar. As the length of the dark period becomes shorter than the maximum length of the sensitivity curve, the length of the curve will be decreased until the minimum length is reached. After this, no further decrease can take place. If the length of a dark period becomes so short that the light is interrupting the positive portion of the sensitivity curve twice, the amount of delay received will be so great that entrainment will no longer be possible.

The model as proposed, therefore, appears to be consistent with the essential phenomenon of the "clock" as described by the biological data.

CHAPTER FOUR

THE ELECTRONIC MODEL

4.1 Oscillator Models.

The oscillator models for the "clock" that have been proposed by other authors (19, 23, 24) have two basic limitations when actual electronic simulation is attempted. First, it is very difficult to control the phase and in some cases the period of an oscillator without creating undesirable transients. The second problem, which was mentioned previously in the discussion of the mathematical model, is the interaction of the onset and the termination of activity inherent in the oscillator models.

The electronic model presented in this chapter circumvents these difficulties. This model parallels very closely the mathematical model in relation to determining the times for onset and termination of activity as separate problems involving the sensitivity curve.

4.2 General Philosophy of Proposed Model.

The general philosophy of the proposed model is shown in pictorial form in Figure 4.1. Basically, the model consists of three functional units: (1) endogenous clock unit, (2) conditional response unit and (3) decision unit.

The endogenous clock unit is used to pace the model's internal rhythm and to supply information concerning the present state of the

Figure 4.1 Block Diagram of Proposed Model

"clock" to the conditional response and decision units.

The conditional response unit comprises the analog portion of the model and is used to determine the amount of phase shift that should be applied to the beginning of the sensitivity curve and the changes in the length of the curve. The decision or logic unit makes all of the required decisions for the model in terms of past and present information concerning the endogenous clock unit and external stimuli.

4.3 General Block Diagram of Electronic Model.

The model, presented in this chapter, is designed for light stimulus only and runs at approximately 0.1 cycle per second, corresponding to twenty-four hours of actual time. This speed appears best when recording equipment, circuit complexity and ease of entering external stimuli are taken into account.

The logical levels were defined so that the logical "1" level corresponds to ground, while the logical "0" corresponds to -12 volts. These levels were chosen because of the availability of equipment with these levels.

No actual circuit diagrams have been included in this chapter since to do so would have multiplied the length of the chapter many times. However, actual circuit diagrams can be found in Appendix A.

Several symbols and notations have been employed on the block diagrams for simplicity. In general, these symbols have been defined previously in Tables I and VII. Table VIII lists symbols and notations that have not been previously defined.

Figure 4.2 shows a general block diagram of the proposed electronic model for the biological clock. The following description of the operation of the model is very general and should serve only to indicate the manner in which the separate parts of the model are related. A more detailed discussion of each of the separate blocks will be found following this general discussion.

The advancing or delaying signal from the conditional response controller is sent to the master clock and trigger which is pacing the model's internal rhythm. This phase shifting signal causes the onset of the sensitivity curve to be advanced or retarded. When the correct time is reached, the circuit triggers and sends a signal to the sensitivity curve simulator and the conditional response controller indicating that the time has been reached for the beginning of the sensitivity curve.

This trigger signal causes the sensitivity curve simulator to start the generation of the sensitivity curve. The data concerning the sensitivity curve is sent to the conditional response controller in analog form and to the decision circuits in digital form. At some fixed time after the beginning of the sensitivity curve, the sensitivity curve simulator indicates that the correct time for the onset of activity has been reached. The model then changes to the active state, remaining in this state until the end of the sensitivity curve. The length of the sensitivity curve is determined by a signal from the conditional response controller.

The decision circuits determine whether the onset of the

Figure 4.2. General Block Diagram of Proposed Electronic Model

sensitivity curve should be advanced or delayed and whether the length of the curve should be decreased. After the decision circuits determine that a phase shifting signal should be computed, the conditional response controller will determine the amount of delay or advance to be sent to the master clock and trigger and the length of the sensitivity curve for the next day.

SYMBOL	DEFINITION
Output point
Input point
T	Trigger signal, indicating the beginning of the sensitivity curve
CRC	Conditional response controller
DC	Decision circuits
MCT	Master clock and trigger
SCS	Sensitivity curve simulator
"AND" gate
"OR" gate
Logical inverter
H	Adjust length of sensitivity curve

Table VIII. Symbols and Notations

4.4 Master Clock and Trigger.

A block diagram of the master clock and trigger is shown in Figure 4.3. The basic function of the master clock and trigger is to retain the animal's natural period and to determine the time for the beginning of the sensitivity curve by use of advancing and delaying phase shift from the conditional response controller. The following is a description of the equipment in each individual block:

Block 1: Analog adder capable of adding a d.c. voltage level and a voltage sweep.

Block 2: A Schmitt trigger circuit with an adjustable trigger level. (See Appendix A, Circuit 1.)

Block 3: A monostable sweep generator which develops a linear voltage sweep of approximately two seconds' duration upon being triggered.

Block 4: A count-down circuit capable of counting to 512.

Block 5: A bistable multivibrator (Circuit 2.)

Block 6: A diode "AND" gate. (Circuit 6.)

Block 7: A square wave generator with frequency of 60 cps. (Circuit 3.)

When the output of the adder reaches the trigger level, the Schmitt trigger circuit will set the bistable multivibrator to the "1" state. This trigger signal is also sent to the conditional response controller and the sensitivity curve simulator. As the bistable multivibrator changes to the "1" state, it allows the square wave clock to pass through the "AND" gate to the count-down circuit. When the

Figure 4.3. Block Diagram of Master Clock and Trigger Circuit

count-down circuit has reached its maximum count (512 or 8.5 seconds), it triggers the sweep generator and resets the bistable multivibrator. As the circuit resets, it will stop the clock pulses from passing through the "AND" gate, thus stopping the count-down circuit. The sweep generator will send a voltage sweep to the adder. By adding the phase shift from the conditional response controller in the form of a d.c. voltage signal, the time at which the output of the adder reaches the trigger level will be shifted appropriately.

The output, H , from the count-down circuit will occur approximately midway between the termination and onset of the sensitivity curve. This signal will be used by the conditional response controller for adjusting the length of the sensitivity curve.

Figure 4.4 shows waveforms for typical operation of the master clock and trigger. The onset of the sensitivity curve was advanced on the first day due to the positive (advancing) phase shift, while the onset on the second day was delayed.

4.5 Sensitivity Curve Simulator.

A block diagram of the sensitivity curve simulator is shown in Figure 4.5. The purpose of the sensitivity curve simulator is to generate a curve similar to Figure 2.9 when triggered by the master clock and trigger. The following is a description of equipment in each individual block:

Block 1: A single-pole, double-throw relay.

Block 2: A Schmitt trigger with a trigger level at a small positive voltage (~ 0 volts). (Circuit 1.)

Figure 4.4. Typical Waveforms during the Operation of the Master Clock and Trigger

Figure 4.5. Block Diagram of the Sensitivity Curve Simulator

Block 3: Two d.c. amplifiers, giving a net positive gain.

Block 4: A Schmitt trigger with a trigger level at a positive voltage of approximately twelve volts. (Circuit 1.)

Block 5: A bistable multivibrator. (Circuit 2.)

Block 6: A Schmitt trigger with a trigger level at a small negative voltage (~ 0 volts). (Circuit 1.)

Block 7: A two-gang, single-pole, double-throw relay.

Block 8: A monostable multivibrator with a pulse duration of approximately one-half second. (Circuit 4.)

Block 9: A digital inverter. (Circuit 5.)

Block 10: Same as Block 9.

Block 11: A Schmitt trigger with a trigger level that is adjustable by a voltage signal. (Circuit 1.)

Block 12: A bistable multivibrator. (Circuit 2.)

Block 13: A single-pole, double-throw relay.

Block 14: A digital inverter. (Circuit 5.)

The sensitivity curve of Figure 2.9 can be divided into three distinct regions: (1) The rapid rising region from onset until the positive maximum is reached, (2) the slowly decaying region from the positive maximum to the negative minimum and (3) the region from the negative minimum to termination.

The generation of the sensitivity curve is divided into three similar regions. In the first region, an output capacitor is charged by a network with a relatively short time constant to a large positive

voltage. This gives the rapid rising section of the curve.

After the circuit has essentially completed the charging progress, the output capacitor is switched to another network with a much longer time constant. The output capacitor is discharged through this network toward a large negative voltage. This will give the long, nearly linear central portion of the curve.

When the voltage on the output capacitor reaches some negative value, the voltage connected to the second network is rapidly increased to a large positive value. Again, the output capacitor begins to charge slowly. However, as it reaches zero this time, the voltage charging the circuit is rapidly reduced to zero causing the termination of the curve.

A typical output waveform is shown in Figure 4.6 with each of the three regions marked. The similarity of this curve to Figure 2.9 should be noted.

By varying the negative value which divides regions II and III, effective and fairly linear control can be obtained over the length of the sensitivity curve. Another method of controlling the length of the sensitivity curve is also present. This method involves varying the negative voltage to which the circuit is discharging in region II. The first method will be the technique employed for this model.

Figure 4.7 shows typical waveforms during the operation of the sensitivity curve simulator. Operation begins with the trigger signal from the master clock and trigger which causes the monostable multivibrator to change to the quasi-stable logical "1" state. This change, in turn, causes relay A to operate, charging the output

Figure 4.7. Typical Waveforms during the Operation of the Sensitivity Curve Simulator

capacitor through network 1 to +32 volts. This network has a time constant of 50 msec. In approximately ten time constants (0.5 sec.), the charging process will be essentially complete. This is the period of the monostable multivibrator.

During this charging process, the output voltage will cross first the trigger level of trigger A and then trigger B. Thus, the logical sensitivity function and then the activity state is changed to the logical "1" state. As the bistable multivibrator B changes to the "1" state (onset of activity) relay C is operated connecting point B to point E.

As the monostable multivibrator resets at the end of its period, bistable multivibrator A will be set to the logical "1" state causing relay B to operate. Relay A ceases to operate when the monostable multivibrator resets. These two changes of relay states will cause the output capacitor to be disconnected from network 1 and connected to the larger time constant network 2. Network 2, in turn, has been connected to -32 volts; thus the output capacitor is discharged with a long time constant toward that level. (The time constant of network 2 is approximately 835 msec.)

As the output voltage again crosses the trigger level of trigger A, the trigger circuit returns the logical sensitivity function to the "0" state.

As output voltage crosses the negative trigger level of trigger C, bistable multivibrator A is reset to the logical "0" state. The voltage signal, V_T , adjusts the trigger level of trigger C thereby

fixing the length of the sensitivity curve. This voltage signal is computed by the conditional response controller. When the bistable multivibrator A is reset, relay B ceases to operate and the output capacitor charges toward +32 volts. Just before the output voltage reaches zero volts, it crosses the trigger level of trigger D. The trigger circuit resets bistable multivibrator B to the logical "0" state, indicating the termination of activity. This change causes relay C to cease operation, and the output capacitor is connected to the ground, ending the sensitivity curve.

4.6 Conditional Response Controller.

A block diagram of the conditional response controller is shown in Figure 4.8. The primary function of the conditional response controller is to compute as directed by the decision circuits the amount of phase shift to be received and the length of the sensitivity curve. The following is a description of the equipment in each block:

- Block 1: A function generator which develops the function, I_e^K , from I.
- Block 2: A two-quadrant analog multiplier.
- Block 3: A monostable multivibrator. (Circuit 4.)
- Block 4: A positive-peak-holding circuit.
- Block 5: A negative-peak-holding circuit.
- Block 6: Same as Block 3.
- Block 7: Same as Block 3.
- Block 8: Same as Block 3.
- Block 9: A digital inverter. (Circuit 5.)

Figure 4.8. Block Diagram of the Conditional Response Controller

Block 10: Same as Block 9.

Block 11: A single-pole, double-throw relay.

Block 12: Same as Block 11.

Block 13: An analog integrator with adjustable saturation.

Block 14: Same as Block 13.

Block 15: Same as Block 11.

Block 16: A bistable multivibrator. (Circuit 2)

Block 17: A bistable multivibrator. (Circuit 2)

Block 18: A single-pole, double-throw relay.

Block 19: A monostable multivibrator. (Circuit 4)

Block 20: A single-pole, double-throw relay.

Block 21: An analog integrator with adjustable saturation.

The amount of phase shift will be given by the following equation:

$$\text{Phase shift} = \left| I_e^K c(t) \right| \text{ max.}$$

The signal developed at the output of the multiplier is equal to $I_e^K c(t)$.

For a delaying phase shift, the output of the multiplier is positive since the sensitivity curve is positive. The peak-holding circuit A will therefore hold the peak value during the light shock. At the end of the shock, the monostable multivibrator A is triggered to its quasi-stable logical "1" state. This causes relay A to become operated. The integrator A integrates the output of the peak-holding circuit during the pulse duration of the monostable multivibrator A. Thus a signal proportional to the voltage on the peak-holding circuit is placed on

the integrator.

As monostable multivibrator A resets, it triggers monostable multivibrator B which resets the peak-holding circuit. Monostable multivibrator B then resets and the circuit has returned to its original state except that a phase shifting signal has been stored on the integrator.

If multiple shocks are received, the integrator causes the desired summation of peak values of phase shift. The saturation of the integrator simulates the constraint on the maximum amount of phase shift to be received on any given day. At the onset of the sensitivity curve for the next day, the integrator is reset.

The operation of the peak-holding circuit B and its related circuits for advancing phase shifts is identical to the discussion above and therefore will not be repeated.

If a delaying phase shift has been received, the bistable multivibrator A has been reset to the logical "0" state. The relay C is not operated and the output of integrator A, V_D , is applied to the master clock and trigger as a phase shift.

If an advancing phase shift (and hence no delaying phase shift) has been received, the bistable multivibrator A has been set to the logical "1" state. The relay C is operated and the output of integrator B, V_A , is applied to the master clock and trigger as a phase shift.

If the length of the sensitivity curve is to be decreased, the bistable multivibrator B is set to the logical "1" state and

relay D is operated. Thus when the monostable multivibrator E is triggered by the master clock and trigger, the output of integrator B will be integrated by integrator C for the pulse duration of monostable multivibrator E. This causes the output of integrator C to be increased which, in turn, decreases the length of the sensitivity curve.

If no signal to decrease the length of the sensitivity curve is received, the bistable multivibrator remains in the logical "0" state where it was reset by the trigger signal, T, from the master clock and trigger. The fixed negative voltage passing through relay D and finally relay E causes the output of the integrator E to be decreased which, in turn, increases the length of the sensitivity curve. Saturation of integrator C limits the maximum and minimum length of the sensitivity curve.

Figure 4.9 shows the output waveforms for several circuits during typical operation of the conditional response controller. Two light shocks are received during the positive portion of the sensitivity curve while a third light shock is received during the negative portion of the curve. The delaying phase shift is used to delay the onset of the sensitivity curve and the advancing phase shift is used to decrease the length of the sensitivity curve.

Figure 4.9. Waveforms for Example of Operation of the Conditional Response Controller

4.7 Logic or Decision Circuits.

A block diagram of the decision circuits is shown in Figure 4.10. The function of the decision circuit is to determine when advancing or delaying phase shifts should be computed and when the length of the sensitivity curve should be shortened. The following is a description of the equipment in each block:

Blocks 1, 2, 3, 4, 5, 6: Diode "AND" gates. (Circuit 6.)

Block 7: A diode "OR" gate. (Circuit 7.)

Blocks 8, 9, 10: A digital inverter. (Circuit 5.)

Block 12: A digital gate booster. (Circuit 9.)

The following logical equations have been developed:

$$A = slc^0$$

$$A_0 = slc^0 f^0$$

$$A_T = slc^0 f$$

$$D = lc$$

$$F = sf + cf + lc$$

The electronic realization uses diode gates since there are only two levels of logic. Gain is achieved in the secondary state feedback loop by the use of a digital gate booster. The necessary delay is inherent in the practical circuits.

Figure 4.11 shows an example of typical operation of the decision circuits. Two light shocks are received during the positive portion of the sensitivity curve, while a third shock is received during the negative portion.

Figure 4.10. Block Diagram of the Decision Circuits

Figure 4.11. Example of Operation of the Decision Circuits

The first light shock causes a delay output and also causes the secondary state to become activated. The secondary state remains in the logical "1" state as long as the model is in the active state.

The second light shock again causes a delay output but does not change the secondary state. The third light shock does not cause an advancing phase shift for the onset of the sensitivity curve since the secondary state is in the logical "1" state. It does cause an advancing signal to be computed and an output indicating that the length of the sensitivity curve should be decreased on the next day.

4.8 Light Source.

Figure 4.12 is a block diagram of the light source. The basic function of the light source is to provide either from internal or external light sources the logical light variable and the analog intensity signal. The following is a description of the equipment in each block on the diagram:

- Block 1: An astable multivibrator, running at a frequency of approximately 20 cps with a 10 per cent variation of frequency. (Circuit 8)
- Block 2: A diode "OR" gate. (Circuit 7)
- Block 3: A count-down circuit capable of counting to 256.
- Block 4: A three-pole, three-gang switch.
- Block 5: A four-gang, single-pole-double-throw, break-before-make switch.
- Block 6: A Schmitt trigger with an adjustable trigger level.
(Circuit 1)

Figure 4.12. Block Diagram of Light Source

Block 7: An adjustable d.c. voltage.

Block 8: A single-pole-double-throw, break-before-make relay.

Block 9: A bistable multivibrator. (Circuit 2.)

Block 10: A low-pass filter.

For a dark-dark internal cycle, switch B is set to DD, and switch A is set to INT. By setting the switch to DD, the bistable multivibrator is reset to zero, indicating the absence of light, and the "OR" gate prevents the passage of pulses to the count-down circuit and resets it to zero. When the bistable multivibrator is reset, the relay is operated setting the light intensity to zero.

For a light-light internal cycle, switch B is set to LL, and switch A is set to INT. By setting the switch to LL, the bistable multivibrator is set to the logical "1" state, thereby indicating the presence of light. The "OR" gate, however, still prevents the passage of pulses to the count-down circuit. The relay remains unoperated and the light intensity is equal to the value of the adjustable d.c. voltage.

For a light-dark internal cycle, switch B is set to LD, and switch A is set at INT. The square wave clock passes through the "OR" gate to the count-down circuit. When some preset number of pulses has been counted, the bistable multivibrator is set to the "1" state indicating the onset of light. The relay will be inoperative while the bistable multivibrator is in the "1" state, and the intensity of light will be equal to the adjustable d.c. voltage allowing the control of the light intensity.

When the count-down circuit reaches the maximum count (256), the bistable multivibrator will be reset, turning off the light. By varying the number of pulses counted before setting the bistable multivibrator and the frequency of the square wave generator, it is possible to vary the ratio of light to dark and the total length of day respectively.

If it is desired to use an external light source, switch A is set to EKT. The trigger sets the bistable multivibrator when the intensity of light is greater than some preset value. Before this value is reached, even though light is present, the intensity is too low for recognition. The low-pass filter insures that very rapid changes of light stimulus has no effect on the model.

4.9 Example of Operation.

The interaction of the various parts of the model can probably best be explained by an example. The model will be subjected to three light shocks while running in constant darkness. The first two shocks will occur during the positive portion of the sensitivity curve, while the last shock will occur during the negative portion of the sensitivity curve. Figure 4.13 shows the relative position in time of the light shocks and the sensitivity function.

It should be noted that this example is similar to the example used to explain the operation of the decision circuits and the conditional response controller. Reference should be made to those sections for more detailed information about the operation of those two portions of the model.

Figure 4.13. Time Relations for Example of Operation

Qualitatively there are ten steps in the operation of the model for this stimuli:

- (1) Onset of sensitivity curve.
- (2) Onset of activity.
- (3) Occurrence of first light shock.
- (4) Occurrence of second light shock.
- (5) The end of the positive portion of the sensitivity curve.
- (6) Occurrence of the third light shock.
- (7) Termination of activity and the sensitivity curve.
- (8) The length of the sensitivity curve for the next day is decreased.
- (9) The sweep voltage is triggered.
- (10) Phase shift is applied to the onset of the sensitivity curve for the next day.

4.9.1 Step One.

Since the model is running in constant darkness, the onset of the sensitivity curve is determined only by the endogenous period. There is no advancing or delaying phase shift present (i.e., the output voltages of integrators A and B in the conditional response controller are equal to zero). When the length of time since the last onset of the sensitivity curve is equal to the endogenous period, the Schmitt trigger in the master clock and trigger sends a trigger signal to the conditional response controller and the sensitivity curve simulator indicating that the time for the onset of the sensitivity curve has been reached.

4.9.2 Step Two.

When the output of the sensitivity curve simulator reaches some preset value, the model is triggered to the active state, corresponding to the animal's onset of activity. The model remains in this state until the end of the sensitivity curve is reached.

4.9.3 Step Three.

When the first light shock occurs, the delay output becomes "1", indicating to the conditional response controller that a delaying phase shift should be computed. The secondary state also becomes "1" indicating that a delaying signal has been received. It remains in this state until the termination of activity.

At the end of the shock, the output voltage of the peak-holding a circuit is transferred to the integrator. The peak-holding circuit is then reset, ready for the next light signal.

4.9.4 Step Four.

The second light shock also causes the delay output to become "1". At the end of the shock, the output voltage of the peak-holding circuit is transferred to the integrator and added to the previous delay signal. The peak-holding circuit is again reset.

4.9.5 Step Five.

As the output of the sensitivity curve reaches the trigger level of trigger A in the sensitivity curve simulator, on its downward sweep, the logical sensitivity function is returned to the "0" state.

4.9.6 Step Six.

The occurrence of the third light shock has no effect on the onset of the sensitivity curve, but it causes an advancing phase shift to be computed. This phase shift is used to decrease the length of the sensitivity curve for the next day.

4.9.7 Step Seven.

As the sensitivity curve returns to zero after passing through the negative portion, the trigger D in the sensitivity curve simulator will reset the model to the inactive state, corresponding to the termination of activity and the end of the sensitivity curve.

4.9.8 Step Eight.

At a time approximately midway between the termination of activity and the onset of the sensitivity curve for the next day, the master clock and trigger will trigger the output, H. This output indicates that it is time to make an adjustment in the length of the sensitivity curve for the next day. For this example, the advancing phase shift will be used to decrease the length of the curve for the next day.

4.9.9 Step Nine.

When a time of approximately 8.5 seconds after the onset of activity is reached, the count-down circuit in the master clock circuit triggers the sweep circuit. The master clock bistable multivibrator is also reset to the logical "0" state. The negative output voltage of the integrator is then added to the positive voltage sweep. This causes the time at which the output of the adder reaches the trigger

level to be retarded as desired.

4.9.10 Step Ten.

When the output of the adder reaches the trigger level, the Schmitt trigger circuit in the master clock will trigger indicating the onset of the sensitivity curve. It also causes the integrators A and B and the bistable multivibrator B in the conditional response controller to be reset.

Except that the length of the sensitivity curve is decreased, all effects of the light shocks have now ended. The length of the sensitivity curve will return to its maximum length after a day or two of running in constant darkness. The time between successive onsets of the sensitivity curve is again equal to the endogenous period.

4.10 Parameters for the Model.

Before the model may be used to simulate any given animal or species of animals, it is necessary to determine experimentally the following parameters for the model:

- (1) The endogenous period.
- (2) The exact form of the sensitivity curve.
- (3) The value of "K" to be used in equation 3.5.
- (4) The maximum amount of phase shift to be received for any given day or from any single light shock.
- (5) The proportionality constant involved with the decreasing of the length of the sensitivity curve by advancing phase shifting signals.

- (6) The constant amount by which the length of the sensitivity curve will be increased in the absence of a decreasing signal.
- (7) The maximum and minimum length of the sensitivity curve.

This information may be obtained by experiments of the type described in Chapter Two. The parameters will be employed in the model in the following manner.

The master clock and trigger circuit will be adjusted such that with no phase shifting signal present, the time between successive onsets of the sensitivity curve will be equal to the endogenous period.

By adjustment of the period of the monostable multivibrator and the time constants in the sensitivity curve simulator it should be possible to generate sensitivity curves of the type shown in Figure 2.9. If it is necessary to generate a curve of a vastly different form, it may be necessary to design a different sensitivity curve simulator.

The maximum amount of phase shift to be received for any given day will determine the saturation level of integrators A and B in the conditional response controller. The maximum amount of phase shift to be received from any single light shock will fix the maximum voltage at the output of the peak-holding circuit. The value of "K" will determine the form of the function generator in the conditional response controller.

The proportionality constant for the length of the sensitivity curve will be used to fix the gain of the integrator C in the conditional response controller. The fixed amount of increasing signal will determine the value of the voltage V in the conditional response controller. The maximum and minimum length of the sensitivity curve will determine the saturation levels of the integrator C.

It should be noted that insofar as this model is concerned a given animal or species of animals is completely characterized by the seven parameters given above. However, there appears to be no animal or species of animals for which all of the parameters have been developed at present. Therefore, it will be necessary to use reasonable parameters for a given animal for the initial simulation by the model.

CHAPTER FIVE

CONCLUSIONS

5.1 The Proposed Model.

This thesis has presented the development of an electronic simulation model for the biological clock phenomenon. However, there is no reason to believe that this model is the only or, in fact, the best model. There does, however, appear to be a good functional correspondence between the biological data and the model.

The electronic model is composed of a sequential logic unit, a conditional response unit and an endogenous clock unit. The sequential logic unit makes all decisions for the model in terms of external stimuli and the present and past state of the "clock." The conditional response unit, which is essentially analog in nature, determines the amount of control to be exerted on the endogenous clock unit. The endogenous clock unit maintains the present state of the model and applies all control signals for state changes.

Thus, it appears that the model represents a rather complex hybrid (digital-analog) adaptive control system, a type of system which hopefully may lead to improvements in the performance of many existing control and communications systems.

The model has been designed to match the actual biological data at certain known "points." It is hoped that the model will be capable of interpolating or extrapolating the data between these points. The

model can, however, relate the behavior of the "clock" to various stimuli more accurately than an intuitive idea of the "clock's" functioning could.

The electronic model of the biological clock is also valuable because of its role as an organizer. The model collects together a large variety of facts into a compact diagram and interrelates them, one to another. The model serves to consolidate the experience obtained thus far with the biological prototype and simplifies the task of formulating new biological experiments.

The proposed model has several limitations which have been made necessary by inadequacies of the biological data or for simplicity of the model. It was not possible to include the effects of temperature stimuli because there were not enough data available to justify the formulation of a model. Also, while induced and spontaneous shifts in period are known to occur in animals, it is not known if there are only certain discrete periods or a continuum of possible periods with some more likely than others. Therefore, a constant period has been assumed for any one animal in the present model.

On the other hand, in the interest of simplicity only single day transients were included in the model. However, some multiple day transients and "learning effects" have been observed in biological experiments. These were not included because they would have involved a memory which was a great deal more extensive than that presently included in the model.

In other instances, "gaps" in the biological information have

been removed by logical deduction. For example, since little is known of the action of the biological system under conflicting delaying and advancing signals, the most logical relationship was utilized in the model. The manner in which the sensitivity curve is shortened under conflicting signals was determined in this way. As new biological data become available, some modification will no doubt have to be made in the model to bring about an exact correspondence.

In constructing this model, primary emphasis was placed on the functional as opposed to the anatomical or elemental aspects of the system. It is doubtful, therefore, that there is a "one-to-one" correspondence between the elements of the biological system and the model.

5.2 Suggestions for Future Work.

Besides the obvious necessity of further biological experimentation to extend the validity of the model and to remove inadequacies in the data, there are also several possibilities for future electronic work:

One area would involve the development of some form of a memory which would enable the simulation of multiple day transients and "learning effects." It would also be desirable to improve the light source. Although this section of the model has little biological significance, it will be very valuable for performing actual simulation experiments.

It would be advisable to improve the elemental or "one-to-one" correspondence of the model and the actual biological system. Perhaps

this could be combined with biological investigation of the "clock" at the cellular level. The advantages of this to both the biological and engineering sciences should be great.

As the model is evolved through successive prototypes, the functional correspondence will become better. It is expected that the acquisition of new biological information will lead to even greater refinements in the model.

BIBLIOGRAPHY

1. Lobban, M. C., "The Entrainment of Circadian Rhythms in Man," Symposia on Quantitative Biology, Vol. XXV, Long Island Biological Association, Long Island, New York, 1960, pp. 325-332.
2. Halberg, F., "Physiologic 24-Hour Periodicity in Human Beings and Mice, The Lighting Regimen and Daily Routine," Photoperiodism and Related Phenomena in Plants and Animals, Publication No. 55, American Association for the Advancement of Science, Washington, D. C., 1959, pp. 803-878.
3. Pittendrigh, C. S., "Circadian Rhythms and the Circadian Organization of Timing Systems," Symposia on Quantitative Biology, Vol. XXV, Long Island Biological Association, Long Island, New York, 1960, pp. 159-184.
4. Pittendrigh, C. S., "On Temperature Independence in the Clock System Controlling Emergence Time in Drosophila," Proceedings of the National Academy of Science, Vol. 40, No. 10, October, 1954, pp. 1018-1029.
5. DeCoursey, P. J., Daily Activity Rhythms in the Flying Squirrel, Doctoral Thesis, University of Wisconsin, Madison, Wisconsin, 1959, p. 146.
6. Pittendrigh, C. S., "Perspective in the Study of Biological Clocks," Symposium on Perspectives in Marine Biology, University of California Press, 1958, pp. 239-268.
7. Justice, K. E., Nocturnalism in Three Species of Desert Rodents, Doctoral Thesis, University of Arizona, Tucson, Arizona, 1960, p. 31.
8. Roberts, S. K., "Circadian Activity Rhythms in Cockroaches," Journal of Cellular and Comparative Physiology, Vol. 55, No. 1, February, 1960, pp. 99-110.
9. DeCoursey, P. J., "Effect of Light on the Circadian Activity Rhythm of the Flying Squirrel, Glaucomys Volans," Zeitschrift fur vergleichende Physiologie, Vol. 55, 1961, pp. 331-354.

10. Roberts, S. K., Circadian Activity Rhythms in Cockroaches, Doctoral Thesis, Princeton University, Princeton, N. J., 1959, p. 17.
11. DeCoursey, P. J., "Phase Control of Activity in a Rodent," Symposia on Quantitative Biology, Vol. XXV, Long Island Biological Association, Long Island, New York, 1960, pp. 49-55.
12. DeCoursey, P. J., Daily Activity Rhythms in the Flying Squirrel, Doctoral Thesis, University of Wisconsin, Madison, Wisconsin, 1959, p. 87.
13. Ibid., pp. 91-98.
14. Ibid., p. 99.
15. Ibid., pp. 93-108.
16. Bruce, V. C., "Environmental Entrainment of Circadian Rhythms," Symposia on Quantitative Biology, Vol. XXV, Long Island Biological Association, Long Island, New York, 1960, pp. 29-48.
17. DeCoursey, P. J., Daily Activity Rhythms in the Flying Squirrel, Doctoral Thesis, University of Wisconsin, Madison, Wisconsin, 1959, pp. 107-117.
18. Ibid., pp. 117-129.
19. Pittendrigh, C. S., and Bruce, V. C., "An Oscillator Model for Biological Clocks," Rhythmic and Synthetic Processes in Growth, Princeton University Press, Princeton, N. J., 1957, pp. 75-109.
20. Roberts, S. K., Circadian Activity Rhythms in Cockroaches, Doctoral Thesis, Princeton University, Princeton, N. J., 1959, pp. 25-29.
21. Pittendrigh, C. S., et.al., "On the Significance of Transients in Daily Rhythms," Proceeding of the National Academy of Sciences, Vol. 44, No. 9, September, 1958, pp. 965-973.
22. Caldwell, S. H., Switching Circuits and Logical Design, John Wiley and Sons, Inc., New York, 1958.
23. Klotter, K., "Theoretical Analysis of Some Biological Models," Symposia on Quantitative Biology, Vol. XXV, Long Island Biological Association, Long Island, New York, 1960, pp. 189-196.

24. Wever, R., "Possibilities of Phase Control, Demonstrated by an Electronic Model," Symposia on Quantitative Biology, Vol. XXV, Long Island Biological Association, Long Island, New York, 1960, pp. 197-206.
25. Korn, G. A., and Korn, T., Electronic Analog Computers, McGraw Hill Book Co., 1957.

APPENDIX A
CIRCUIT DIAGRAMS

Circuit	Title	Page
1	Schmitt Trigger	93
2	Bistable Multivibrator	93
3	60 cps. Square Wave Generator	94
4	Monostable Multivibrator	95
5	Digital Invertor	96
6	Diode "AND" Gate	97
7	Diode "OR" Gate	97
8	Astable Multivibrator	98
9	Digital Gate Booster	99

Note:

(1) R_1 — Determines the effect of Control Input.

(2) R_2 — Adjusts the trigger level with no Control Input.

Circuit 1. Schmitt Trigger

Note:

- (1) Circuit uses positive 12 volt pulses.
- (2) A positive input at the "SET" input causes the "Q" output to become "1" (0 volts)

Circuit 2. Bistable Multivibrator

Circuit 3. 60 cps Clock Plus "AND" Gate

Note:

(1) RC determine period.

Circuit 4. Monostable Multivibrator

Circuit 5. Invertor

Circuit 6. "AND" Gate

Circuit 7. "OR" Gate

Note:

(1) Varying R changes the period.

Circuit 3. Astable Multivibrator

Circuit 9. Gate Booster