JOYNER, ROGER DALE. The Effects of Temperature and Single Frequency, Audio Sound on the Germination Rate of Seeds. (1973) Directed by: Dr. Gaylord T. Hageseth.

It was the purpose of this study to determine the effects, if any, of temperature and single frequency, audio sound on the germination rate of seeds. These data were then used in the development of a mathematical model which describes this action. With the aid of this model, the activation energy was also determined, as well as certain other parameters.

In all the experiments a single variety of turnip seed was used. All samples were randomly selected and were germinated under identical conditions, with the exceptions of sound and temperature.

All the data were collected by the same person by visual observation of the seeds during germination. A constant temperature board, with a tight fitting lid for control of humidity, was used to obtain the required environmental conditions.

The data were graphically and mathematically analyzed using various computer programs. The theoretical curves obtained from the model were fitted to the experimental data points by the least-squares method, with the chi-square test being used as the criteria for goodness of fit.

It was found that the rate of germination depends upon the ambient temperature and that there is a difference

between the noise group rates and the control (quiet) group rates at the same temperature. Further, a mathematical model was developed which fit the experimental data within acceptable limits.

THE EFFECTS OF TEMPERATURE AND SINGLE FREQUENCY AUDIO SOUND ON THE GERMINATION RATE OF SEEDS

by

Roger D. Joyner

A Thesis Submitted to
the Faculty of the Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Greensboro
1973

Approved by

G T Haggerty

Thesis Adviser

APPROVAL PAGE

This thesis has been approved by the following committee of the Faculty of the Graduate School at the University of North Carolina at Greensboro.

Thesis Adviser G T Hogerty

Oral Examination Committee Members Ralph L. Lamneson

Gerald W. Meisner

8-25-73

Date of Examination

ACKNOWLEDGMENTS

The author wishes to thank the physics department of The University of North Carolina at Greensboro for providing the space and equipment necessary for this research. The invaluable assistance of Dr. Gaylord T. Hageseth is gratefully acknowledged. The author also expresses appreciation to Mr. Joseph Nelson for his help in construction of some of the experimental equipment, to Mr. W. Keith Yokley for his aid in the reading of this thesis and to Dr. R. T. Whitlock for his assistance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES
(Continued)

LIST OF FIGURES
(Continued)

CHAPTER I

INTRODUCTION

Studies concerning the effects of various parameters such as light and sound on the germination and growth of seeds and plants have been conducted for many years. The effect of light on seeds and plants has been thoroughly documented and is understood (Borthwick, Hendricks, Toole, and Toole, 1954). In particular considerable work in this area has been done by E. Toole, V. Toole, and others at the United States Department of Agriculture (Toole, 1959), (Toole, Hendricks, Borthwick, and Toole, 1956).

However, the same is not true with respect to the effects of sound upon seeds and plants. From Darwin's attempts to stimulate mimosa plants with notes from a bassoon in the 19th century (Strong, 1966) to present experiments with sophisticated electronic equipment, the results have, for the most part, been inconclusive and contradictory. In most of these experiments the sample sizes have been too small for good statistical results, and adequate control of the parameters was not present. Some definitive experiments have been carried out, such as the work of Anderson (1972) and others at Colorado State University.

One area which has been almost totally overlooked by researchers in this field is the time rate of change of the number of seeds germinated. It is this germination rate which forms the basis of this thesis. In particular we have studied the effects of temperature and applied sound pressure level on the germination rate of turnip seeds. This was accomplished by counting the number of seeds germinated during each hour for groups of seeds at various temperatures. At each temperature one group of seeds was run in a quiet environment and one group was subjected to 100 db of 4000 Hz sound continuously.

From this data a mathematical model for seed germination was developed and is presented in Chapter II. The method of data collection and parameter control are discussed in Chapter III. The data analysis and results appear in Chapter IV, and Chapter V includes comments about further experimentation as well as the final conclusions.

CHAPTER II

THEORY

The process of seed germination, although very complex, can be thought of as just the end result of a series of chemical reactions. For the most part these reactions involve enzyme-substrate complexes in the presence of catalysts and inhibitors. The kinetics and thermodynamics of enzyme reactions have been under investigation for many years and are now understood. Thorough treatments of the subject can be found in many textbooks, such as the one by Laidler (1958). In this thesis it is to be assumed that the same mathematics used to describe autocatalytic enzyme reactions can be directly applied to the germination rates of seeds. In particular the treatment used by Kunitz (1948) will be closely followed.

According to Stevens (1970) an autocatalytic reaction is one in which one of the products catalyzes the reaction itself. If two substrates A and B combine to form the complex AB,

$$A + B \rightleftarrows AB, \qquad (1)$$

it is possible for AB to react further to form the final product F by two alternate reactions. F can be formed by a first-order enzyme reaction,

$$AB \rightleftarrows F + B, \qquad (2)$$

or by a second-order autocatalytic enzyme reaction,

$$AB + F \rightleftarrows 2F + B \qquad (3)$$

In the case of a first-order reaction, it can be stated that the initial rate of F formation is

$$\frac{d[F]}{dt} = k_1[A] \qquad (4)$$

where [F] is the concentration of the product F, [A] is the concentration of enzyme A, and k_1 is the rate constant. Whereas for the second-order autocatalytic reaction the rate is given by

$$\frac{d[F]}{dt} = k_2([A]_o - [F])([F]_o + [F]) \qquad (5)$$

where $[A]_o$ is the initial concentration of A, $[F]_o$ is the initial concentration of F, and k_2 is the reaction rate constant. Integrating equation (5) gives the S-shaped curve that is typical of this type of reaction.

In the case of seed germination $[A]_o$, $[F]_o$, [A], and [F] are enzyme concentrations within the seed and can be directly measured only by chemical methods. Therefore, it is necessary to find some external parameters which can be correlated to these concentrations.

Inside the seed F has some initial concentration, $[F]_o$, and increases until some critical concentration $[F]_c$ is reached, at which time germination, the emergence of the radical, occurs. Thus, it is possible to estimate the concentration of F for all the seeds present by counting the number of germinated seeds. If we assume that each germinated seed contributes one unit of $[F]_c$ and non-germinated seeds contribute zero, then for a total of 1,000 seeds the average concentration of F is equal to the number of germinated seeds times the critical concentration of F, divided by 1,000. If $[F]_c$ divided by 1,000 is chosen to be the unit of enzyme concentration and is set equal to one, then the average concentration of F is equal to the number of germinated seeds N, and

$$\frac{d[F]}{dt} = \frac{dN}{dt} \qquad (6)$$

so that equation (5) can be used to describe the rate of seed germination.

The physical meaning of the parameters in equation (5) now takes on new meaning. $[F]$ is equal to the number of seeds germinated and $[A]_o$ is equal to the number of seeds that will germinate under the given conditions.

If the germination rates of seeds is studied at various temperatures, then the thermodynamics of the reversible denaturation of proteins as developed by Kunitz (1948) as well as Anson and Mirsky (1934), Stern (1938), and Eyring and Stern (1939), can be applied to the problem. By application of this method it is possible, after calculating the equilibrium constant at each temperature, to determine the change in enthalpy, the Gibb's free energy, and entropy for the germination process.

In any reversible reaction there is a tendency toward the establishment of a state of equilibrium between the concentrations of the initial and final products of the reaction. This fact is expressed by an equilibrium constant which is independent of the direction in which the process is initiated (Kunitz, 1948).

Let us again consider reactions (1), (2), and (3). The equilibrium constant for reaction (1) is

$$K_1 = \frac{[AB]_o}{[A]_o\,[B]_o} \tag{7}$$

where $[AB]_o$ is the initial concentration of the complex AB. The second reaction (2) has a concentration which is given by

$$K_2 = \frac{[B]_o\,[F]_o}{[AB]_o}. \tag{8}$$

The second-order autocatalytic reaction (3) has an equilibrium constant given by

$$K_3 = \frac{[F]_o^2\,[B]_o}{[AB]_o\,[F]_o} = \frac{[F]_o\,[B]_o}{[AB]_o}. \tag{9}$$

Note that the equilibrium constants for the first-order and second-order reactions are equal, so that

$$K_3 = K_2.$$

If (1) and (2) or (1) and (3) are consecutive, we may define a new equilibrium constant for use in describing both reactions:

$$K_{12} = K_1K_2 = \frac{[F]_o}{[A]_o}. \tag{10}$$

The thermodynamic parameters Gibb's free energy (G), enthalpy (H), and entropy (S), are related according to the equation

$$\Delta G = \Delta H - T\Delta S \tag{11}$$

where T is the absolute temperature. The symbol ΔG denotes the change in free energy of the system when one mole of enzyme A is converted into one mole of enzyme F. Likewise, ΔH is the change in enthalpy per mole and ΔS is the change in entropy per mole. Partial differentiation of equation (8) with respect to T gives

$$\frac{d(\Delta G)}{dT} = -\Delta S. \tag{12}$$

The change in free energy can be calculated by using

$$\Delta G = RT(\ln K_{12}) \tag{13}$$

or

$$K_{12} = e^{-\Delta G/RT} \tag{14}$$

where R is the universal gas constant and is equal to 1.98 calories. By substituting the expression for ΔG in equation (13) into equation (11), we arrive at the expression

$$-\ln K = \frac{\Delta H}{R}\frac{1}{T} - \frac{\Delta S}{R}. \tag{15}$$

Now by differentiating with respect to (1/T), we have

$$\frac{-d(\ln K)}{d(1/T)} = \frac{\Delta H}{R} \tag{16}$$

which is known as van't Hoff's equation. If lnK is plotted as a function of (1/T) the slope at any point is equal to $\Delta H/R$. Over small temperature ranges the change in enthalpy is nearly constant, so that

$$\ln \frac{K(T_2)}{K(T_1)} = \frac{-\Delta H}{R}\left[\frac{1}{T_1} - \frac{1}{T_2}\right]. \tag{17}$$

If the slope is negative, the change in H is positive and the reaction is endothermic. On the other hand, if it is positive, the change in H is negative and the reaction is exothermic.

If ΔG is plotted as a function of T, the change in entropy can be determined. Equation (11) should be a straight line, with its slope equal to the change in entropy and the intercept should be equal to the change in enthalpy. The change in enthalpy from equation (11) should be equal to that calculated from equation (17).

CHAPTER III

EXPERIMENTAL CONSIDERATIONS

In these experiments it was necessary to control four parameters which could affect the results. These parameters were temperature, sound field, humidity, and light, with the first two being the experimental variables. It was decided that the temperature and humidity could best be controlled through the use of a "growth box," inside of which the seeds would be germinated. Control of the sound field depended on two basic elements: (1) reduction of "background noise" and (2) control of the applied sound pressure level and frequency. Control of the light parameter was met by insuring that all seeds were subjected to equal light intensities of the same duration.

The "growth box" consisted of a box with dimensions of approximately 43.5 cm x 38.5 cm x 3.5 cm. All parts of the box were made from 1/4 inch Plexiglass except for the top, which was a 1/16 inch thick sheet of aluminum. Inside of this box was a grid of 3/8 inch copper tubing, through which the water used in controlling the temperature was circulated. Attached to the top of this box was another smaller (34 cm x 38 cm x 2 cm) box which formed the actual growth chamber. The chamber had a tight-fitting, removable lid constructed from 1 inch x 1 inch angle aluminum with one sheet of clear acetate plastic glued to the inside and one glued to the outside, forming a double layer window. The entire assembly was mounted on a plywood support and was then insulated on all sides except the top with one inch of rigid foam. During the experiments the bottom box was filled with water.

The other apparatus used in temperature control were a Haake model E 12 0-100°C heater-pumping unit, a Haake precision 0-100°C thermometer, a Polyscience Corporation KR30 refrigeration unit, a Keithly Instruments model 600A electrometer, and a copper-constantan thermocouple. The heater-pumping unit was mounted on top of an insulated water reservoir. The heater-pumping unit was capable of maintaining the temperature of the water in the reservoir to within 0.1°C of the desired temperature. The temperature of the water in the reservoir was monitored with the Haake thermometer, the least count of which was 0.25°C.

The water was pumped from the reservoir through the copper grid in the lower section of the growth chamber, through the refrigeration unit, then back to the reservoir (see figure 1). At temperatures above 24°C the refrigeration unit was bypassed. The copper grid acted as a heat exchanger, bringing the water in the lower section of the growth chamber to the same temperature as the reservoir. The water in the lower section heated or cooled the upper section so that the entire unit reached equilibrium with the reservoir. By this

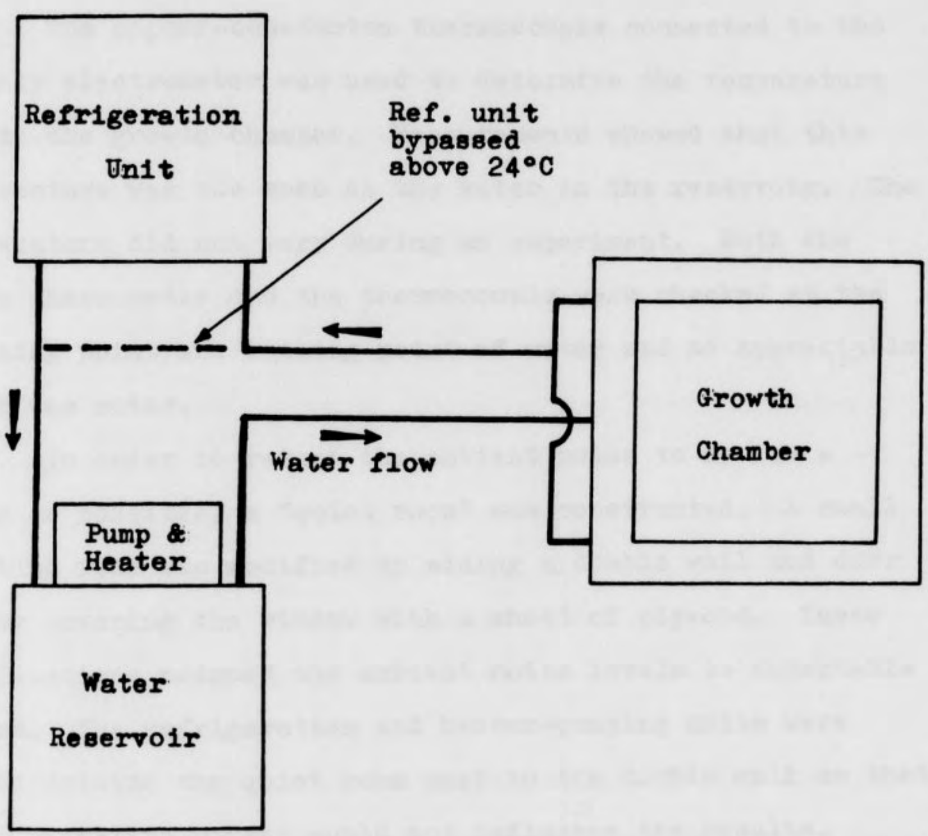

Figure 1: Block diagram of temperature control units.

method it was possible to control the temperature inside the growth chamber to within 0.1°.

The copper-constantan thermocouple connected to the Keithly electrometer was used to determine the temperature inside the growth chamber. Measurements showed that this temperature was the same as the water in the reservoir. The temperature did not vary during an experiment. Both the Haake thermometer and the thermocouple were checked at the freezing point and boiling point of water and no appreciable error was noted.

In order to reduce the ambient noise to as low a value as possible, a "quiet room" was constructed. A small existing room was modified by adding a double wall and door and by covering the window with a sheet of plywood. These modifications reduced the ambient noise levels to acceptable values. The refrigeration and heater-pumping units were placed outside the quiet room next to the double wall so that their operating noises would not influence the results.

Based upon previous work by G. Hageseth and others at The University of North Carolina at Greensboro, all the noise experiments were run at a sound pressure level of 100 db and a frequency of 4000 Hz. This applied sound was generated by a Hewlett-Packard 200AB audio oscillator. The signal was amplified by a Bogen MX60A 60-watt amplifier before being fed to a University Sound loudspeaker with two ID60 driver units. In some of the initial experiments a McIntosh 75-watt amplifier was used. The loudspeaker was placed at a distance of 43 cm above the growth chamber.

A Hewlett-Packard model 8062A Impulse Sound Level Meter with a calibrated Hewlett-Packard 15119A Condensor Microphone Assembly and a Hewlett-Packard model 8055A Filter Set (31.5 Hz - 1600 Hz) were used to check the sound pressure level during the experiments. The level was set at about 106 db at the top of the growth chamber lid, for it was found that a 6 db loss occurred due to the acetate window. Because of the small size of the room some standing waves were present, producing nodes and antinodes in the area of the growth chamber. These nodes and antinodes caused variations in the applied field such that the actual field varied with position from 102 db to 110 db at the top of the chamber lid. Therefore the field within the chamber was 100 ± 4 db.

The seeds were in total darkness except when readings were being made. During readings illumination was provided by one 40-watt incandescent bulb and one high intensity 15-watt lamp both 33 cm from the seeds. These lamps were on for approximately 5 minutes each hour in all experiments. Therefore the light received by the seeds in all experiments was the same and this constant parameter should have no effect upon the results.

All experiments at a given temperature were run in pairs. The control (quiet) group was run first and the experimental (noise) group second. The temperature controlling units were turned on several hours before the start of the experiment so that the various parts would have sufficient time to reach thermal equilibrium. Two layers of Schleicher-Schuell grade 604 filter paper were placed on the bottom of the chamber and approximately 125 ml of distilled water was added at the start of each experiment. Additional distilled water was added as was necessary in order to keep the seeds sufficiently wet. In all the experiments the humidity was 100%. At the higher temperatures considerable condensation could be seen on the inside of the lid, indicating very high humidity.

At the start of each experiment the seeds were placed in the growth chamber simultaneously. They were then put in rows of 50 seeds each. In noise experiments the sound was turned on before the wetting of the seeds and was not turned off until after the last reading. The seeds used were Turnip Seven from FCX lot number 1-14901.

After a certain period of time, depending on the expected dead-time (the time from wetting until germination), the seeds were visually examined and those which had germinated were removed and their number recorded. Germination was considered to have occurred whenever the tip of the root could be seen. The seeds were examined every hour after the initial reading and all seeds germinated were removed and the number recorded. This was continued for approximately 25 to 31 hours after wetting.

The first experimental run was at 40°C and the temperature was decreased by about 4°C for each subsequent experiment. One minor experimental problem noted was that it was not always easy to obtain the exact temperature equilibrium desired, and it was necessary to use some other value. This was particularly true at 21-21.5°C, 27-28°C, and 39-40°C.

The only major experimental problem involved the ambient noise level. After completing the 18°C experiment the results indicated that the refrigeration unit might be affecting the seeds. A check was made and it was found that the refrigeration unit raised the background noise level from 63 db linear to 73 db linear. To determine whether or not this increase in background noise could affect the seeds, the 24°C run was repeated without the refrigeration unit or heater-pumping unit. Although the actual temperature was room temperature (23°C), the two should have produced very similar results. However, when the results of the repeated experiment were compared to those of the original one, their difference indicated that the additional noise was affecting the experiments. Therefore the refrigeration unit and heater-pumping unit were moved to an adjacent room. In this location the background noise level was 63 db with the refrigeration unit in operation. The experiments at 21°C and 18°C were then repeated.

After the 14°C experiment, runs were made at 37.5°C and 33°C in order to provide additional data points. These additional data points were chosen in order to provide further useful information in areas not adequately covered by the previous data. Also, the noise experiment at 40°C was repeated. This was done to see if germination might actually occur at a time later than the 20.5 hour cut-off time of the first 40°C experiment. This decision was in part prompted by the fact that in the 14°C experiments germination did not start until about 23 hours after wetting.

CHAPTER IV

DATA ANALYSIS AND RESULTS

Each set of data collected (see Appendix I) was analyzed by the use of a computer program which performed a least-square and minimum chi-square fit to the germination rate data. The fitting involved varying the parameters k_2, $[A]_0$, and $[F]_0$ until the best fit was obtained. These parameters, the theoretical values, the chi-square for each point, and the total chi-square for the curve were printed out. A listing of the values appears in tables 1 and 2. The calculated values of k_2, $[A]_0$, and $[F]_0$ were then used in the same computer program to obtain the theoretical values for the time-integrated germination rate curves. No fitting was performed on the integrated curves. The two theoretical curves and the corresponding data points for each experiment were plotted and can be found in Appendices II and III.

Appendix II contains the germination rate curves. These curves are plots of the number of seeds germinated during a given hour versus the mid-time of that hour. In each case the solid line represents the theoretical curve, open circles represent quiet data points and closed circles noise data points. This same format holds for all curves in this thesis. The short vertical bar through each point represents the uncertainty of that point. The uncertainty was determined by taking the square root of each data point.

Table 1: Tabulated Experimental Values

QUIET DATA

Temp. (°C)	k_2^a	$[A]_o$	$[F]_o^b$	χ^2	No. Pnts.
40.0	28.50	155	0.6	5.6	6
37.5	5.14	844	16.7	6.7	11
35.0	3.19	719	110.6	8.0	11
33.0	1.31	1015	902.5	8.3	11
31.0	1.98	855	372.0	4.0	11
27.0	4.41	827	67.3	8.5	11
24.0[c]	5.98	803	6.0	10.0	11
23.0	8.28	541	9.2	6.5	7
21.5[c]	7.18	700	0.5	4.6	12
21.0	6.47	792	6.9	5.7	9
18.0[c]	12.96	429	2.2	1.5	7
18.0	10.73	515	1.9	4.8	8
14.0	10.00	414	5.6	6.1	8

a. $\times 10^{-4}$
b. $\times 10^{-3}$
c. broadband noise present

Table 2: Tabulated Experimental Values

NOISE DATA

Temp. (°C)	k_2^a	$[A]_o$	$[F]_o^b$	χ^2	No. Pnts.
39.0	10.50	207	18.2	4.1	12
37.5	2.64	976	150.5	6.8	11
35.0	2.55	713	106.0	6.2	11
33.0	2.29	969	404.1	6.6	11
31.0	3.62	759	144.3	6.5	11
28.0	3.26	910	150.0	15.7	11
24.0[c]	5.27	771	25.3	7.5	9
23.0	6.89	778	5.8	8.9	11
21.5[c]	8.03	677	4.5	6.0	10
21.0	6.59	797	3.3	6.4	10
18.0[c]	7.24	633	3.2	6.0	8
18.0	5.50	779	12.9	5.4	11
14.0	9.90	488	1.2	5.2	10

a. $\times 10^{-4}$
b. $\times 10^{-3}$
c. broadband noise present

The graphs of the germination rate curves and the corresponding total chi-squares indicate a good fit to the data points by the theoretical curve. This suggests that the model is correct. It should be pointed out that in most cases certain data points were not included in the chi-square calculations. This was done because these points were thought to be incorrect. The number of data points actually used appears with the chi-square values.

Appendix III contains the integrated rate curves. These curves are plots of the total number of seeds germinated at a given hour versus that hour. Because no curve fitting was performed with these data and because the necessary integration constants were not included, the fits of the theoretical curves to the data points are generally not as good as for the differential rate curves. However, if the integration constant is added to each theoretical value (see for example the 33°C curve) the fit to the data is quite close. The fact that the solid theoretical lines nearly coincide with the data points of these curves also suggests that the theoretical equation and thus the theoretical model are correct.

By examining the germination rate and integrated rate curves, one can see the effects of noise on the germination rate. Ignoring those curves at 24°C, 21.5°C, and 18°C, in which the broadband noise was present, one finds that noise is beneficial below about 33°C and yet detrimental above this point.

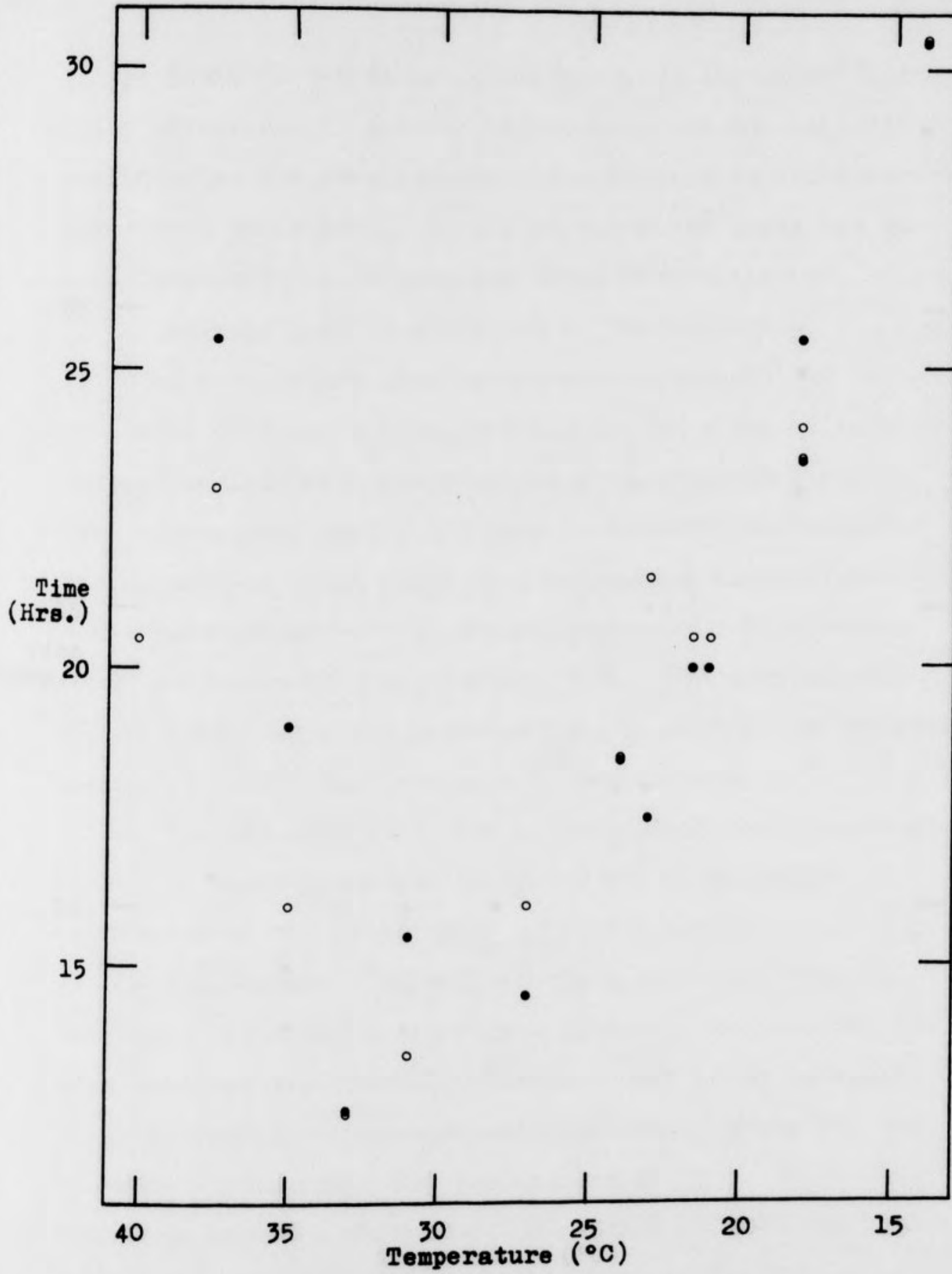

Figure 2: Graph of peak-time versus temperature

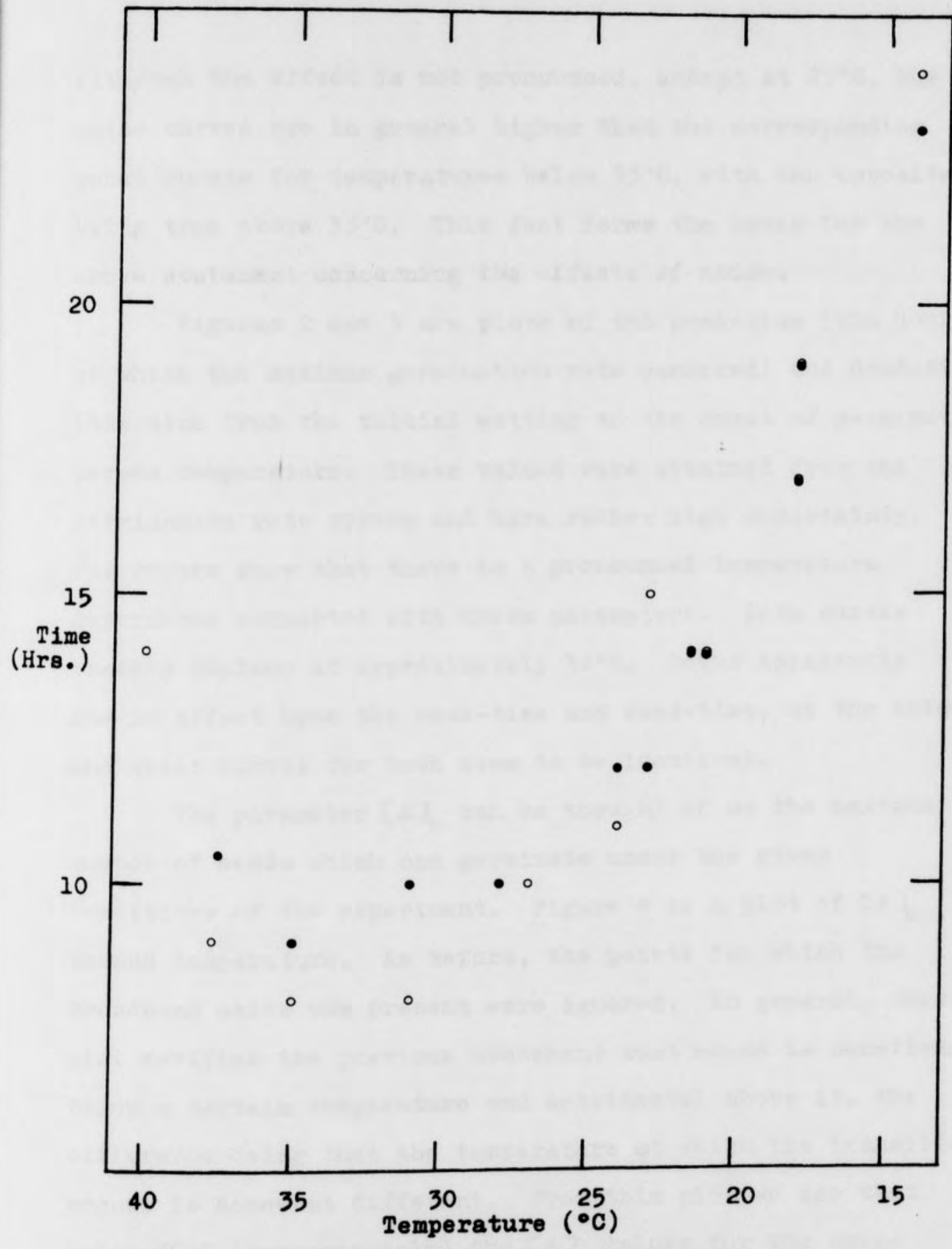

Figure 3: Graph of dead-time versus temperature

Although the effect is not pronounced, except at 23°C, the noise curves are in general higher than the corresponding quiet curves for temperatures below 33°C, with the opposite being true above 33°C. This fact forms the basis for the above statement concerning the effects of noise.

Figures 2 and 3 are plots of the peak-time (the hour at which the maximum germination rate occurred) and dead-time (the time from the initial wetting to the onset of germination) versus temperature. These values were obtained from the germination rate curves and have rather high uncertainty. The curves show that there is a pronounced temperature dependence connected with these parameters. Both curves reach a minimum at approximately 34°C. Noise apparently has no effect upon the peak-time and dead-time, as the noise and quiet curves for both seem to be identical.

The parameter $[A]_0$ can be thought of as the maximum number of seeds which can germinate under the given conditions of the experiment. Figure 4 is a plot of $[A]_0$ versus temperature. As before, the points for which the broadband noise was present were ignored. In general, this plot verifies the previous statement that sound is beneficial below a certain temperature and detrimental above it, the difference being that the temperature at which the transition occurs is somewhat different. From this plot we see that below 30°C (approximately) the $[A]_0$ values for the noise points are higher than for the corresponding quiet points.

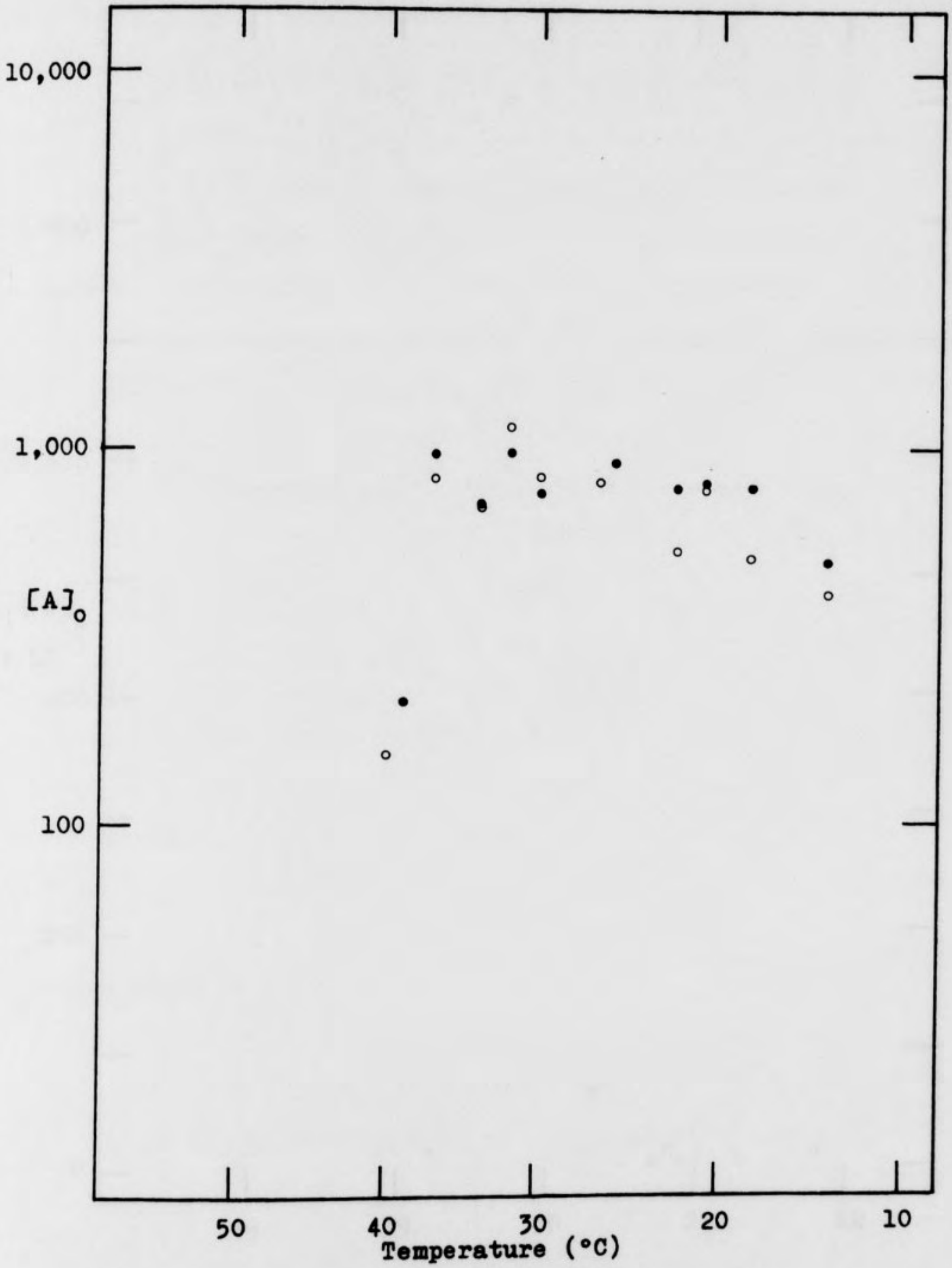

Figure 4: Graph of $[A]_o$ versus temperature

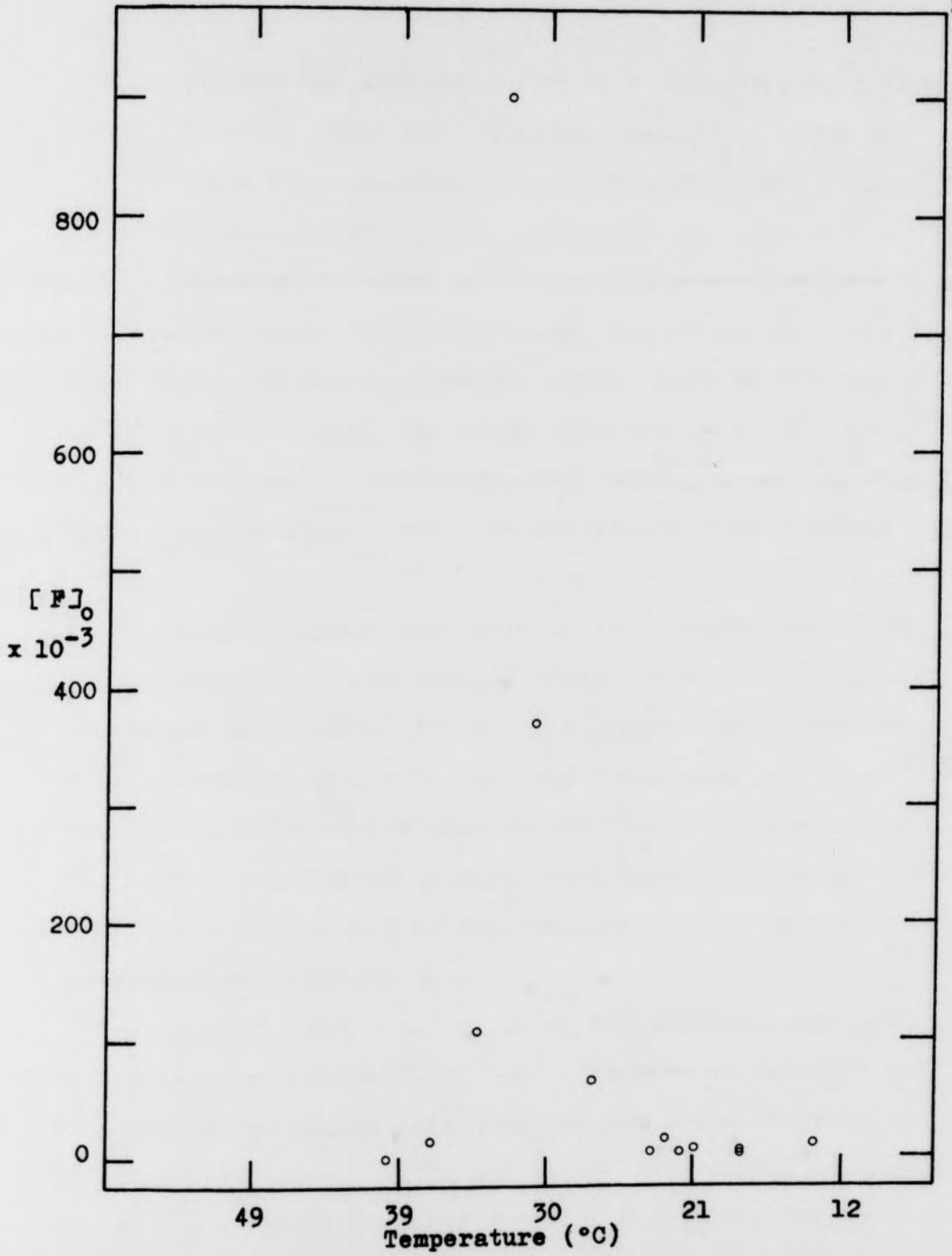

Figure 5: Graph of [F]$_o$ versus temperature for quiet data

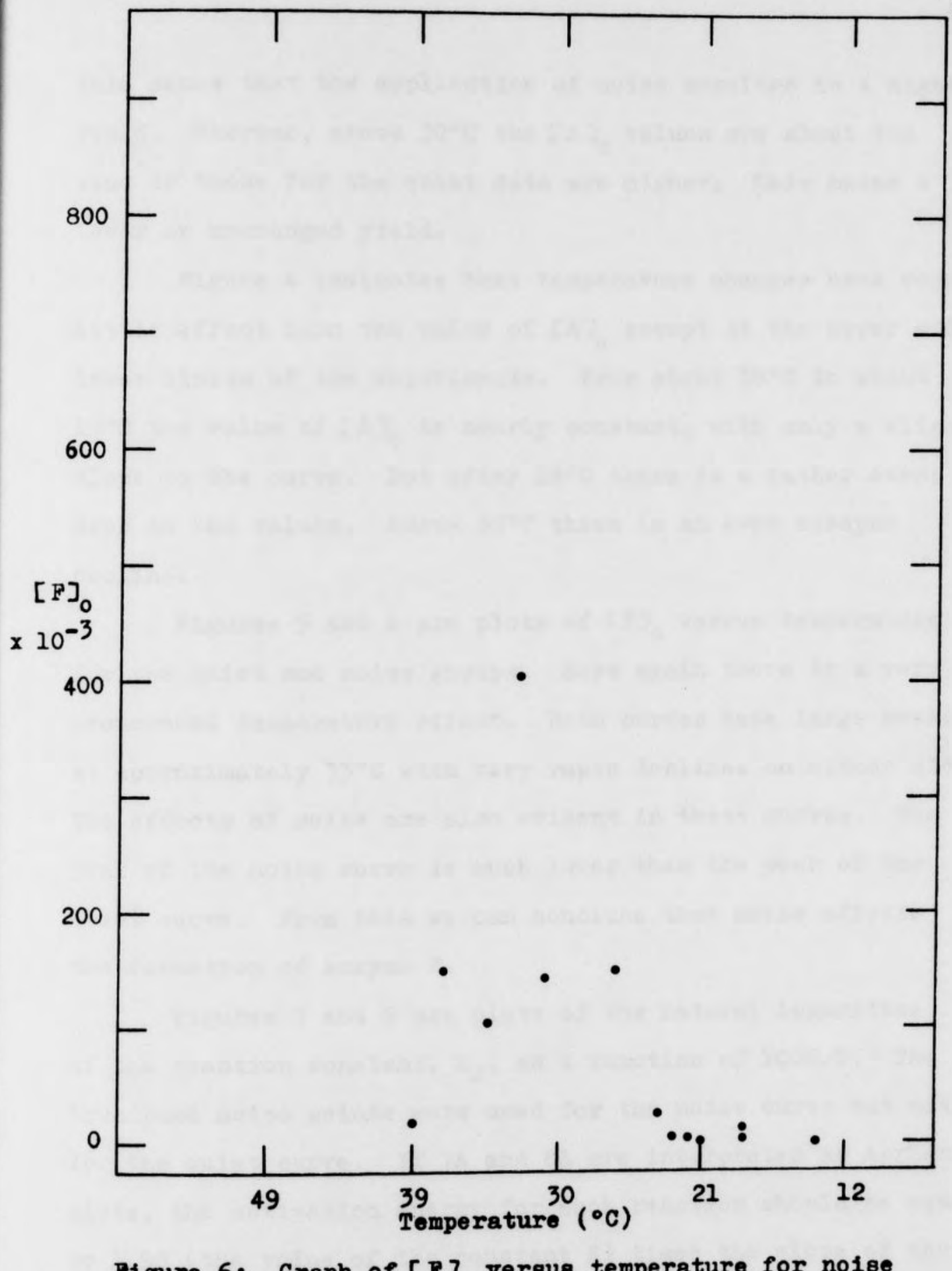

Figure 6: Graph of $[F]_o$ versus temperature for noise data

This means that the application of noise resulted in a higher yield. Whereas, above 30°C the $[A]_0$ values are about the same or those for the quiet data are higher. This means a lower or unchanged yield.

Figure 4 indicates that temperature changes have very little effect upon the value of $[A]_0$ except at the upper and lower limits of the experiments. From about 38°C to about 18°C the value of $[A]_0$ is nearly constant, with only a slight slope to the curve. But after 18°C there is a rather steep drop in the values. Above 38°C there is an even steeper decline.

Figures 5 and 6 are plots of $[F]_0$ versus temperature for the quiet and noise groups. Here again there is a very pronounced temperature effect. Both curves have large peaks at approximately 33°C with very rapid declines on either side. The effects of noise are also evident in these curves. The peak of the noise curve is much lower than the peak of the quiet curve. From this we can conclude that noise affects the formation of enzyme F.

Figures 7 and 8 are plots of the natural logarithms of the reaction constant, k_2, as a function of 1000/T. The broadband noise points were used for the noise curve but not for the quiet curve. If 7A and 8A are interpreted as Arrhenius plots, the activation energy for each reaction should be equal to 1.98 (the value of the constant R) times the slope of the corresponding curve. For the quiet data the activation

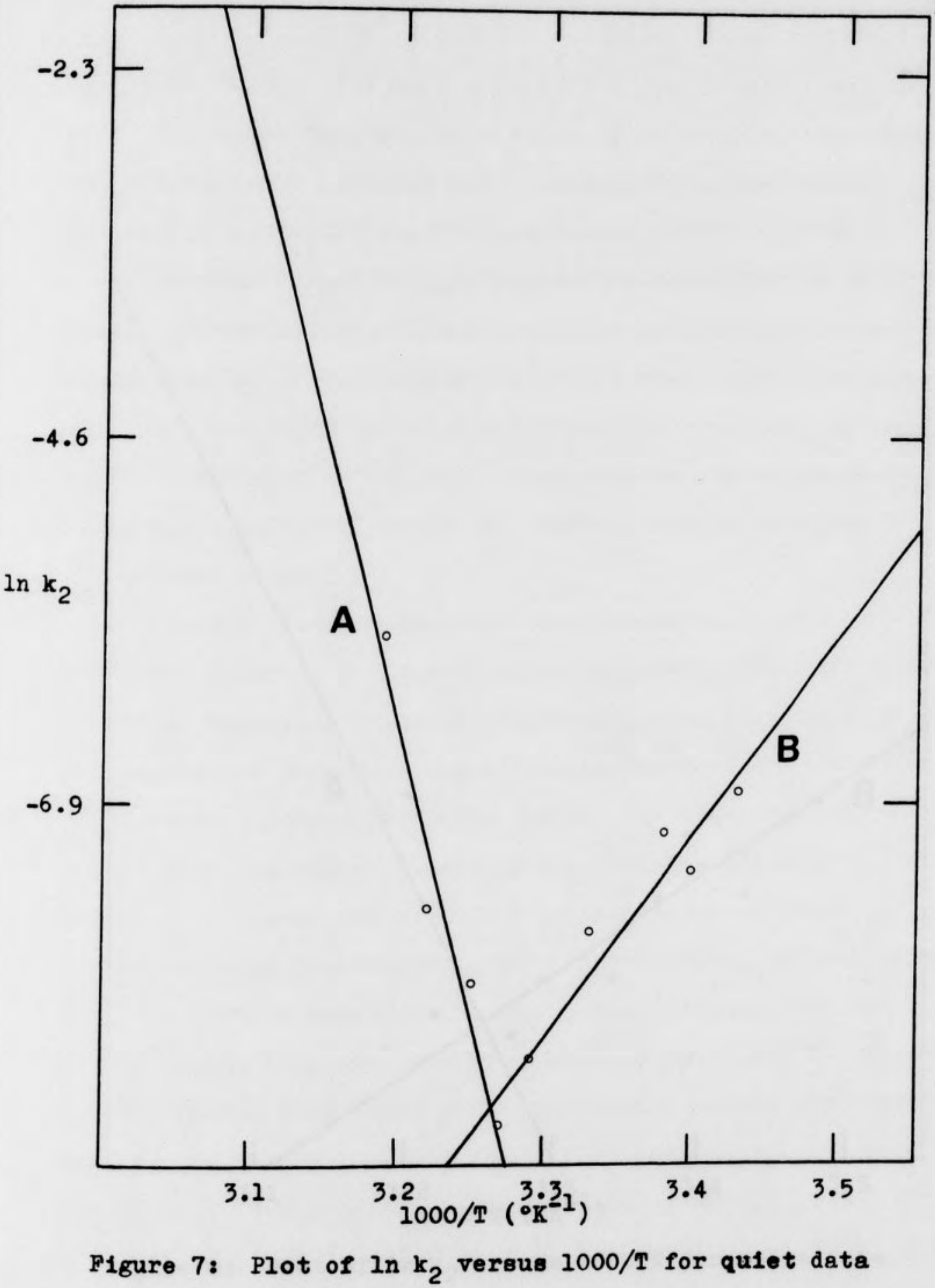

Figure 7: Plot of ln k_2 versus 1000/T for quiet data

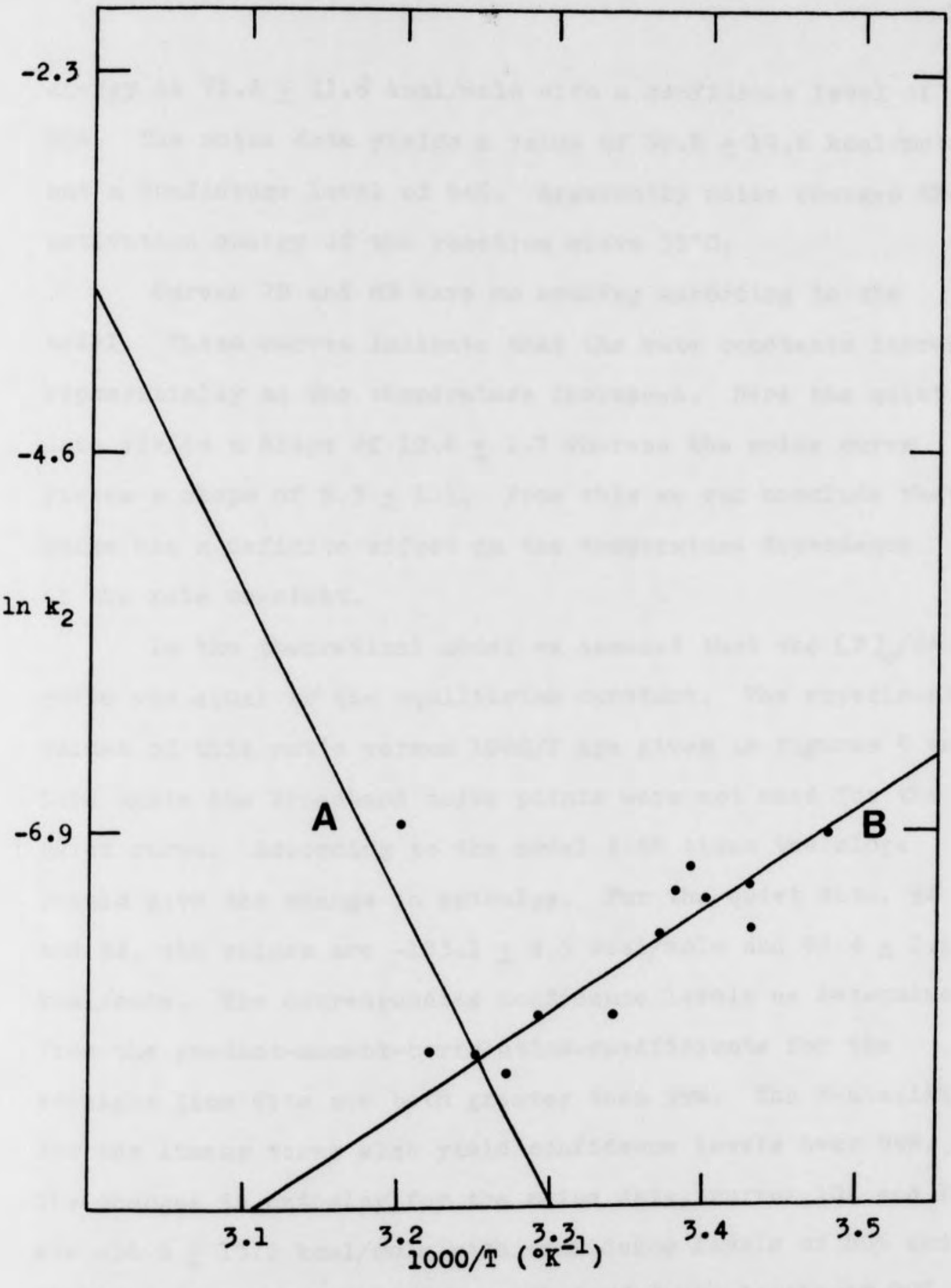

Figure 8: Plot of ln k_2 versus 1000/T for noise data

energy is 71.4 $\pm$ 11.6 kcal/mole with a confidence level of 95%. The noise data yields a value of 36.6 $\pm$ 19.6 kcal/mole and a confidence level of 64%. Apparently noise changes the activation energy of the reaction above 33°C.

Curves 7B and 8B have no meaning according to the model. These curves indicate that the rate constants increase exponentially as the temperature decreases. Here the quiet data yields a slope of 12.6 $\pm$ 1.7 whereas the noise curve yields a slope of 6.3 $\pm$ 1.1. From this we can conclude that noise has a definite effect on the temperature dependence of the rate constant.

In the theoretical model we assumed that the $[F]_o/[A]_o$ ratio was equal to the equilibrium constant. The experimental values of this ratio versus 1000/T are given in figures 9 and 10. Here again the broadband noise points were not used for the quiet curve. According to the model 1.98 times the slope should give the change in enthalpy. For the quiet data, 9A and 9B, the values are -133.1 $\pm$ 9.5 kcal/mole and 68.4 $\pm$ 2.1 kcal/mole. The corresponding confidence levels as determined from the product-moment-correlation-coefficients for the straight line fits are both greater than 99%. The F-statistics for the linear terms also yield confidence levels over 99%. The changes in enthalpy for the noise data, curves 10A and 10B, are -36.8 $\pm$ 13.2 kcal/mole with confidence levels of 80% and 88%, and 50.7 $\pm$ 8.5 kcal/mole with confidence levels of 82% and 99+%.

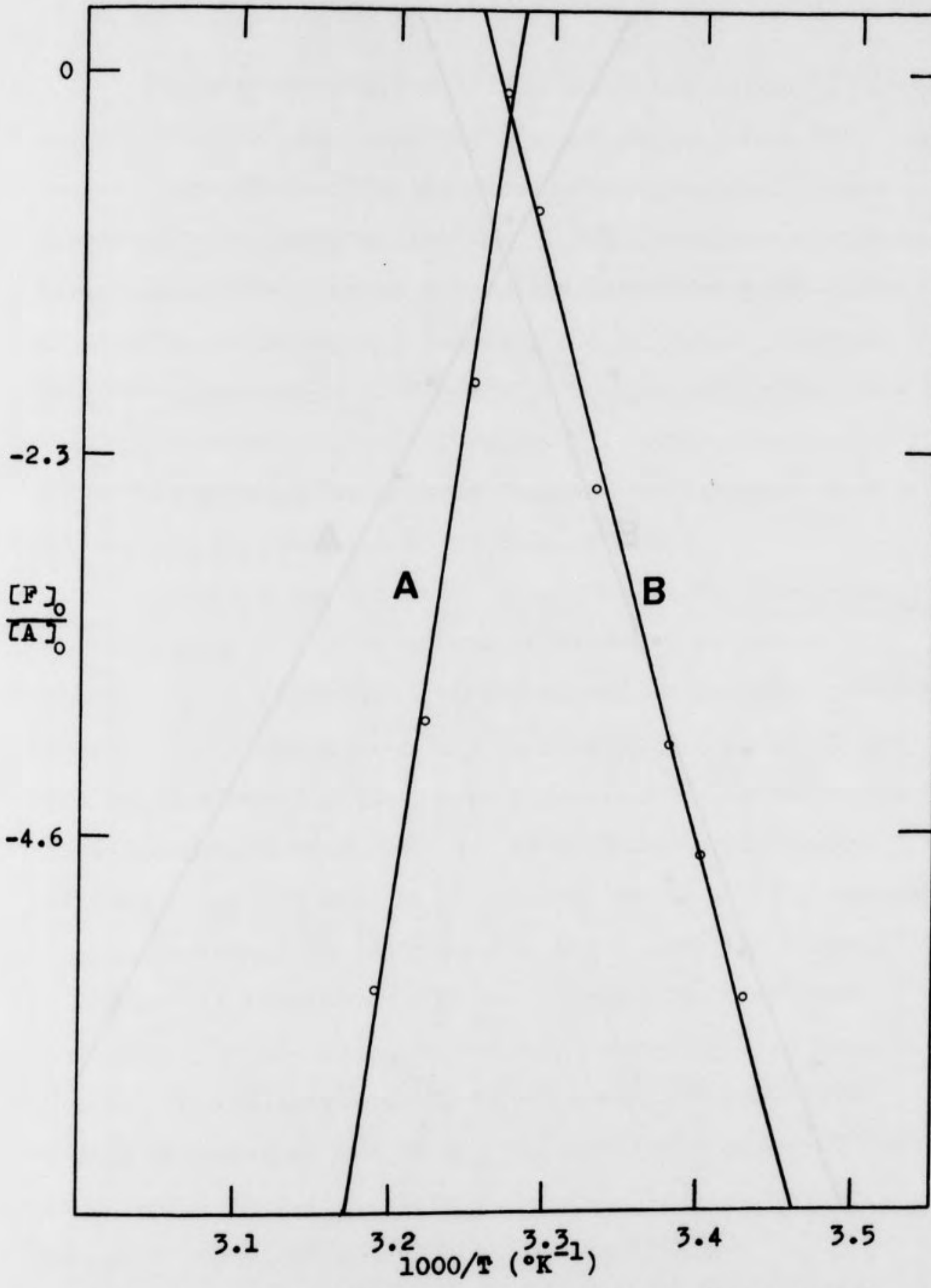

Figure 9: Plot of $[F]_o/[A]_o$ versus 1000/T for quiet data

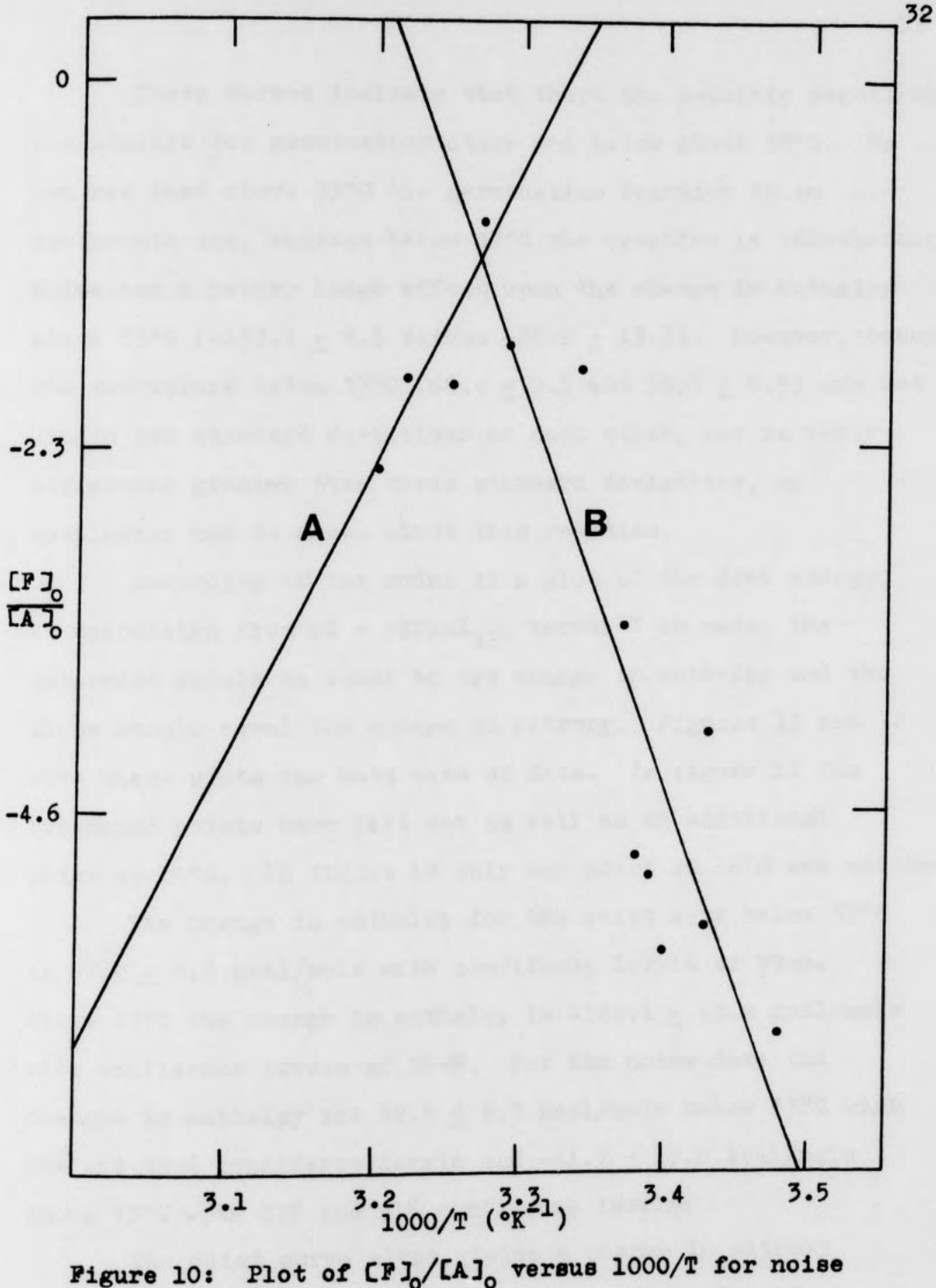

Figure 10: Plot of $[F]_o/[A]_o$ versus 1000/T for noise data

These curves indicate that there are separate reactions responsible for germination above and below about 33°C. We can see that above 33°C the germination reaction is an exothermic one, whereas below 33°C the reaction is endothermic. Noise has a rather large effect upon the change in enthalpy above 33°C (-133.1 $\pm$ 9.5 versus -36.8 $\pm$ 13.2). However, because the two values below 33°C (68.4 $\pm$ 2.1 and 50.7 $\pm$ 8.5) are not within two standard deviations of each other, nor is their difference greater than three standard deviations, no conclusion can be drawn about this reaction.

According to the model if a plot of the free energy, as calculated from $\Delta G = -RT\ln K_{12}$, versus T is made, the intercept should be equal to the change in enthalpy and the slope should equal the change in entropy. Figures 11 and 12 give these plots for both sets of data. In figure 11 the broadband points were left out as well as an additional point at 14°C. In figure 12 only one point at 18°C was omitted.

The change in enthalpy for the quiet data below 33°C is 67.2 $\pm$ 2.5 kcal/mole with confidence levels of 99+%. Above 33°C the change in enthalpy is -149.1 $\pm$ 13.9 kcal/mole with confidence levels of 99+%. For the noise data the changes in enthalpy are 52.9 $\pm$ 6.7 kcal/mole below 33°C with 89% and 99+% confidence levels and -41.7 $\pm$ 13.8 kcal/mole above 33°C with 83% and 91% confidence levels.

The quiet curve slope yields a change in entropy of .219 $\pm$ .008 kcal/mole-°K below 33°C and a value of

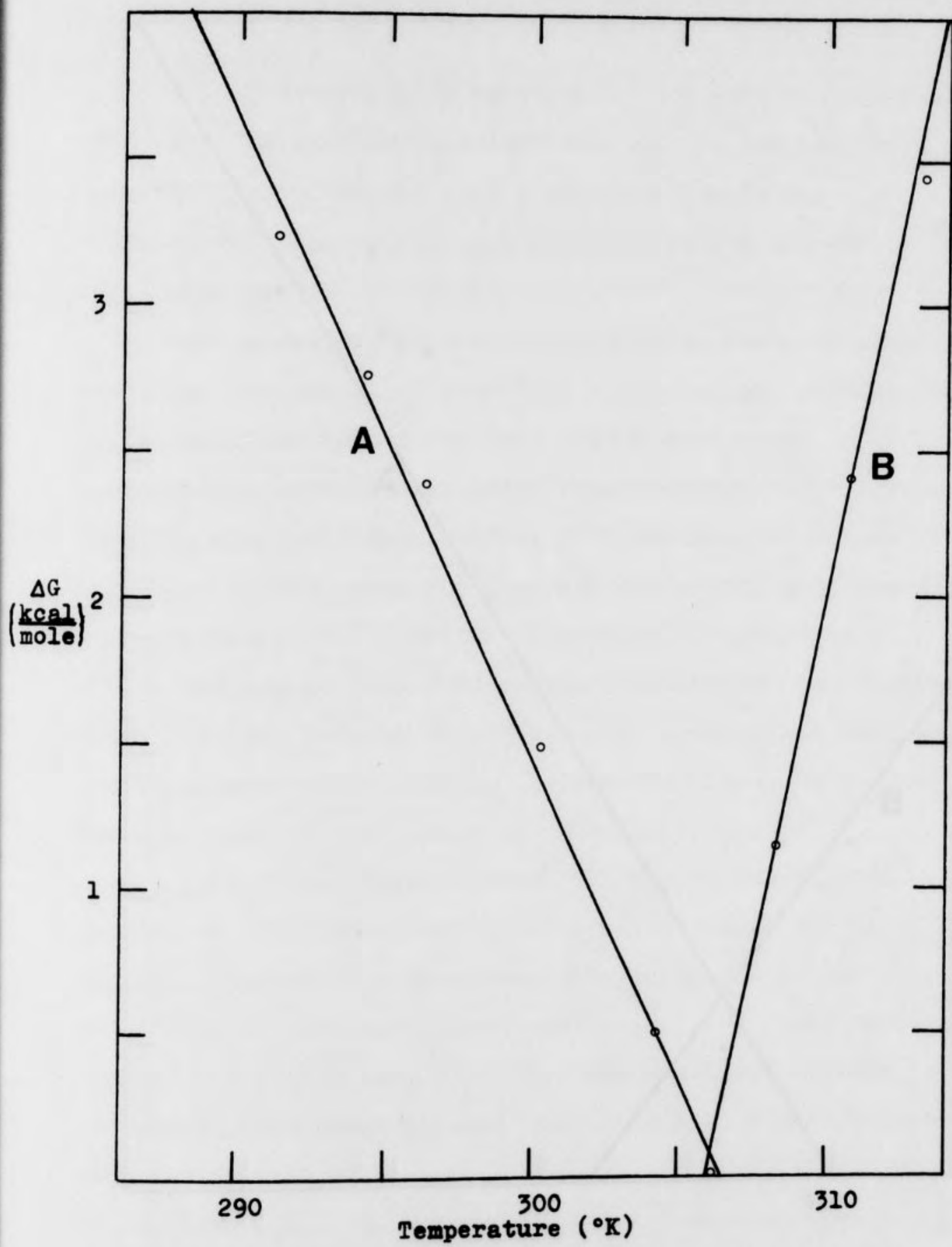

Figure 11: Plot of ΔG versus temperature for quiet data

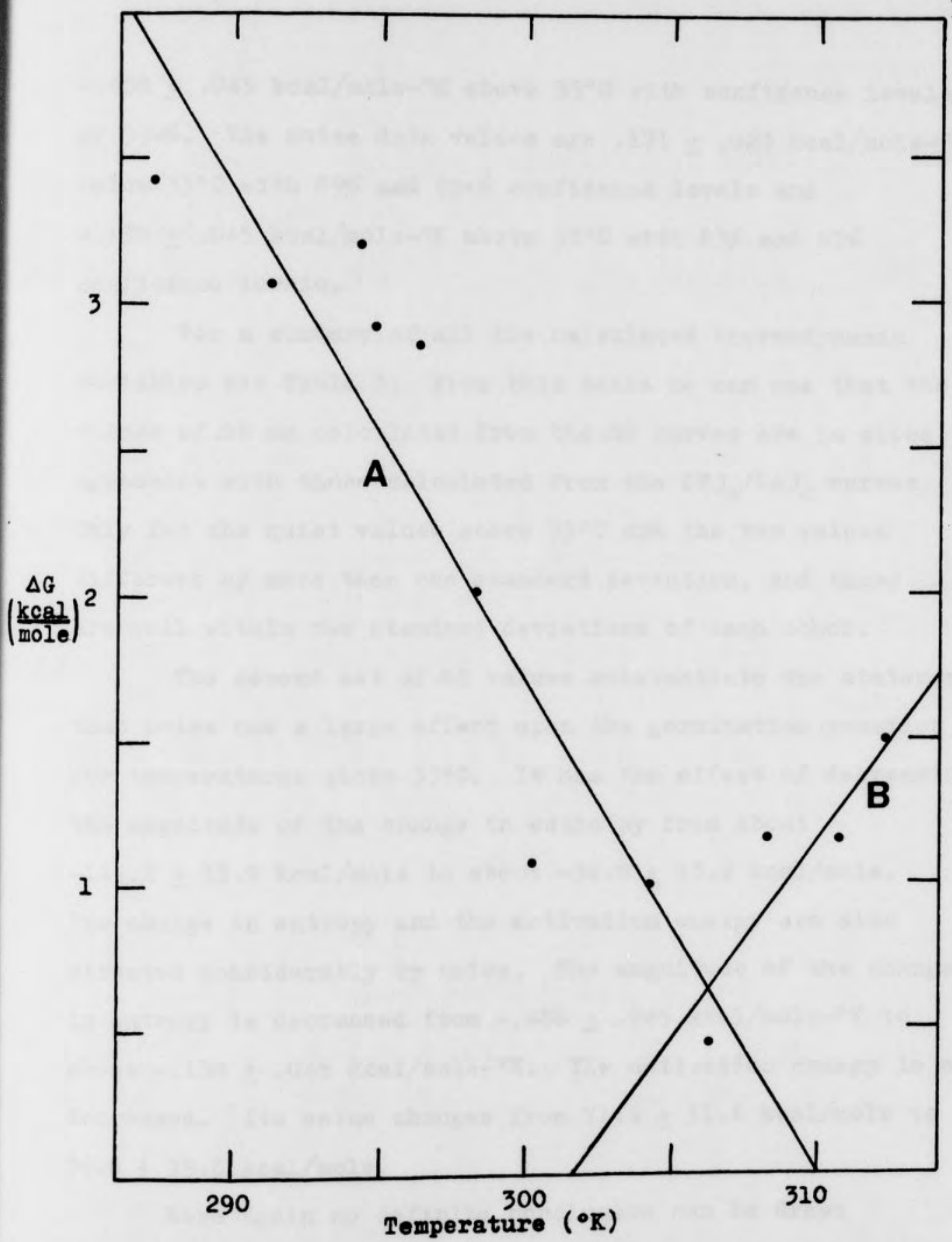

Figure 12: Plot of ΔG versus temperature for noise data

$-.488 \pm .045$ kcal/mole-°K above 33°C with confidence levels of 99+%. The noise data values are $.171 \pm .023$ kcal/mole-°K below 33°C with 89% and 99+% confidence levels and $-.138 \pm .045$ kcal/mole-°K above 33°C with 83% and 91% confidence levels.

For a summary of all the calculated thermodynamic variables see Table 3. From this table we can see that the values of ΔH as calculated from the ΔG curves are in close agreement with those calculated from the $[F]_0/[A]_0$ curves. Only for the quiet values above 33°C are the two values different by more than one standard deviation, and these are well within two standard deviations of each other.

The second set of ΔH values substantiate the statement that noise has a large effect upon the germination reaction for temperatures above 33°C. It has the effect of decreasing the magnitude of the change in enthalpy from about -149.1 ± 13.9 kcal/mole to about -36.8 ± 13.2 kcal/mole. The change in entropy and the activation energy are also affected considerably by noise. The magnitude of the change in entropy is decreased from $-.488 \pm .045$ kcal/mole-°K to about $-.138 \pm .045$ kcal/mole-°K. The activation energy is also decreased. Its value changes from 71.4 ± 11.6 kcal/mole to 36.6 ± 19.6 kcal/mole.

Here again no definite conclusion can be drawn concerning the effects of noise on the thermodynamic variables at temperatures below 33°C. The two quiet values (68.4 ± 2.1

Table 3: Calculated Thermodynamic Variables

Source Curve	ΔH	ΔS	ΔE
	Above 33°C - Quiet Data		
F/A	-133.1 ± 9.5		
ΔG	-149.1 ± 13.9	-.488 ± .045	
k_2			71.4 ± 11.6
	Above 33°C - Noise Data		
F/A	-36.8 ± 13.2		
ΔG	-41.7 ± 13.8	-.138 ± .045	
k_2			36.6 ± 19.6
	Below 33°C - Quiet Data		
F/A	68.4 ± 2.1		
ΔG	67.2 ± 2.5	.219 ± .008	
k_2			
	Below 33°C - Noise Data		
F/A	50.7 ± 8.5		
ΔG	52.9 ± 6.7	.171 ± .023	
k_2			

kcal/mole and 67.2 ± 2.5 kcal/mole) are quite comparable, being only about one-half of a standard deviation apart. The two noise values (50.7 ± 8.5 kcal/mole and 52.9 ± 6.7 kcal/mole) compare even more favorably, being only about one-third of a standard deviation apart. But, when the quiet values are compared to the noise values for either curve, they are slightly more than two standard deviations apart. Therefore, one cannot say that they are definitely different or the same. This holds for the change in entropy values which are also slightly more than two standard deviations apart. It can be said that if noise does affect these parameters, the effect is not nearly so great as it is at temperatures above 33°C.

A survey of the literature was made in an attempt to determine if our changes in enthalpy and entropy were of the right order of magnitude for reactions of this type. According to Anson and Mirsky (1934) the change in enthalpy for the denaturation of trypsin is 67.6 kcal/mole with the corresponding change in entropy being given as .213 kcal/mole-°K. Eisenberg and Schwert (1951) report that the change in enthalpy and entropy for the denaturation of chymotrypsinogen is 143 kcal/mole and .432 kcal/mole-°K. These values are of the same order of magnitude as the ones we have calculated and in some cases compare quite favorably.

CHAPTER V

CONCLUSIONS AND COMMENTS

The germination rate of turnip seeds can be described by the same mathematics used in the study of autocatalytic enzyme reactions. This treatment gives values for the activation energy and the changes in enthalpy, entropy, and free energy. Further, two of the mathematical parameters determined can be interpreted in terms of initial enzyme concentrations within the seed.

In the process of experimentation it was found that there are apparently two reactions with different thermodynamic parameters responsible for the germination of turnip seeds. The ambient temperature affects these reactions such that one predominates above 33°C and the other below 33°C. This temperature effect is also evidenced by changes in the yield, germination rate, peak-time and dead-time of the seeds.

The application of 4000 Hz, 100 db sound also affects the germination rate of turnip seeds. This effect is rather complex and seems to be smaller than the effects of temperature. The application of this sound causes large changes in the thermodynamic variables at temperatures above 33°C but apparently has little effect upon them below 33°C. Sound tends to increase the yield below 30°C and tends to decrease the yield above this temperature.

This field merits considerably more research. Similar work should be done with other seeds to determine whether or not their germination rates fit the model developed here. This work should also be extended to see if there is a threshold as far as the duration or sound pressure level of the applied sound is concerned. It is possible that the further application of this research could result in a more comprehensive understanding of the process of seed germination.

BIBLIOGRAPHY

Anderson, D. M., (1972) "Audio Sound Effects on Seedling Growth" Master Thesis, Colorado State University.

Anson, M. L. and Mirsky, A. E., (1934) J. Gen. Physiol. 17, 393.

Borthwick, H. A., Hendricks, S. B., Toole, E. H., and Toole, V. K., (1954) Botan. Gaz. 115, 205.

Eisenberg, M. A. and Schwert, G. W., (1951) J. Gen. Physiol. 34, 483.

Eyring, H. and Stern, A. E., (1939) Chem. Rev. 24, 253.

Kunitz, M., (1948) J. Gen. Physiol. 32, 241.

Laidler, K. J., (1958) The Chemical Kinetics of Enzyme Action. London: The Oxford University Press.

Stern, A. E., (1938) Enzymeforsch 7, 1.

Stevens, B., (1970) Chemical Kinetics. London: Chapman and Hall Ltd.

Strong, C. L., (1966) Sci. Amer. 215, 100.

Toole, E. H., (1959) Photoperiodism and Related Phenomena in Plants and Animals. Washington: American Association for the Advancement of Science.

Toole, E. H., Hendricks, S. B., Borthwick, H. A., and Toole, V. K., (1956) Ann. Rev. Plant Physiol. 7, 299.

APPENDIX I

Experimental Data

Table 4

EXPERIMENT I

40°C

Time (Hrs.)	Quiet No. Ger.	Quiet Total	Noise[a] No. Ger.	Noise[a] Total
11	0	0	-	-
12	1	1	1	1
13	1	2	1	2
14	4	6	0	2
15	2	8	0	2
16	1	9	0	2
17	6	15	0	2
18	11	26	0	2
19	18	44	0	2
20	21	65	0	2
21	19	84	-	-
22	9	93	-	-
23	11	104	-	-
24	15	119	-	-
25	10	129	-	-

a. Terminated at 20.5 hours

Table 5

EXPERIMENT II

35°C

Time (Hrs.)	Quiet No. Ger.	Quiet Total	Noise No. Ger.	Noise Total
11.0	15	15	8	8
12.0	35	50	12	20
13.0	32	82	33	53
14.0	48	130	36	89
15.0	56	186	40	129
16.0	--	---	49	178
16.5	98	284	--	---
17.0	30	314	33	211
18.0	53	367	30	241
19.0	37	404	45	286
20.0	35	439	41	327
21.0	--	---	43	370
21.5	69	508	--	---
22.0	23	531	34	404
23.0	30	561	42	446
24.0	18	579	35	481
25.0	27	606	33	514

Table 6

EXPERIMENT III

31°C

Time (Hrs.)	Quiet No. Ger.	Quiet Total	Noise No. Ger.	Noise Total
11	37	37	20	20
12	64	101	53	73
13	70	171	48	121
14	88	259	94	215
15	86	345	79	294
16	64	409	64	358
17	62	471	66	424
18	53	524	70	494
19	49	573	42	536
20	49	622	43	579
21	29	651	40	619
22	41	692	30	649
23	27	719	25	674
24	25	744	21	695
25	22	766	16	711

Table 7

EXPERIMENT IV

27°C

Time (Hrs.)	Quiet No. Ger.	Quiet Total	Noise[a] No. Ger.	Noise[a] Total
11	7	7	35	35
12	24	31	48	83
13	49	80	86	169
14	81	161	110	279
15	82	243	92	371
16	99	342	91	462
17	81	423	69	531
18	84	507	74	605
19	66	573	49	654
20	48	621	63	717
21	50	671	53	770
22	35	706	22	792
23	24	730	24	816
24	28	758	22	838
25	23	781	21	859

a. 28°C

Table 8

EXPERIMENT V

21.5°C

Time (Hrs.)	Quiet No. Ger.	Quiet Total	Noise No. Ger.	Noise Total
11.0	1	1	1	1
12.0	1	2	0	1
13.0	1	3	0	1
14.0	2	5	2	3
15.0	11	16	-	-
15.5	--	--	8	11
16.0	24	40	7	18
17.0	36	76	35	53
18.0	52	128	56	109
19.0	85	213	76	185
20.0	70	283	102	287
21.0	90	373	89	376
22.0	64	437	56	432
23.0	73	510	77	509
24.0	55	565	44	553
25.0	42	607	43	596

Table 9

EXPERIMENT VI

18°C

Time (Hrs.)	Quiet No. Ger.	Quiet Total	Noise No. Ger.	Noise Total
11	0	0	-	-
12	0	0	-	-
13	0	0	-	-
14	1	1	0	0
15	0	1	2	2
16	0	1	0	2
17	0	1	1	3
18	1	2	1	4
19	7	9	2	6
20	7	16	10	16
21	25	41	21	37
22	33	74	33	70
23	50	124	39	109
24	60	184	73	182
25	59	243	52	234
26	--	---	79	313
27	--	---	56	369
28	--	---	70	439

Table 10

EXPERIMENT VII

24°C

Time (Hrs.)	Quiet No. Ger.	Quiet Total	Noise No. Ger.	Noise Total
11.0	4	4	0	0
12.0	6	10	2	2
13.0	8	18	13	15
14.0	24	42	15	30
15.0	46	88	--	--
16.0	81	169	105	135
17.0	83	252	107	242
18.0	110	362	68	310
19.0	66	428	65	375
20.0	88	516	76	451
21.0	71	587	68	519
22.0	52	639	--	---
22.5	--	---	96	615
23.0	35	674	15	630
24.0	47	721	38	668
25.0	26	747	40	708

Table 11

EXPERIMENT VIII

23°C

Time (Hrs.)	Quiet No. Ger.	Quiet Total	Noise No. Ger.	Noise Total
11.0	0	0	1	1
12.0	0	0	3	4
13.0	0	0	9	13
14.0	0	0	14	27
15.0	2	2	48	75
16.0	7	9	100	175
17.0	17	26	---	---
17.3	--	--	112	287
18.0	23	49	69	356
19.0	42	91	94	450
20.0	65	156	84	534
21.0	44	200	--	---
21.3	--	---	71	605
22.0	50	250	29	634
23.0	77	327	50	684
24.0	41	368	27	711
25.0	47	415	25	736

Table 12

EXPERIMENT IX

21°C

Time (Hrs.)	Quiet No. Ger.	Quiet Total	Noise No. Ger.	Noise Total
11.0	-	-	1	1
12.0	-	-	0	1
13.0	-	-	1	2
14.0	3	3	3	5
15.0	10	13	9	14
16.0	15	28	24	38
17.0	29	57	27	65
18.0	65	122	67	132
19.0	93	215	96	228
20.0	86	301	106	334
21.0	106	407	94	428
22.0	88	495	--	---
22.5	--	---	130	558
23.0	70	565	38	596
24.0	79	644	51	647
25.0	36	680	56	703
26.0	38	718	--	---

Table 13

EXPERIMENT X

18°C

Time (Hrs.)	Quiet No. Ger.	Quiet Total	Noise No. Ger.	Noise Total
13.00	0	0	-	-
14.00	0	0	-	-
15.00	0	0	-	-
16.00	0	0	-	-
17.00	3	3	11	11
18.00	4	7	17	28
19.00	13	20	28	56
20.00	27	47	34	90
21.00	35	82	67	157
22.00	--	--	62	219
22.25	70	152	--	---
23.00	47	199	90	309
24.00	90	289	95	404
25.00	52	341	84	488
26.00	83	424	52	540
27.00	--	---	58	598
28.00	--	---	49	647
29.00	--	---	39	686

Table 14

EXPERIMENT XI

14°C

Time (Hrs.)	Quiet		Noise	
	No. Ger.	Total	No. Ger.	Total
18	0	0	0	0
19	0	0	0	0
20	1	1	0	0
21	0	1	0	0
22	0	1	1	1
23	0	1	3	4
24	7	8	7	11
25	8	16	8	19
26	11	27	10	29
27	13	40	22	51
28	34	74	45	96
29	28	102	41	137
30	49	151	65	202
31	40	191	56	258

Table 15

EXPERIMENT XII

37.5°C

Time (Hrs.)	Quiet No. Ger.	Quiet Total	Noise No. Ger.	Noise Total
11.00	16	16	--	--
11.50	--	--	38	38
12.00	22	38	27	65
13.00	28	66	53	118
14.00	76	142	90	208
15.00	73	215	96	304
16.00	80	295	85	389
17.00	93	388	--	---
17.25	--	---	101	490
18.00	85	473	50	540
19.00	81	554	74	614
20.00	91	645	46	660
21.00	44	689	57	717
22.00	38	727	46	763
23.00	36	763	41	804
24.00	23	786	33	837

Table 16

EXPERIMENT XIII

33°C

Time (Hrs.)	Quiet No. Ger.	Quiet Total	Noise No. Ger.	Noise Total
12	325	325	362	362
13	102	427	96	458
14	89	516	96	554
15	80	596	87	641
16	92	688	60	701
17	56	744	57	758
18	45	789	53	811
19	41	830	26	837
20	19	849	27	864
21	23	872	26	890
22	35	907	13	903
23	24	931	19	922
24	16	947	10	932
25	12	959	9	941

Table 17

EXPERIMENT XIV

39°C

Time (Hrs.)	No. Ger.	Total
18	10	10
19	5	15
20	8	23
21	14	37
22	12	49
23	11	60
24	12	72
25	11	83
26	13	96
27	15	111
28	10	121
29	14	135
30	10	145

APPENDIX II

Germination Rate Curves

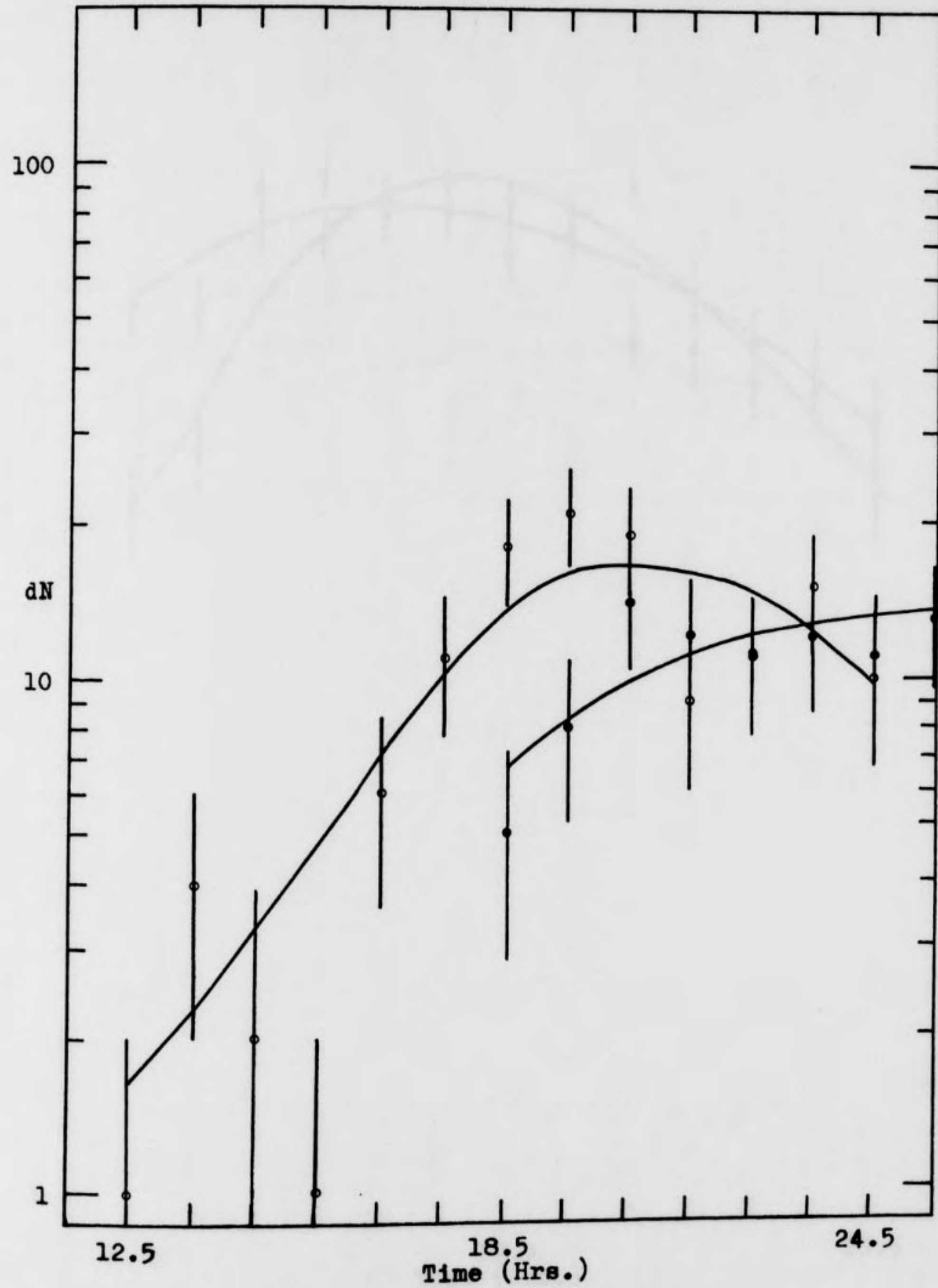

Figure 13: 40°C germination rate curve
39°C germination rate curve

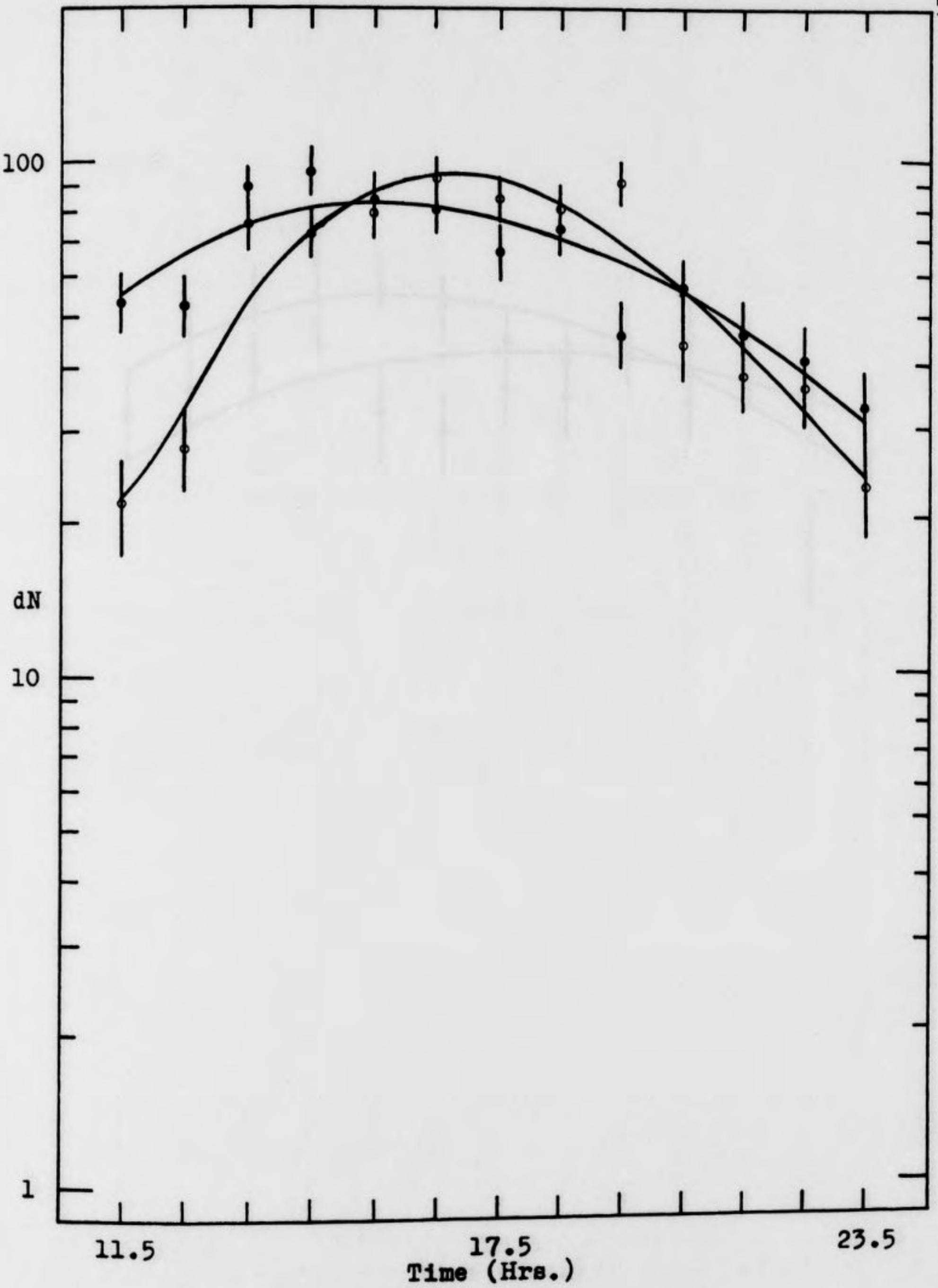

Figure 14: 37.5°C germination rate curves

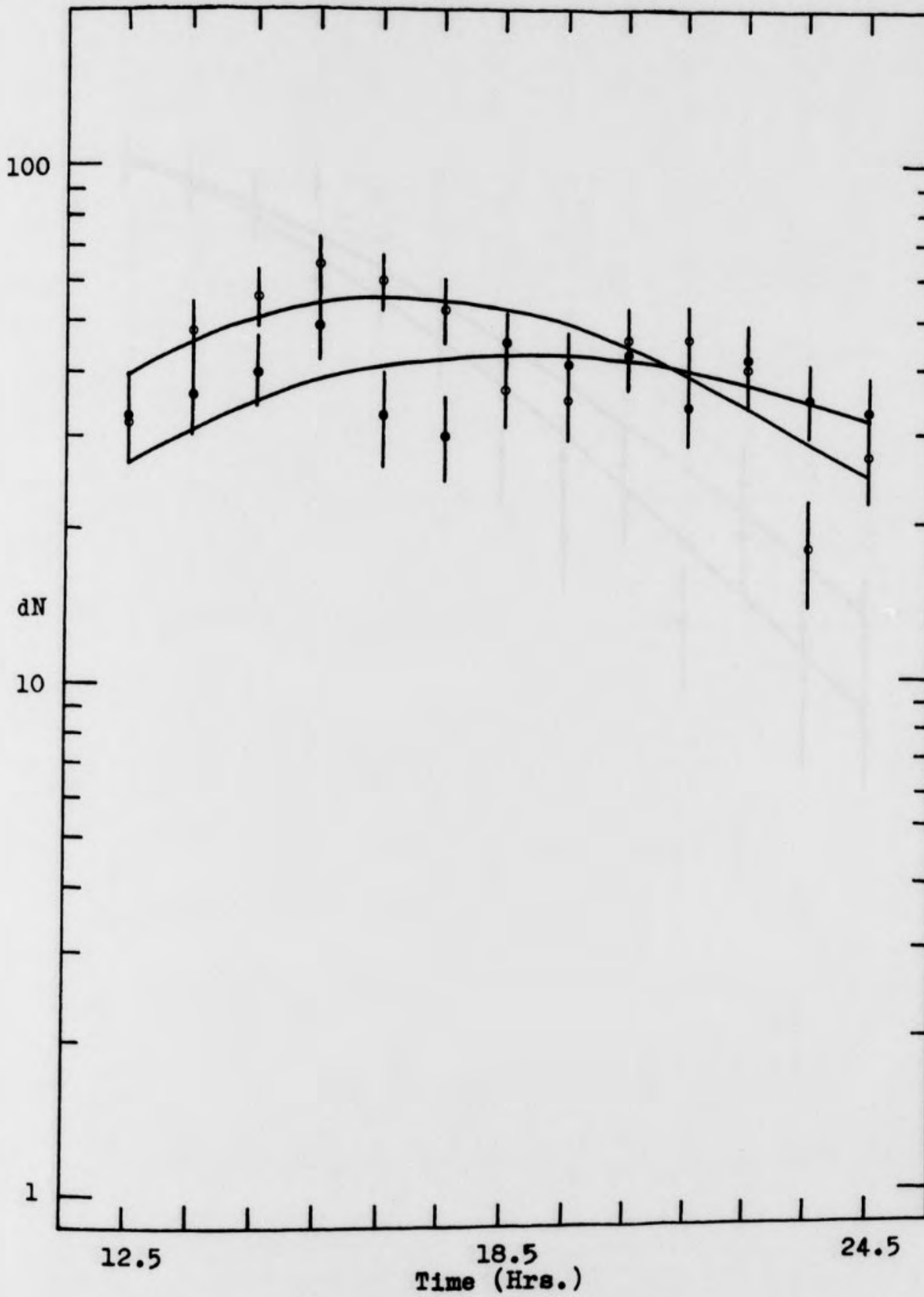

Figure 15: 35°C germination rate curves

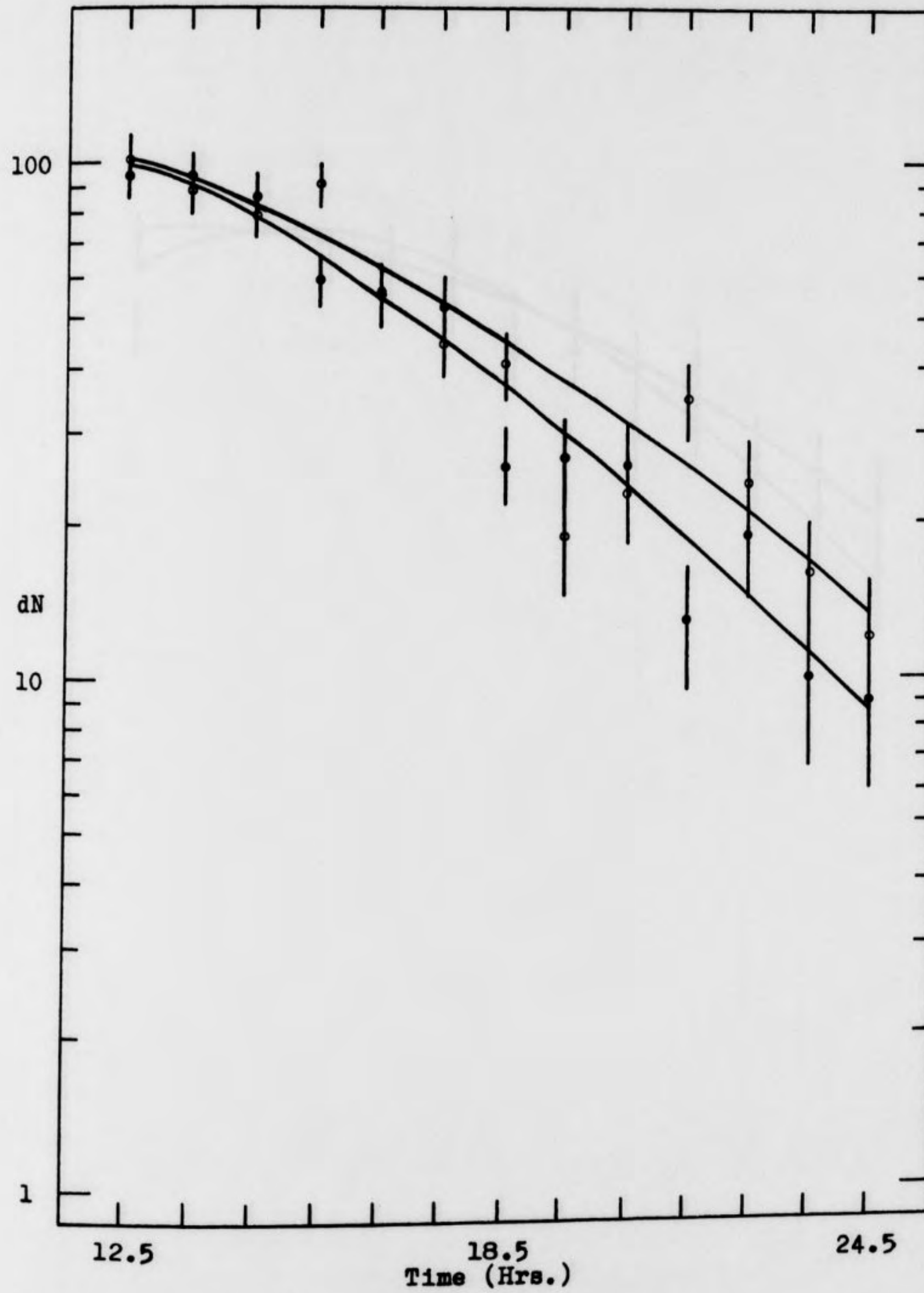

Figure 16: 33°C germination rate curves

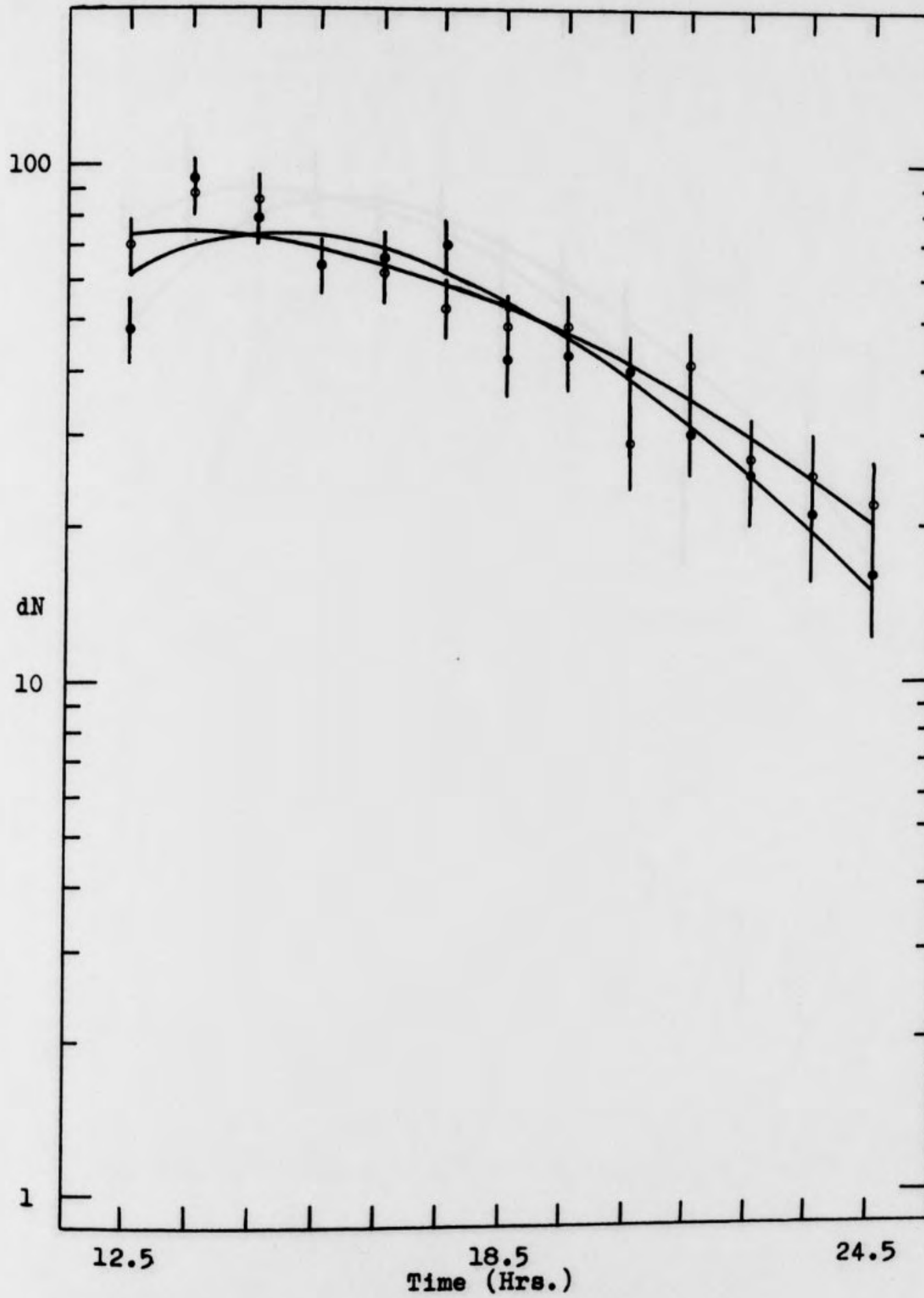

Figure 17: 31°C germination rate curves

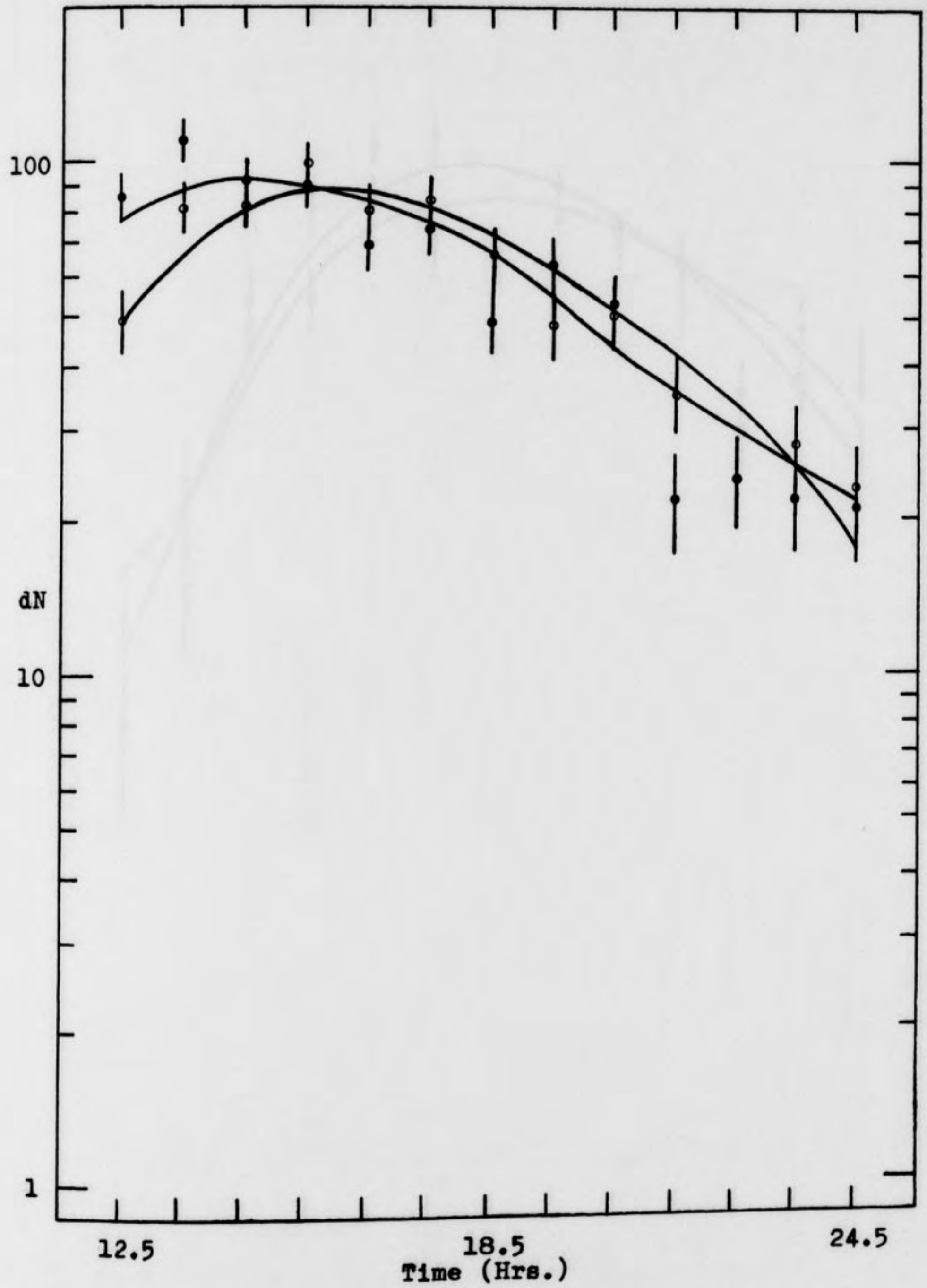

Figure 18: 27°C germination rate curve
28°C germination rate curve

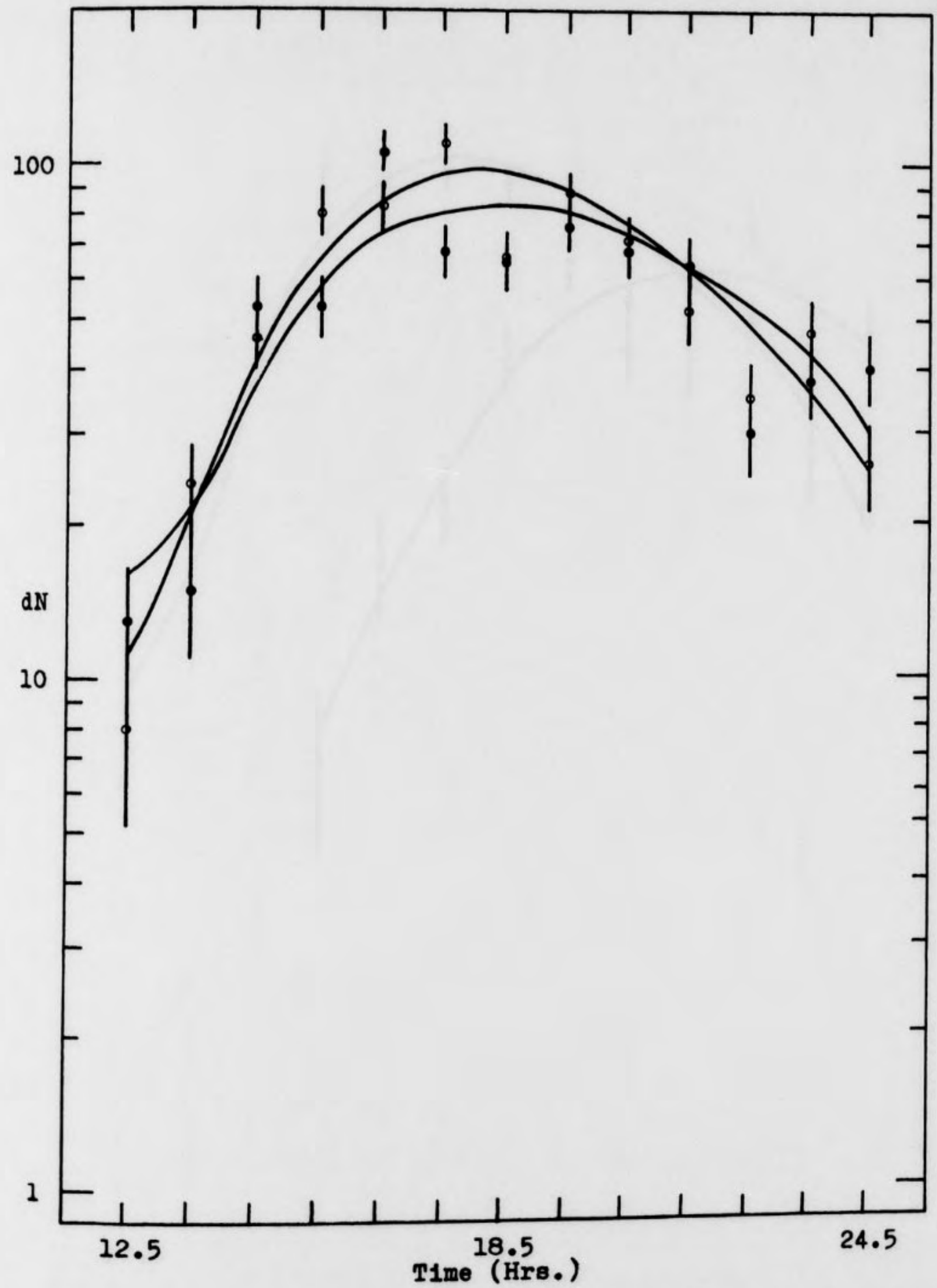

Figure 19: 24°C germination rate curves (broadband noise)

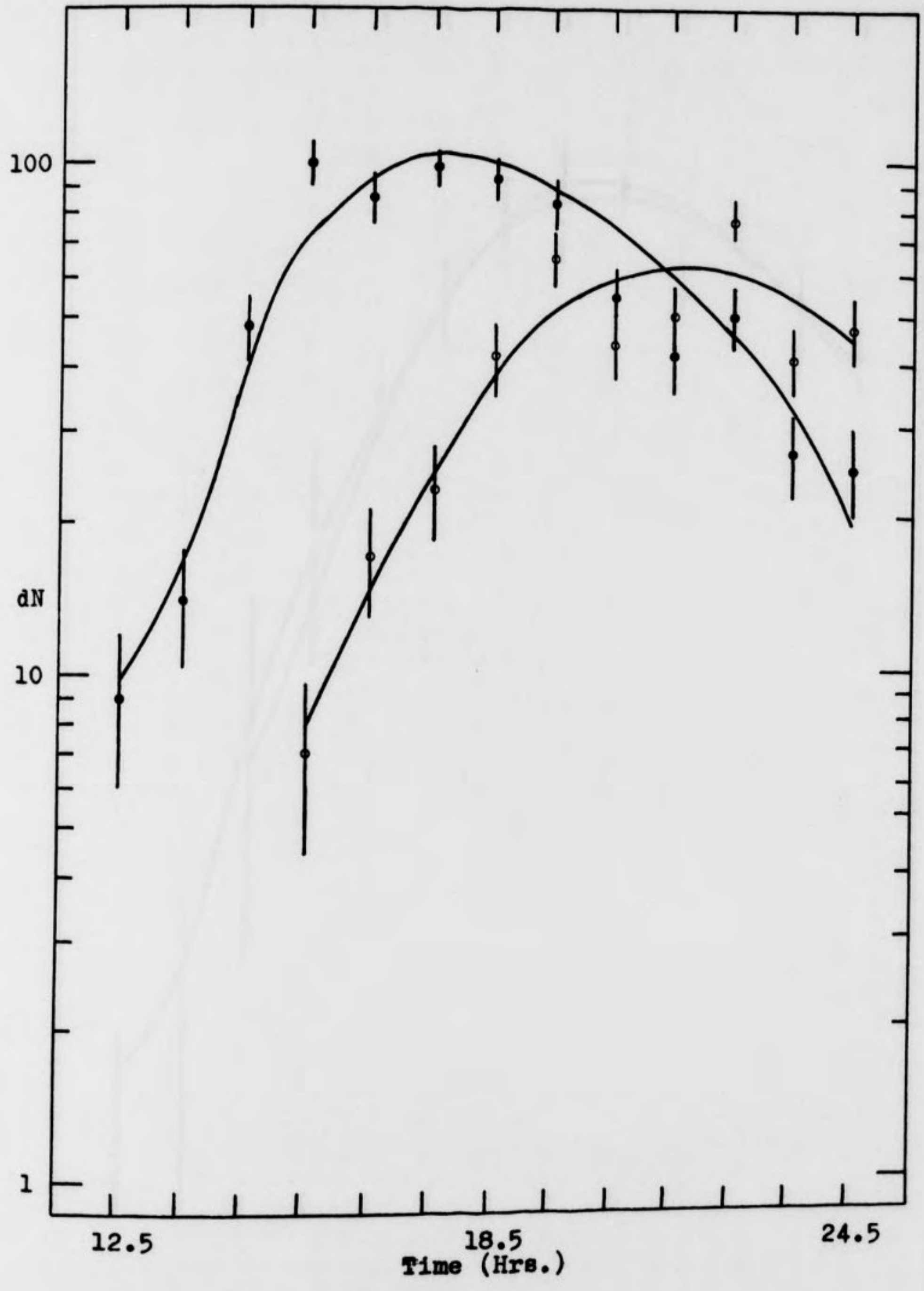

Figure 20: 23°C germination rate curves

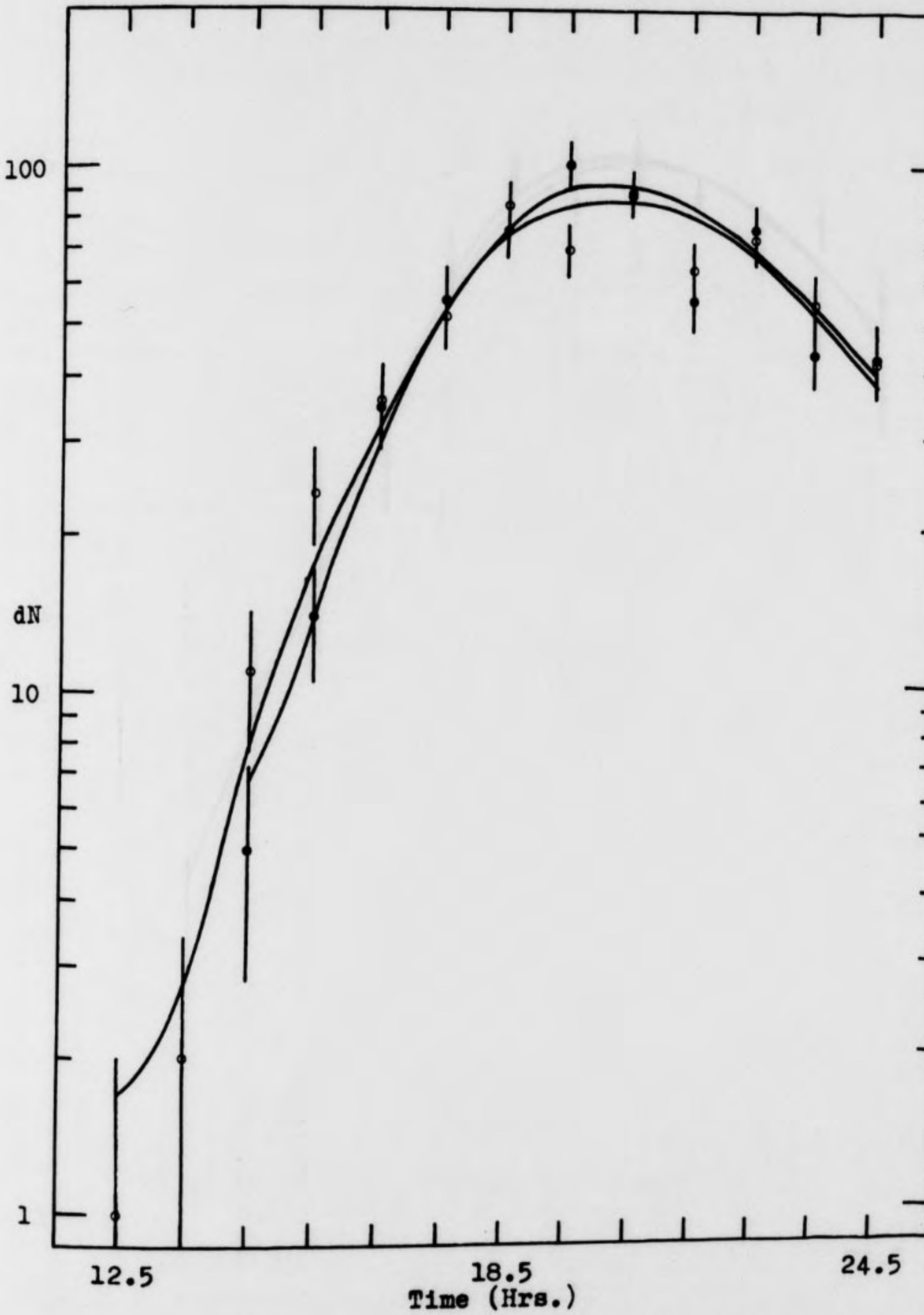

Figure 21: 21.5°C germination rate curves (broadband noise)

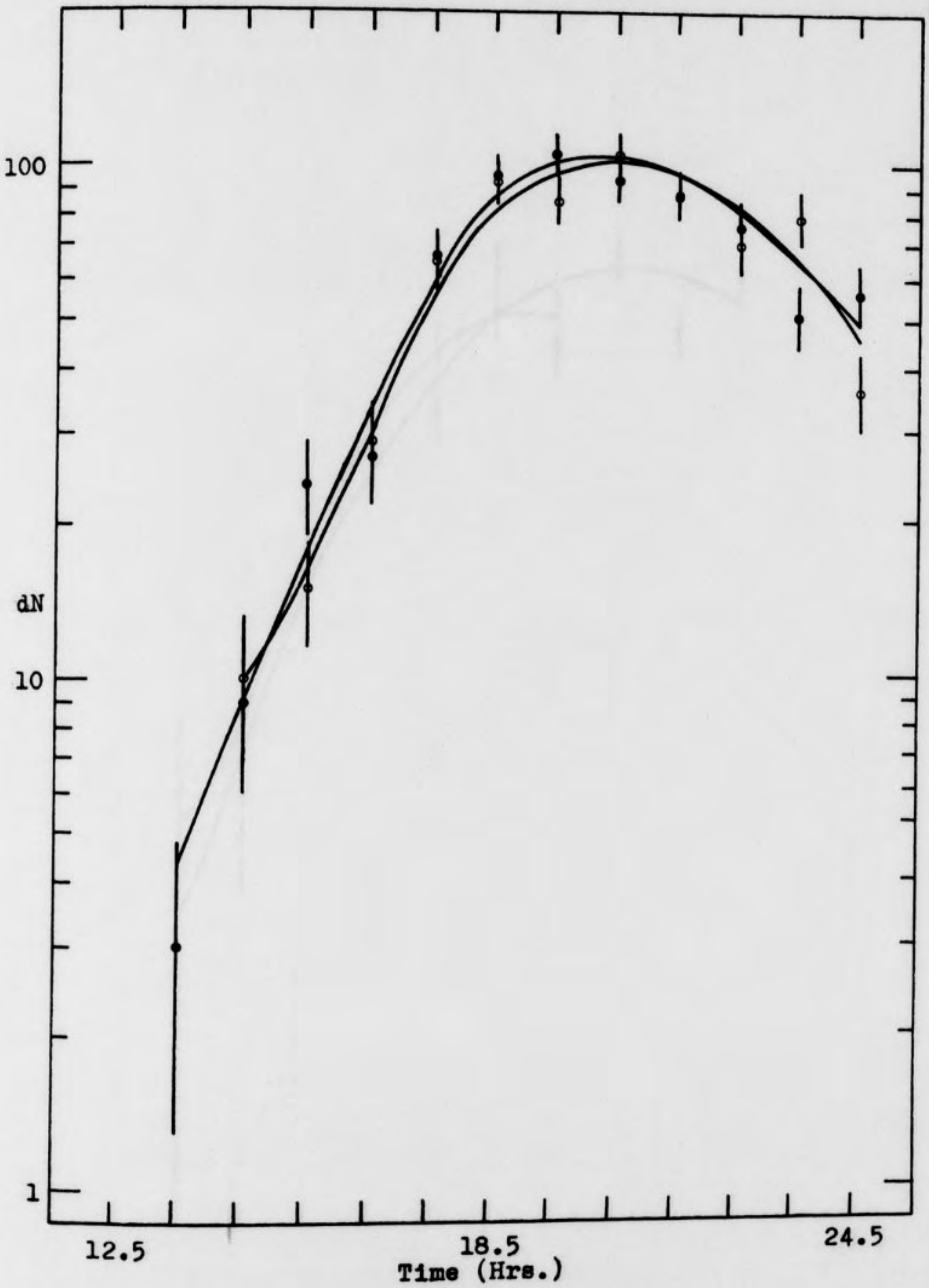

Figure 22: 21°C germination rate curves

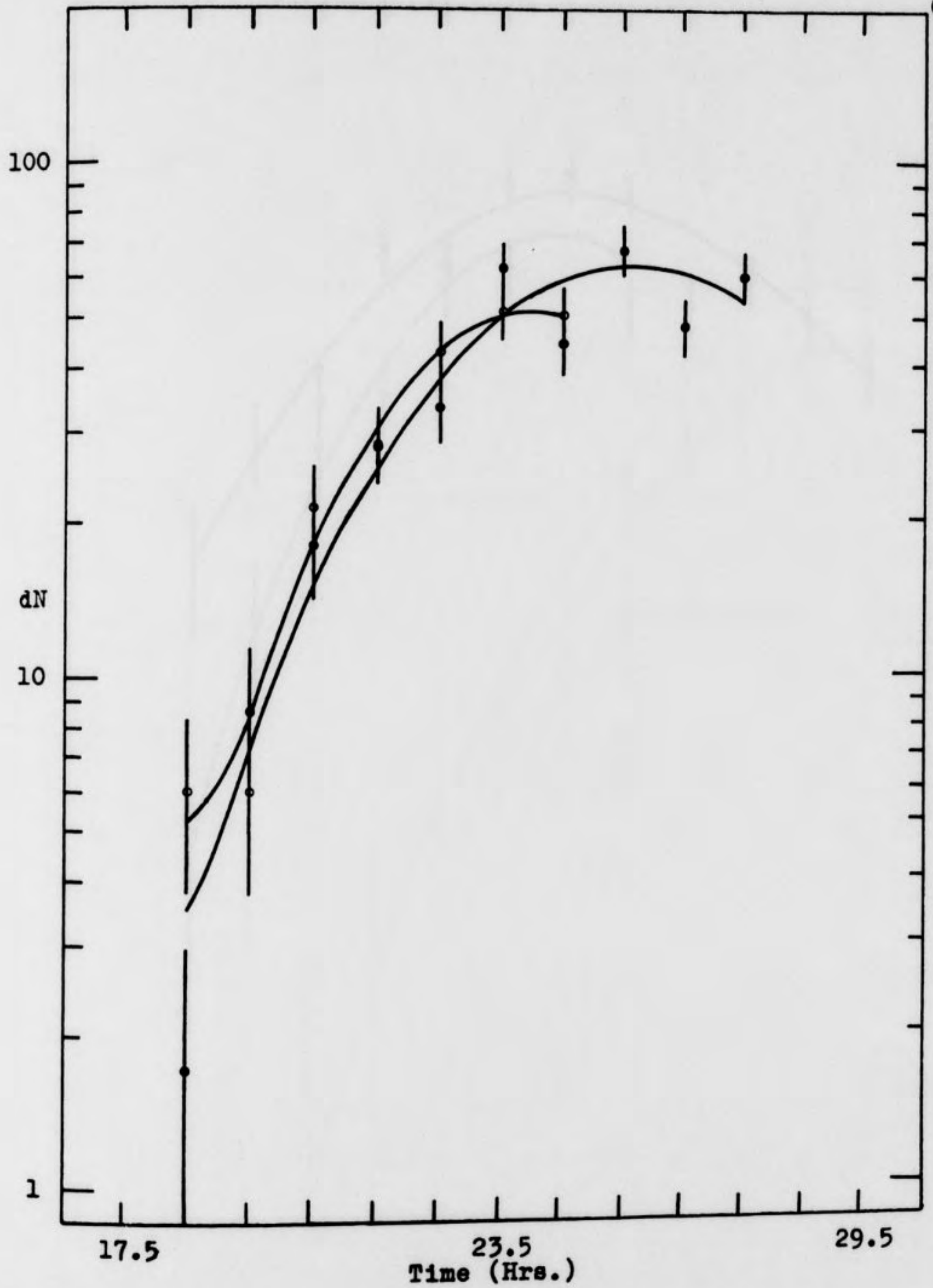

Figure 23: 18°C germination rate curves (broadband noise)

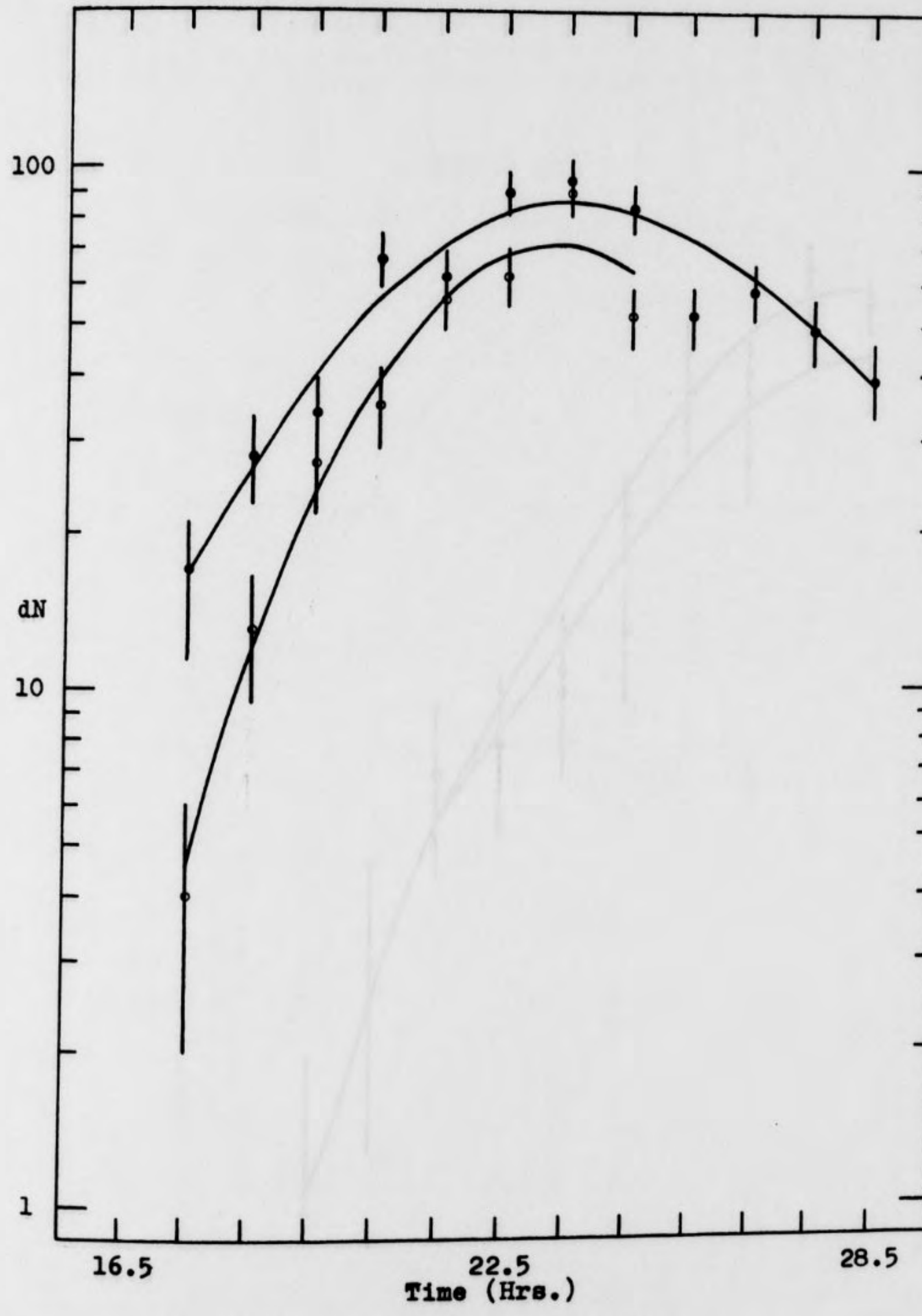

Figure 24: 18°C germination rate curves

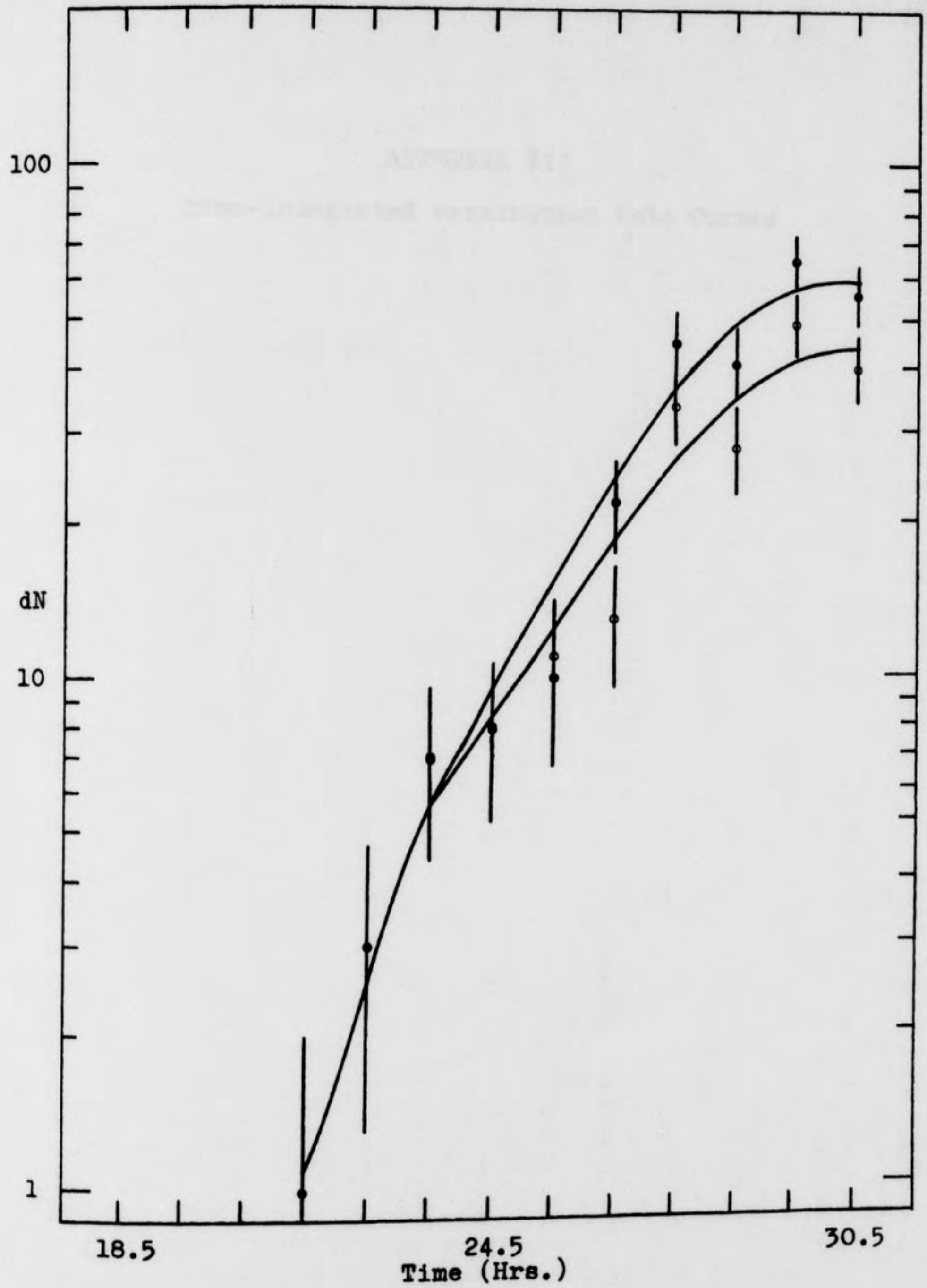

Figure 25: 14°C germination rate curves

APPENDIX III

Time-Integrated Germination Rate Curves

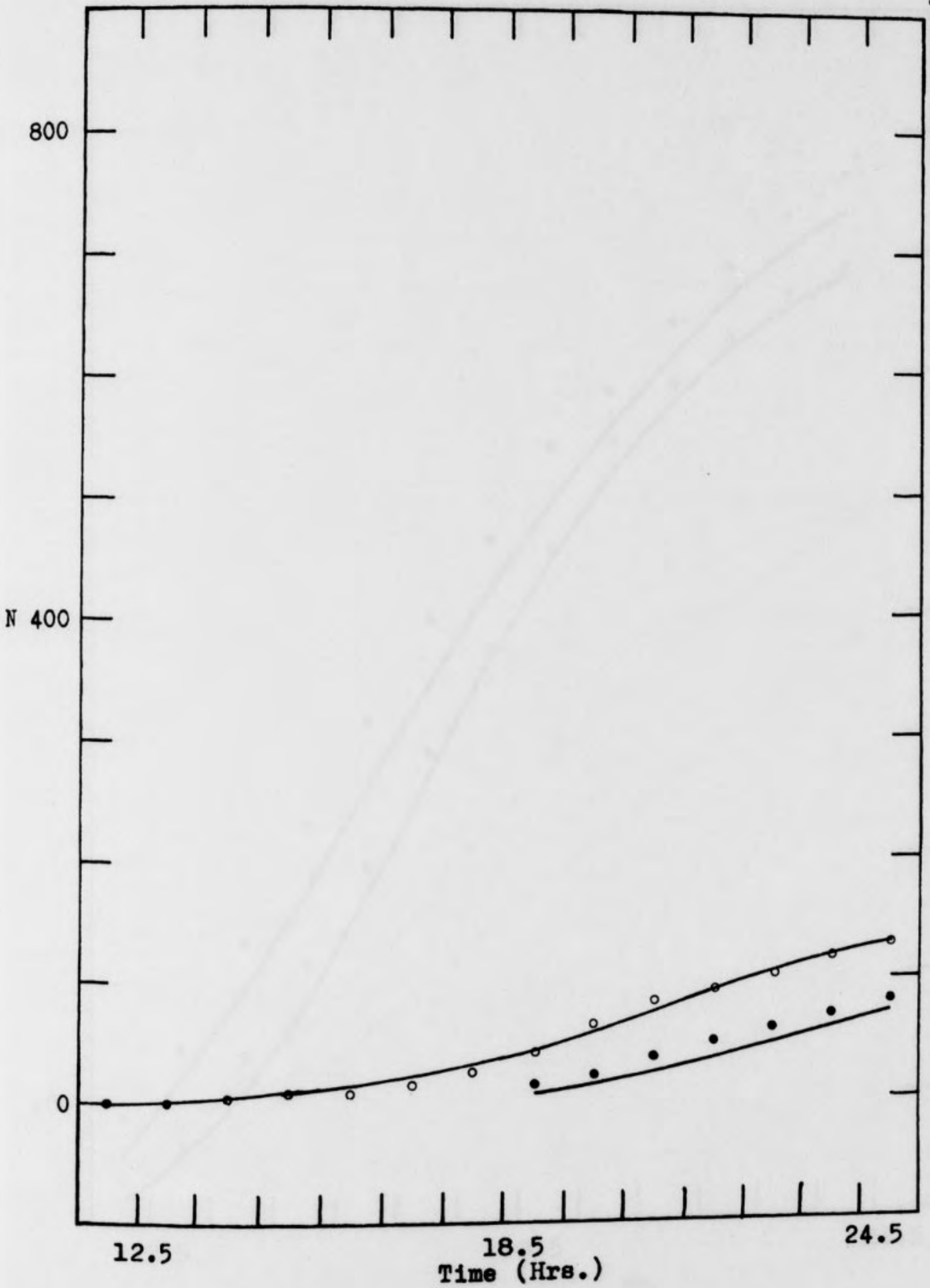

Figure 26: 40°C integrated rate curve
39°C integrated rate curve

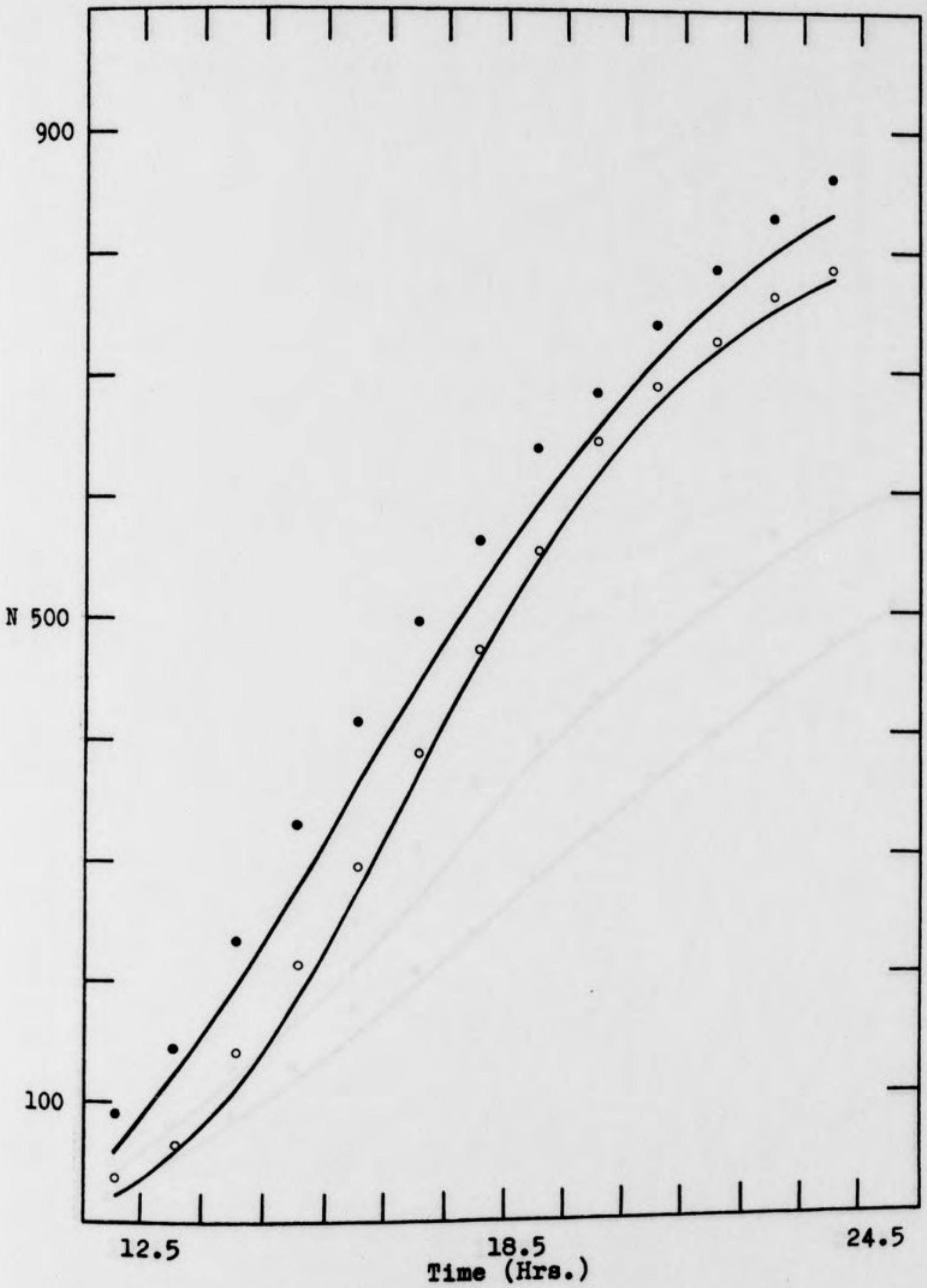

Figure 27: 37.5°C integrated rate curves

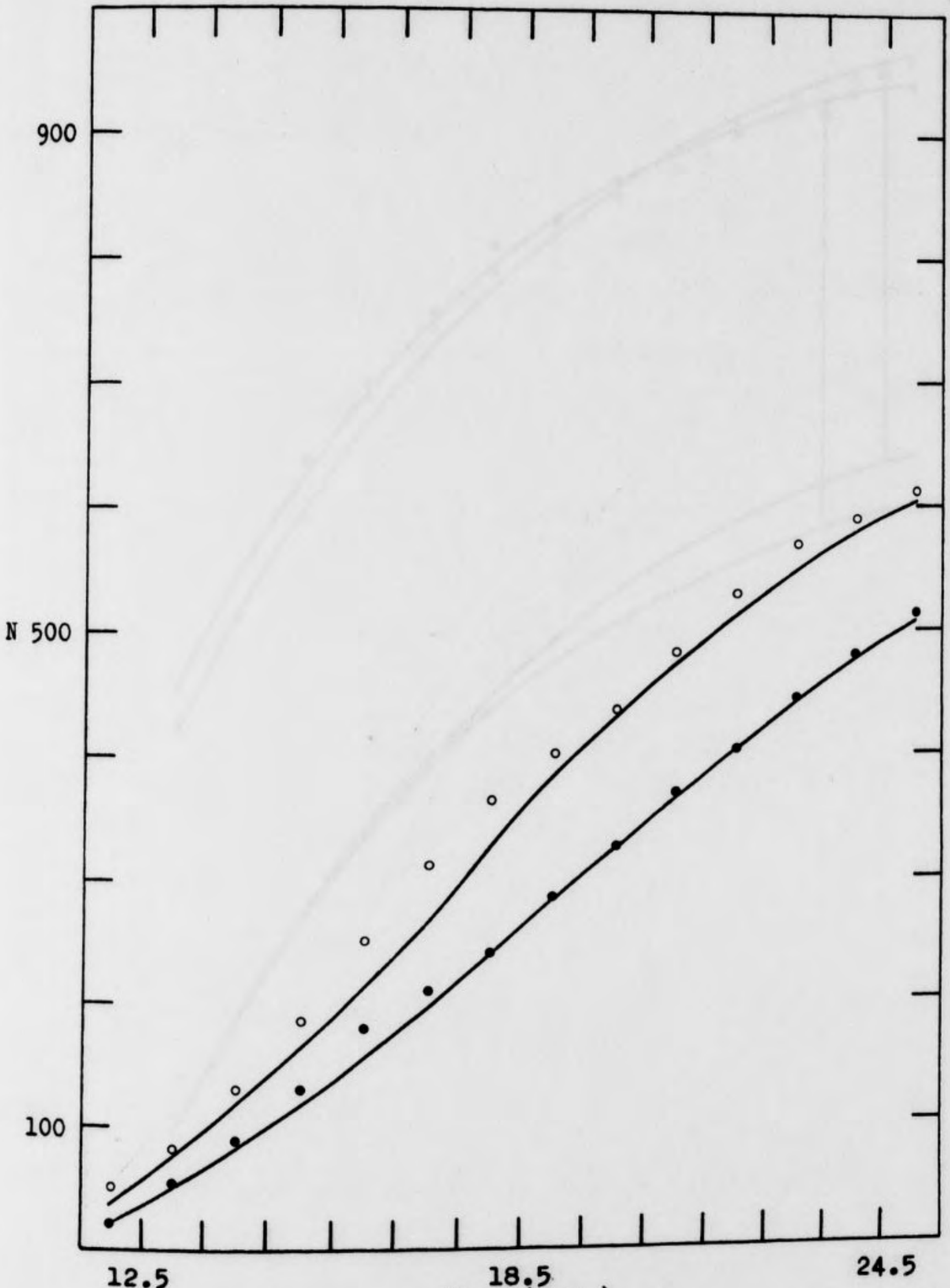

Figure 28: 35°C integrated rate curves

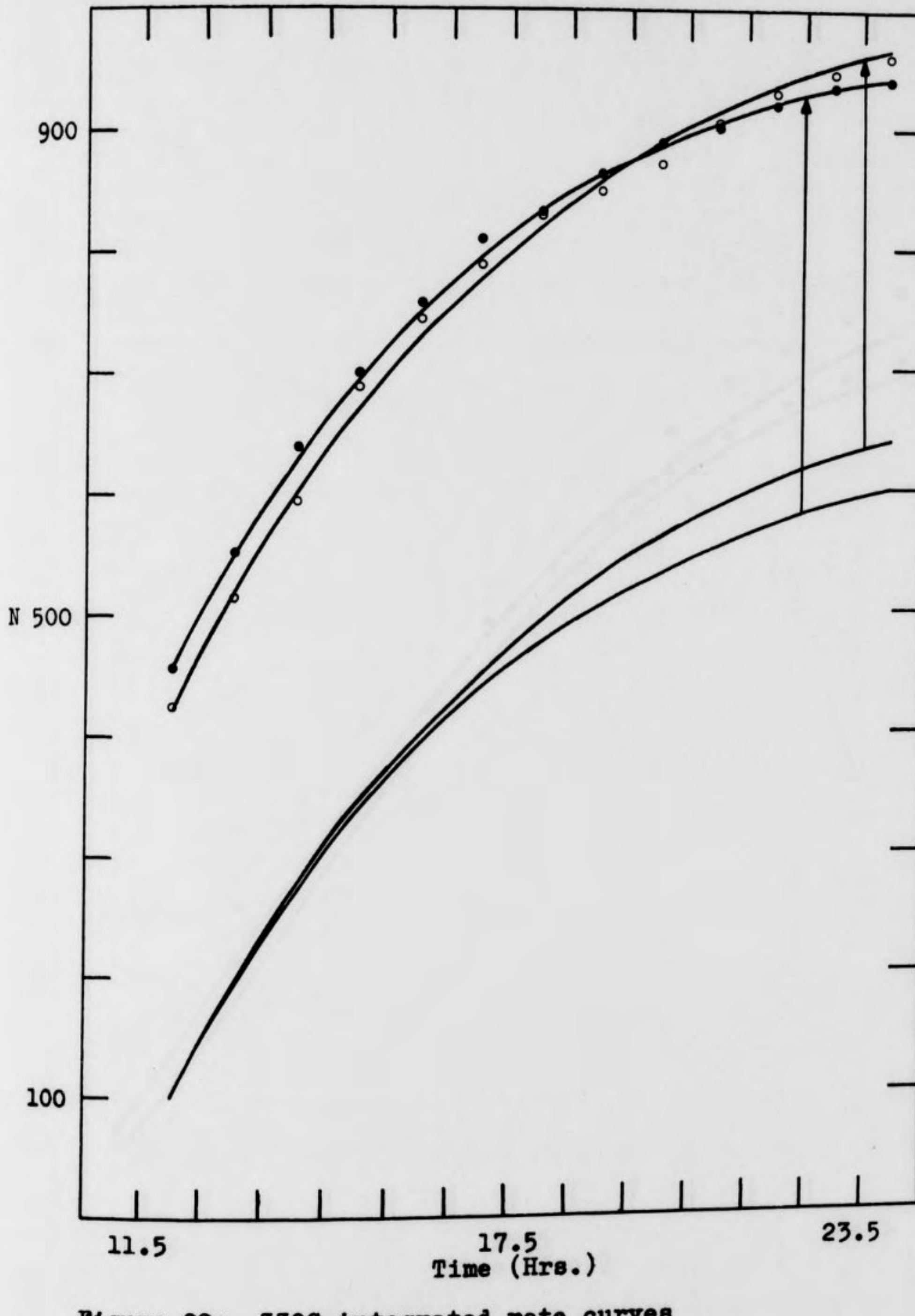

Figure 29: 33°C integrated rate curves

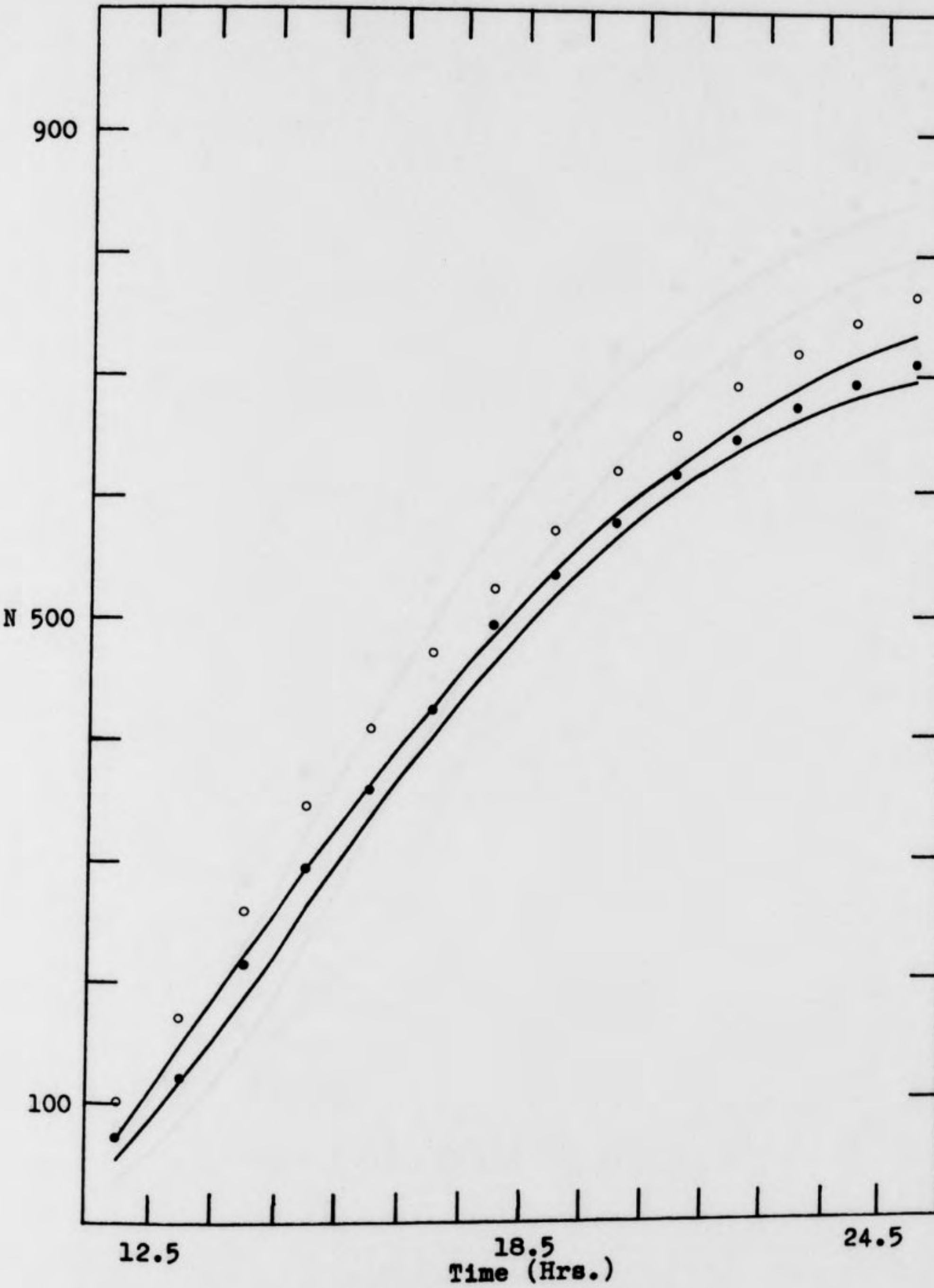

Figure 30: 31°C integrated rate curves

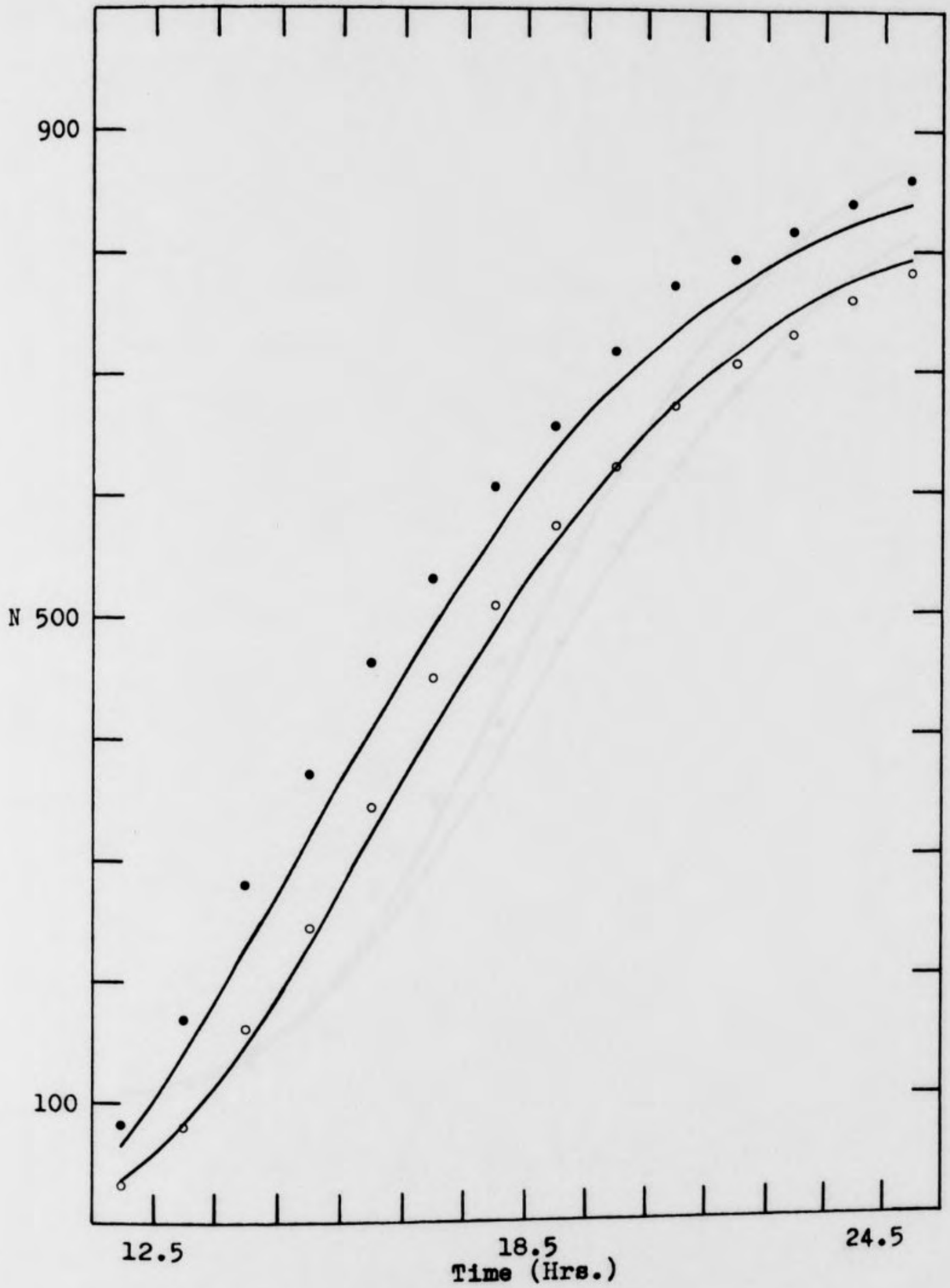

Figure 31: 27°C integrated rate curve
28°C integrated rate curve

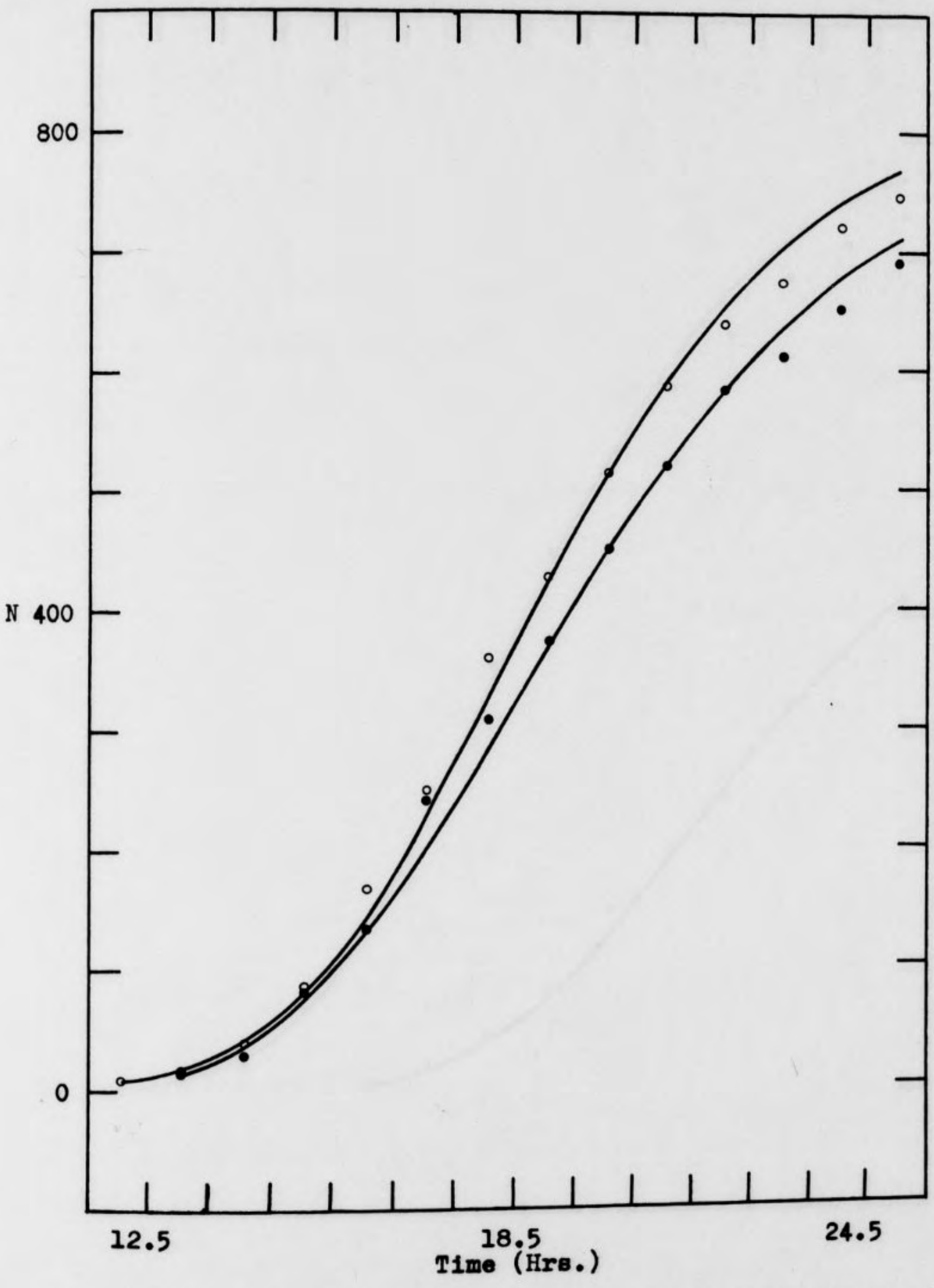

Figure 32: 24°C integrated rate curves (broadband noise)

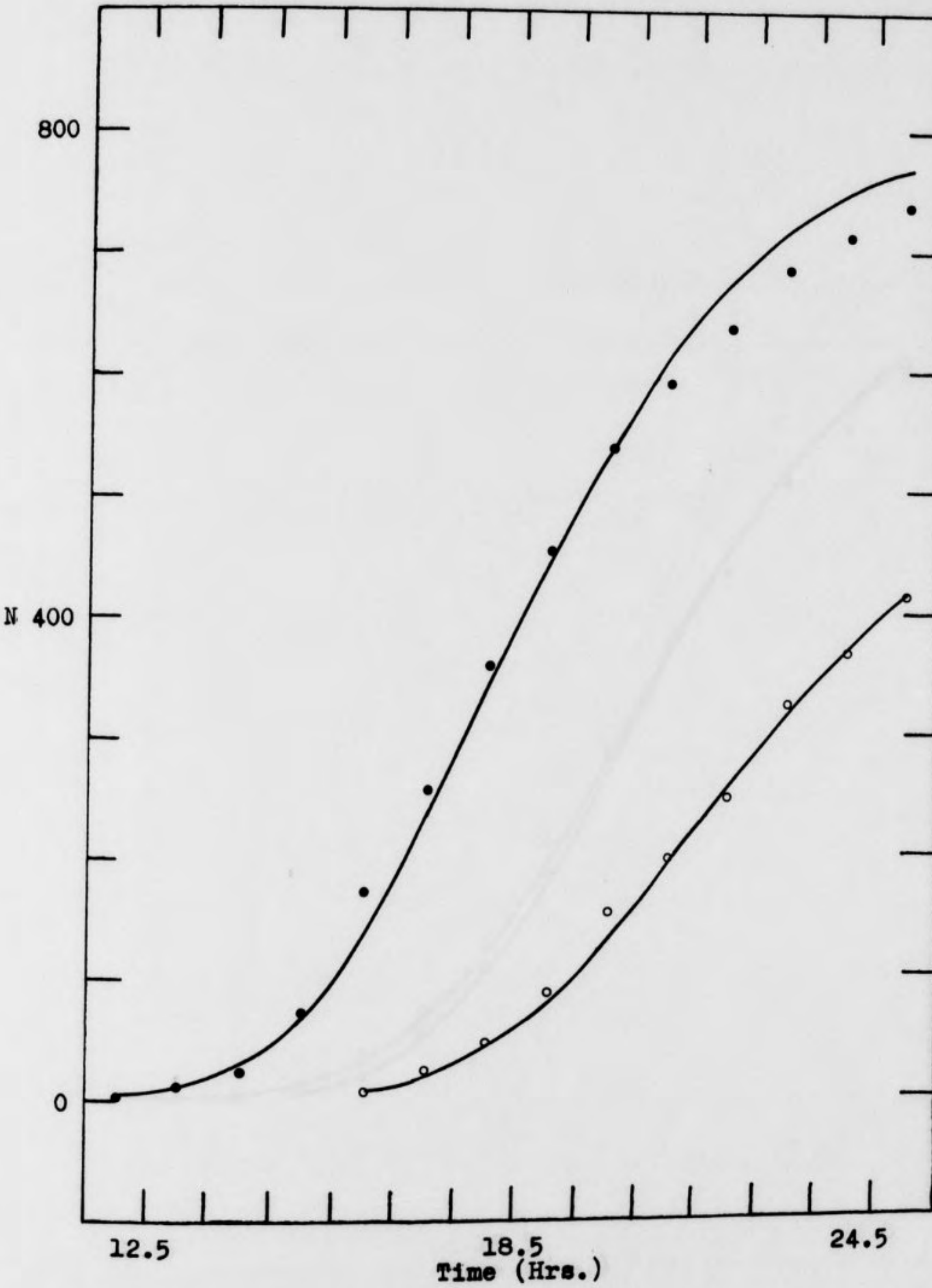

Figure 33: 23°C integrated rate curves

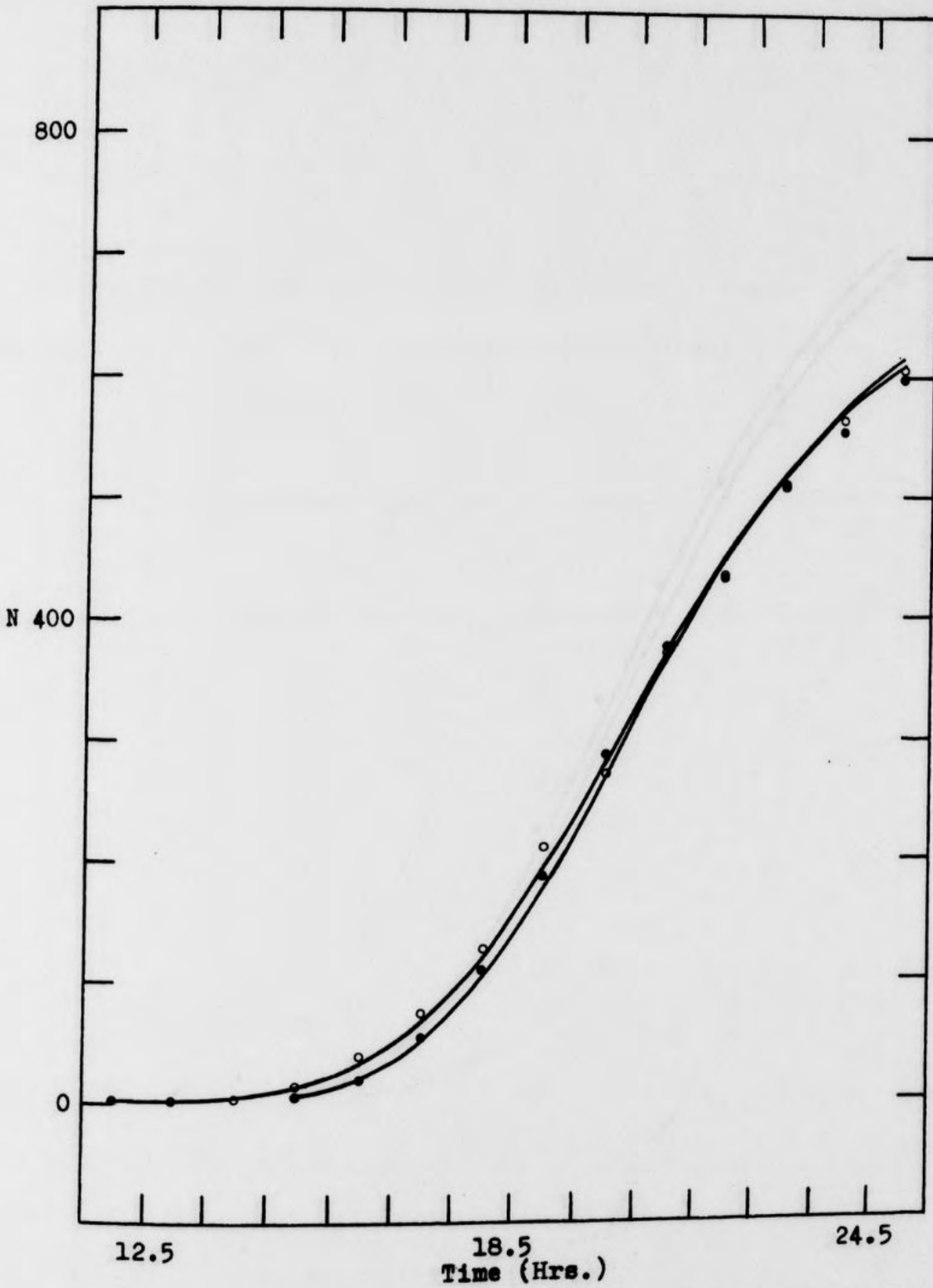

Figure 34: 21.5°C integrated rate curves (broadband noise)

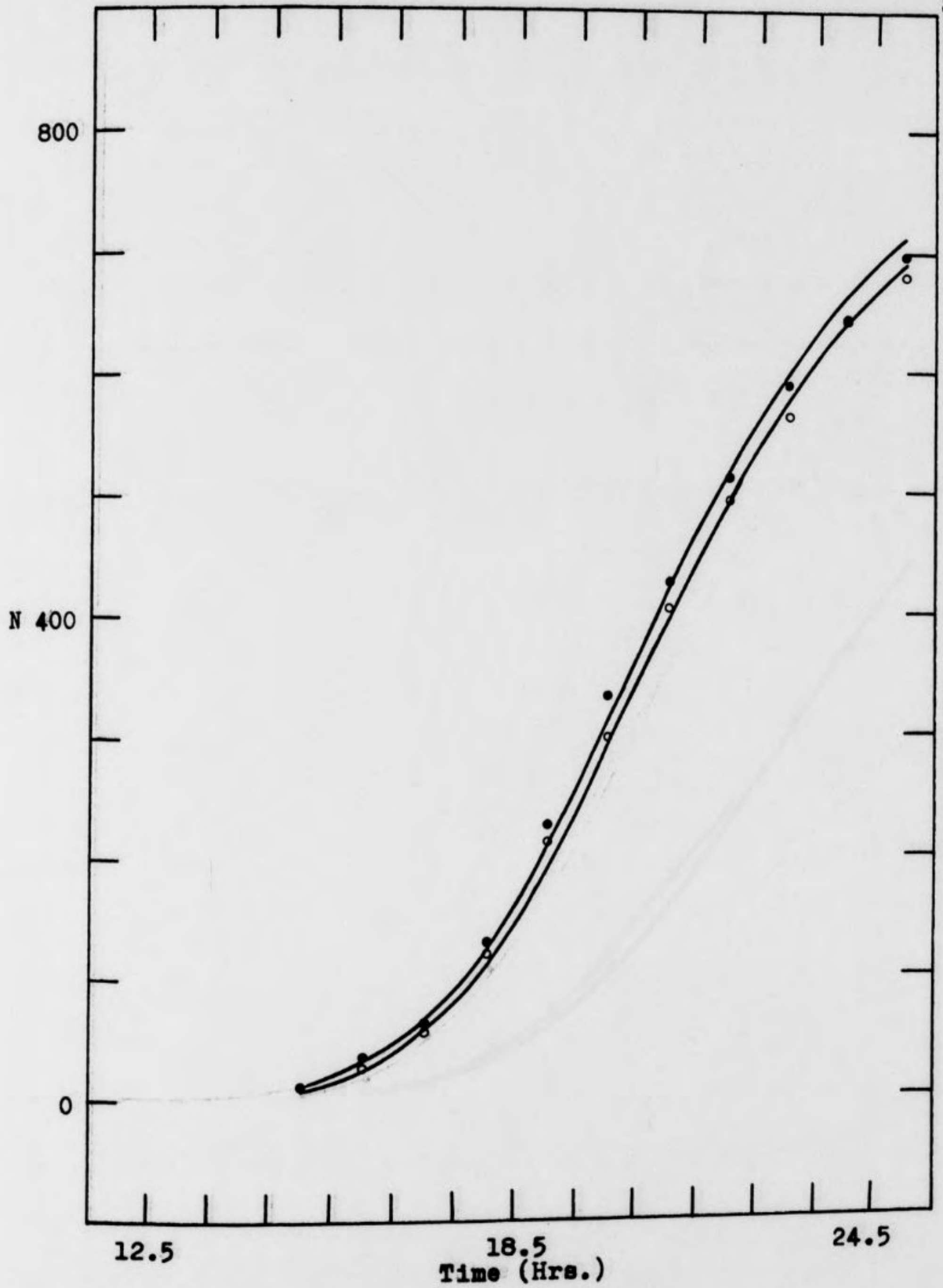

Figure 35: 21°C integrated rate curves

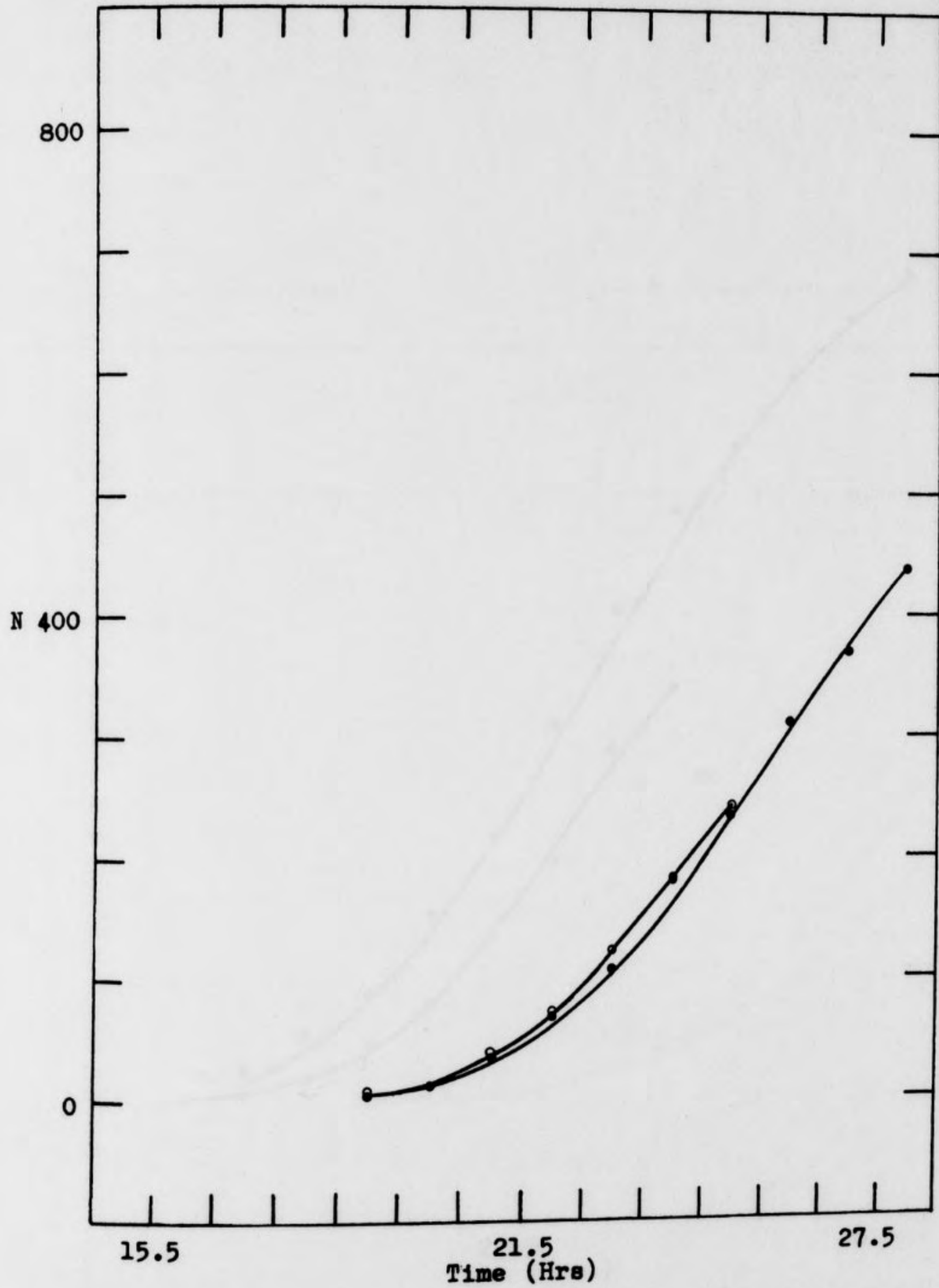

Figure 36: 18°C integrated rate curves (broadband noise)

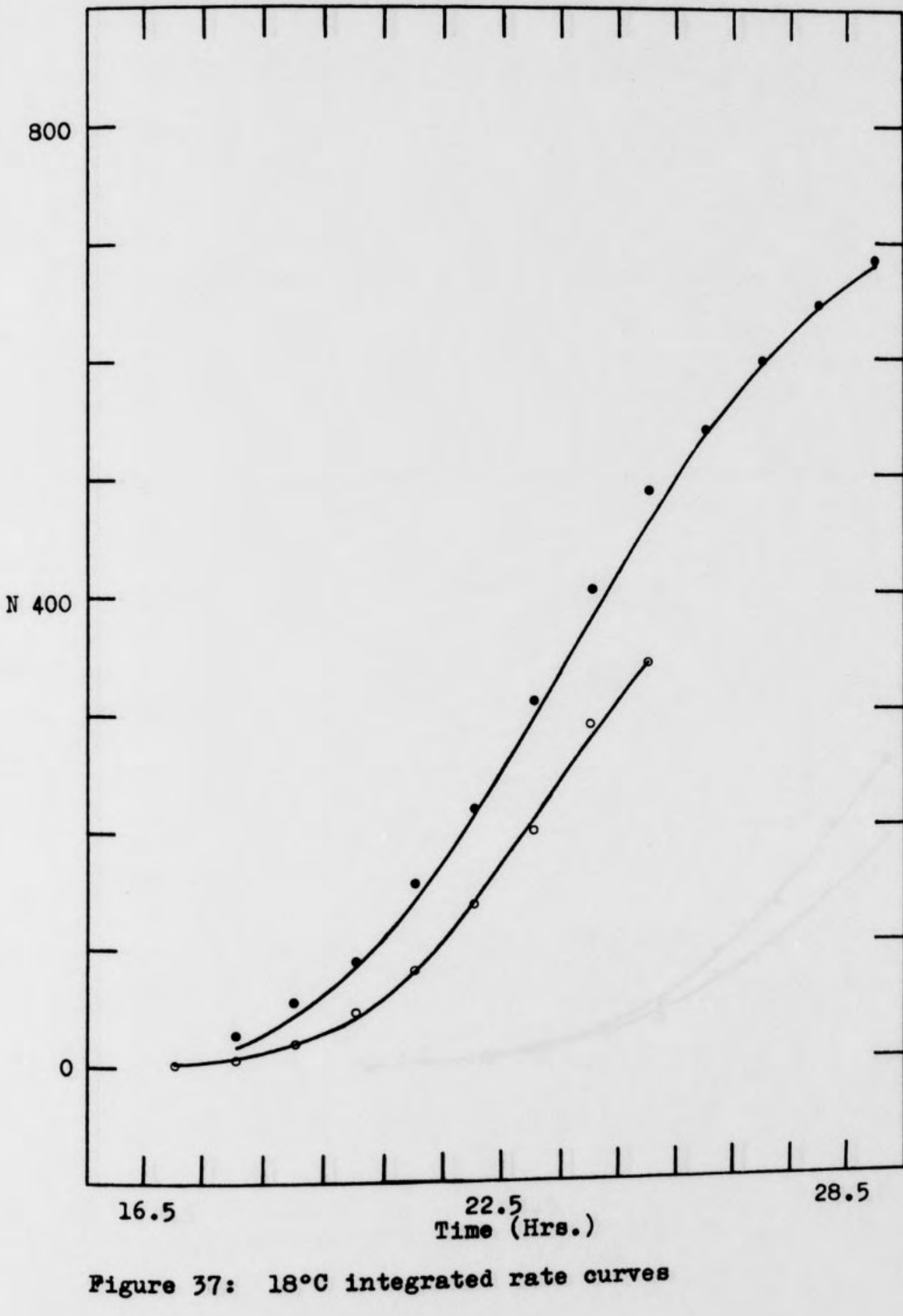

Figure 37: 18°C integrated rate curves

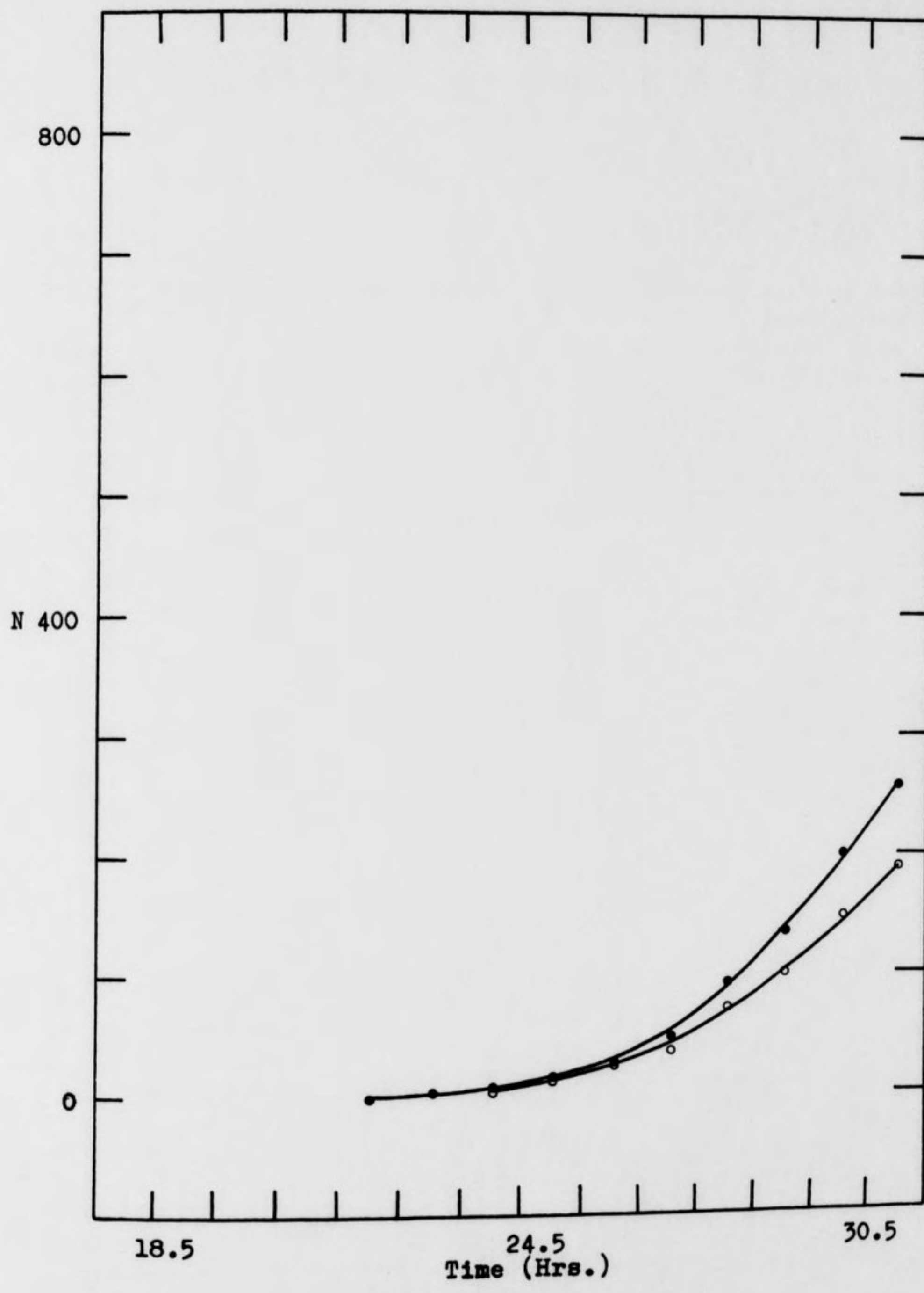

Figure 38: 14°C integrated rate curves