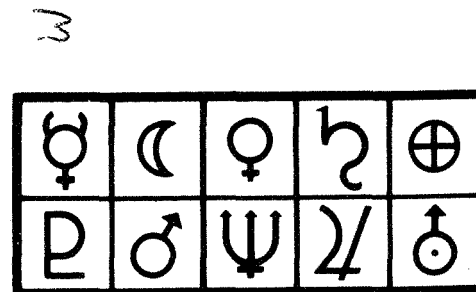


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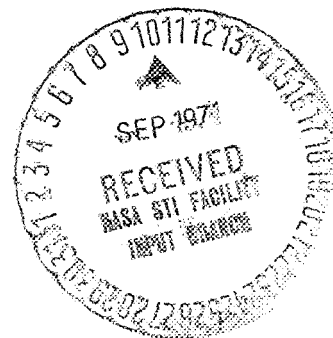


PLANETARY QUARANTINE

SANDIA LABORATORIES QUARTERLY REPORT
PLANETARY QUARANTINE PROGRAM

Prepared by:
Planetary Quarantine Department 1740

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SANDIA LABORATORIES QUARTERLY REPORT - PLANETARY QUARANTINE PROGRAM

NINETEENTH QUARTERLY REPORT OF PROGRESS

For

Period Ending December 31, 1970

Planetary Quarantine Department
Sandia Laboratories, Albuquerque, New Mexico

December 1970

Project No. 0064010

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SANDIA LABORATORIES QUARTERLY REPORT
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Summary of Activities

Thermoradiation Sterilization. Activities this quarter have been primarily devoted to the investigation of the thermoradiation phenomenon in a nitrogen atmosphere since present plans for terminal dry heat sterilization of spacecraft call for a nitrogen environment. The oxygen dependency of radiation inactivation of organisms (at ambient temperatures) is well known, but this effect has not been previously investigated at elevated temperatures. The following series of experiments were run this quarter:

<u>Temperature</u>	<u>Dose Rate</u>
Ambient (23°C)	660 krads/hour
Ambient (23°C)	32 krads/hour
95°C	None
95°C	4 krads/hour
95°C	11 krads/hour
95°C	38 krads/hour
95°C	85 krads/hour

The inactivation rate appears to depend upon dose rate, but in a less striking manner than with a standard atmosphere. A synergism exists and the "optimal" dose rate occurs in the same region previously identified for a standard atmosphere. The work at ambient temperature was in agreement with behavior previously reported in the literature.

Mechanism Studies in Thermoradiation Inactivation. Several approaches are being taken into the investigation of the mechanisms of thermoradiation inactivation. These approaches involve both experimental and modeling efforts.

Assuming that a spore may be viewed as a biological system composed of various classes of biopolymers, one approach is to study the individual response of members of each of these classes to thermoradiation treatment. It is expected that a correlation between at least one of these classes and spore inactivation can be gained by using previously developed models.

As a starting place, a bacterial virus, T4 phage, was exposed to thermoradiation and the kinetic data analyzed using a generalization of the thermoradiation inactivation model previously reported.

The T4 phage was chosen because the work of others suggested that, at least with radiation, the nucleic acid is the constituent primarily involved in inactivation. If so, phage studies present a reasonably efficient way of studying the effect of thermoradiation on this class of biopolymer.

As a result of these studies and analyses, the model of thermoradiation inactivation of spores has been slightly modified to include a term representative of "breakage". Also, one begins to conclude that the underlying causes of spore and phage inactivation by thermoradiation are very similar, possibly not differing in general reaction type, but only in the molecular stability inherent in the system.

Water Activity Experiments. Based on thermal inactivation modeling efforts which describe spore inactivation in chemical kinetic terms, it was postulated in previous reports that the behavior of spore inactivation as a function of water activity (A_w), for low values of A_w , might be of a "spiked" character. A spike in this region would have practical implications because of its proximity to the actual RH value anticipated for spacecraft sterilization. Previous activity has centered on the development of apparatus capable of precise control and measurement of RH in low ranges. Preliminary data was obtained this quarter using this system. These data suggest strongly that such a spike does indeed exist.

Computerized Identification System. This activity was completed during the past quarter, and a document describing it is in press. A computer program, suitable for inclusion in the Lunar Information System, has been written. Using the biological data taken by the PHS Spacecraft Bioassay Laboratory from the Apollo 10, 11, 12 and 13 spacecraft, the program is capable of obtaining the same identification as PHS personnel in excess of 90% of the time. The remaining disagreement is attributable to two causes: errors in data recording or transcription to punched cards, and the fact that occasional identifications are made at the bench with samples in hand that, from the computer's point of view, cannot be identified (being unacceptably different from defined categories based on the information given it).

Thermoradiation Sterilization

- A. Description. The objective of this activity is to thoroughly investigate the sterilizing effects of combinations of heat and radiation, and to assess the practicality of this process for spacecraft sterilization. Thermoradiation offers the possibility of sterilization at temperatures less than 100°C at low dose rates of approximately 10 krad/hour. This is possible because of a synergistic effect in bacterial inactivation which has been observed when combinations of heat and gamma radiation are applied simultaneously. Should any spacecraft components prove to be heat sensitive at high temperatures, thermoradiation offers a potential means of overcoming reliability problems.

There appear to be potentially significant spin-off possibilities for the thermoradiation process in the sterilization of drugs, pharmaceuticals, cosmetics, medical products and food. This is particularly true at the lower temperatures.

B. Progress.

1. Activities this quarter were primarily concerned with the effectiveness of thermoradiation in a nitrogen atmosphere. Since present plans for terminal dry heat sterilization of spacecraft include the use of dry nitrogen as a working gas, it is necessary to determine how thermoradiation inactivation is effected in an oxygen free atmosphere. The oxygen dependency of radiation

inactivation has been established by a number of investigators. For example, Proctor⁽¹⁾ found that an increase of 33% in the inactivation dose was required for B. subtilis spores in dry nitrogen as compared to irradiation in air. Hollaender⁽²⁾ found even greater differences, that is, reduced radiation inactivation in nitrogen as compared to air for E. coli. No previous work was found that was applicable to nitrogen effects on simultaneous gamma radiation and dry heat inactivation.

Materials and Methods. Basic equipment used in the nitrogen experiments was the same as for previous thermoradiation experimentation. Ovens used were sufficiently tight to permit the use of bottled nitrogen without excessive expenditures. A nitrogen flow of 1/4 to 1/2 CFM was maintained through the .45 cu. ft. ovens using commercial grade nitrogen similar to what will most likely be used in terminal spacecraft sterilization. This grade has the following specifications: Nitrogen, liquid pumped, 99.995% pure. Impurities not to exceed, 9 gr H₂O/1000 cu. ft., 30 ppm oxygen, 5 ppm carbon bearing gases, 5 ppm hydrogen, a trace of argon, helium, neon, dewpoint less than -76°F.

(1) Proctor, Bernard E., Goldblith, Samuel A., Oberle, Marilyn and Miller, William C., "Radiosensitivity of Bacillus subtilis under different environmental conditions," Radiation Research 3:295-303, 1955.

(2) Hollaender, A., Stapleton, G. E., and Martin, F. L., "X-Ray sensitivity of E. coli as modified by oxygen tension," Nature, 167, 103 1951.

B. subtilis spores in an ethanol suspension were pipetted on to 1" x 1" aluminum planchettes. After evaporation of ethanol, samples were placed in a vacuum over Drierite for 15 hours prior to sterilization treatment. Experiments were performed using heat alone, radiation alone and then thermoradiation in nitrogen to determine the individual effects on the heat and radiation inactivation components as well as for thermoradiation.

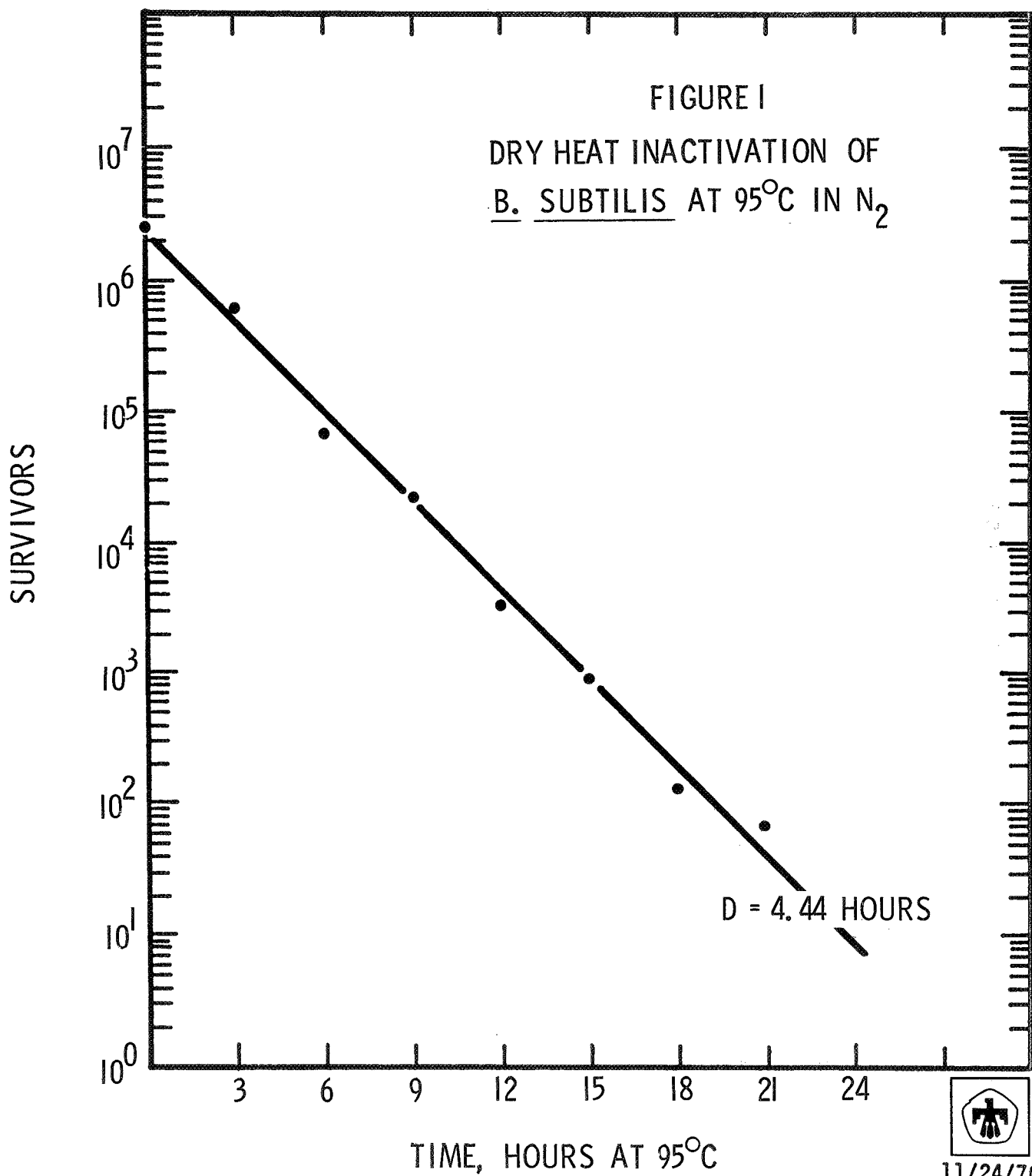
Results. A dry heat experiment at 95°C in N₂ was performed to establish the inactivation rate of heat alone. The results are shown in Figure 1. The D-value obtained from this condition was 4.4 hours.

The next experiments were to determine radiation inactivation in N₂ at room temperature. Experiments were completed at two dose rates with results shown in Figures 2 and 3. D-values obtained ranged from 133 krads per log reduction at the high dose rate, Figure 2, to 145 krads per log reduction at the lower dose rate, Figure 3. The experimentation is not sufficiently complete to establish any definition of the dose rate sensitivity in N₂. It is, however, evident that the dose required for radiation inactivation in N₂ is greater than the radiation dose required in air. This corroborates the work previously cited in References 1 and 2. Experiments are planned at three additional dose rates to define the dose rate characteristic in N₂.

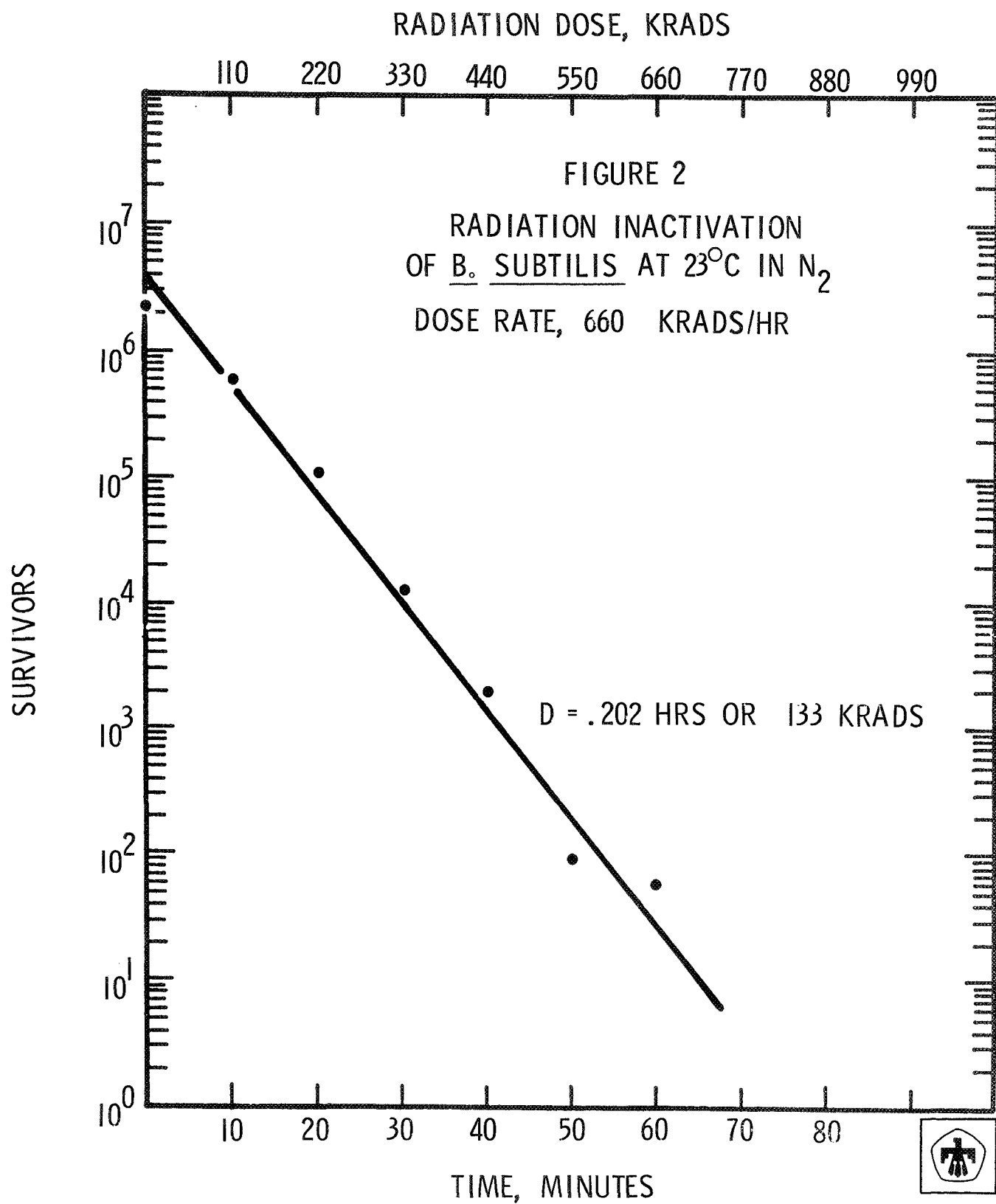
Thermoradiation experiments at 95°C in N₂ were next completed at dose rates of 4 krads/hour, 11 krads/hour, 38 krads/hour and

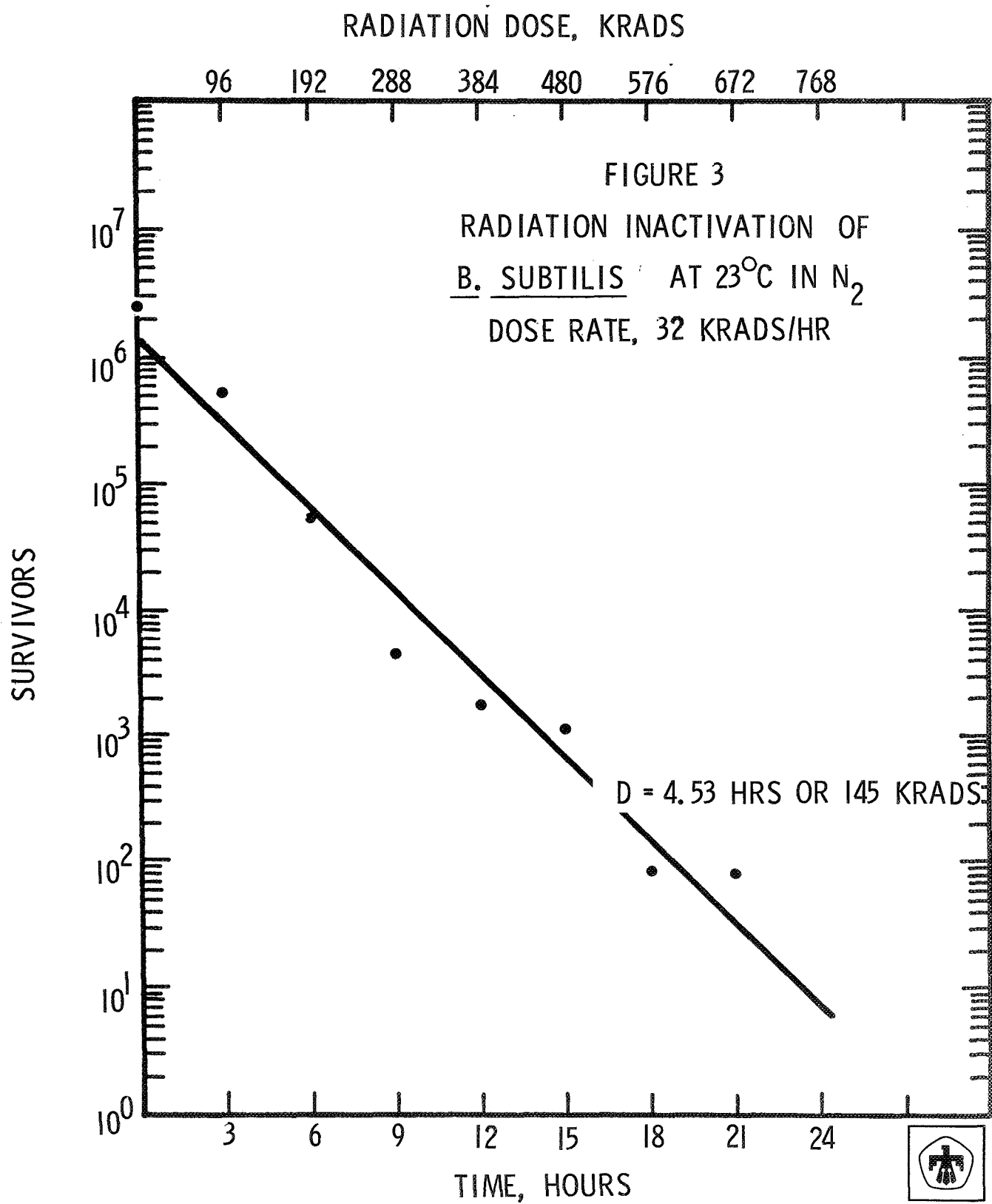
85 krads/hour. The resulting curves are shown in Figures 4, 5, 6 and 7 and are summarized in Figure 8. The D-values varied from about 1 hour at the 85 krad/hour rate to 3.4 hours at 4 krad/hour. An interesting comparison is then made in Figure 9, where the thermoradiation data in N_2 is compared to similar thermoradiation data in air. Although the D-value in N_2 for high dose rates is generally somewhat higher than in air, at the optimal dose rate of 10 krads/hour, there is essentially no difference in D-value.

2. An additional area under investigation is the thermoradiation effectiveness on mated surface contamination. Initial contacts have been made with I. J. Pflug, University of Minnesota, and reports have been obtained on the system used by the University of Minnesota for the dry heat studies of mated surface contamination. We hope to make use of established techniques wherever possible.



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RADIATION DOSE, KRADS

12 24 36 48 60 72 84 96 108

FIGURE 4

THERMORADIATION INACTIVATION FOR

B. SUBTILIS AT 95°C IN N₂

DOSE RATE 4 KRADS/HOUR

SURVIVORS

10⁷
10⁶
10⁵
10⁴
10³
10²
10¹
10⁰

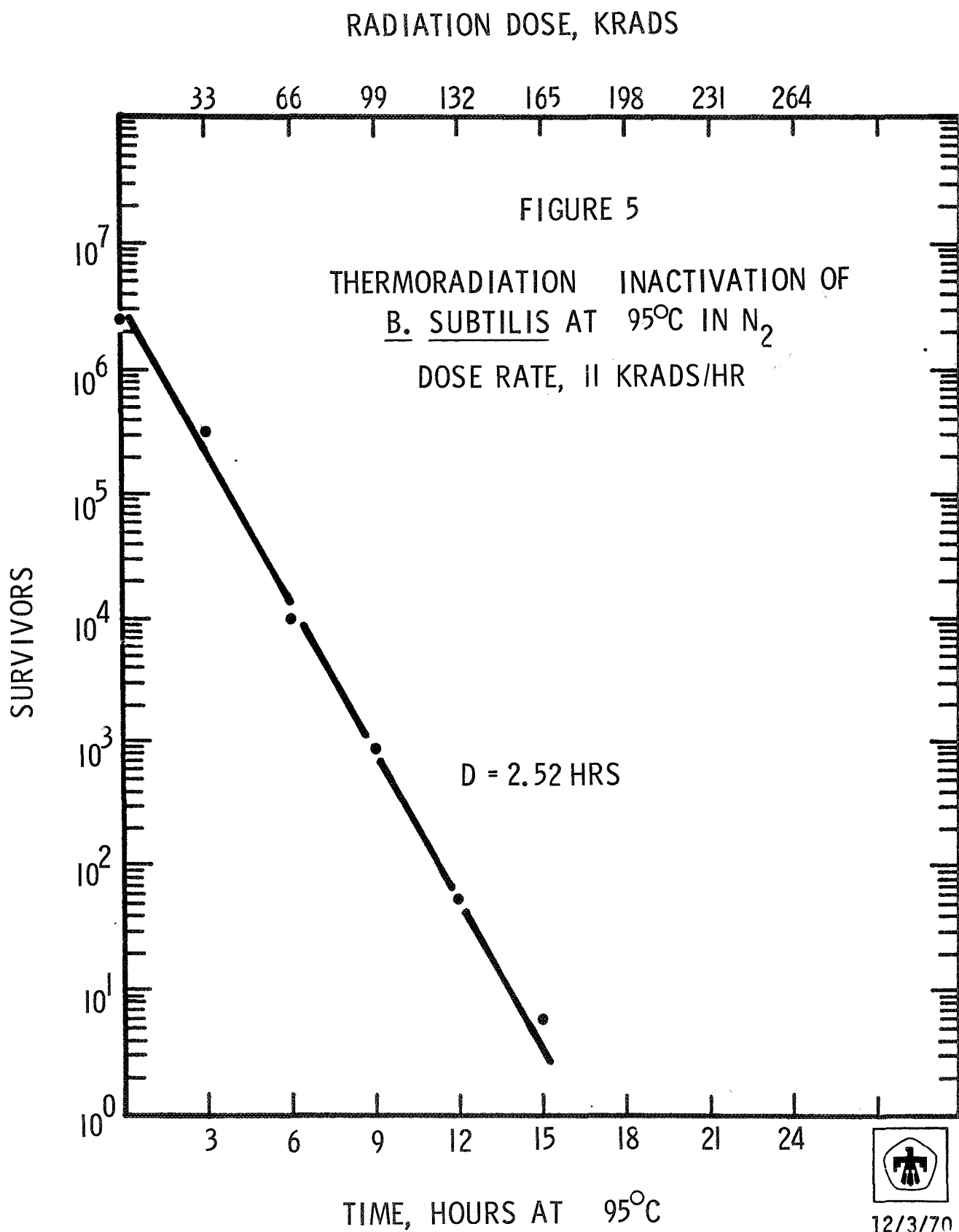
D = 3.42 HRS

TIME, HOURS AT 95°C

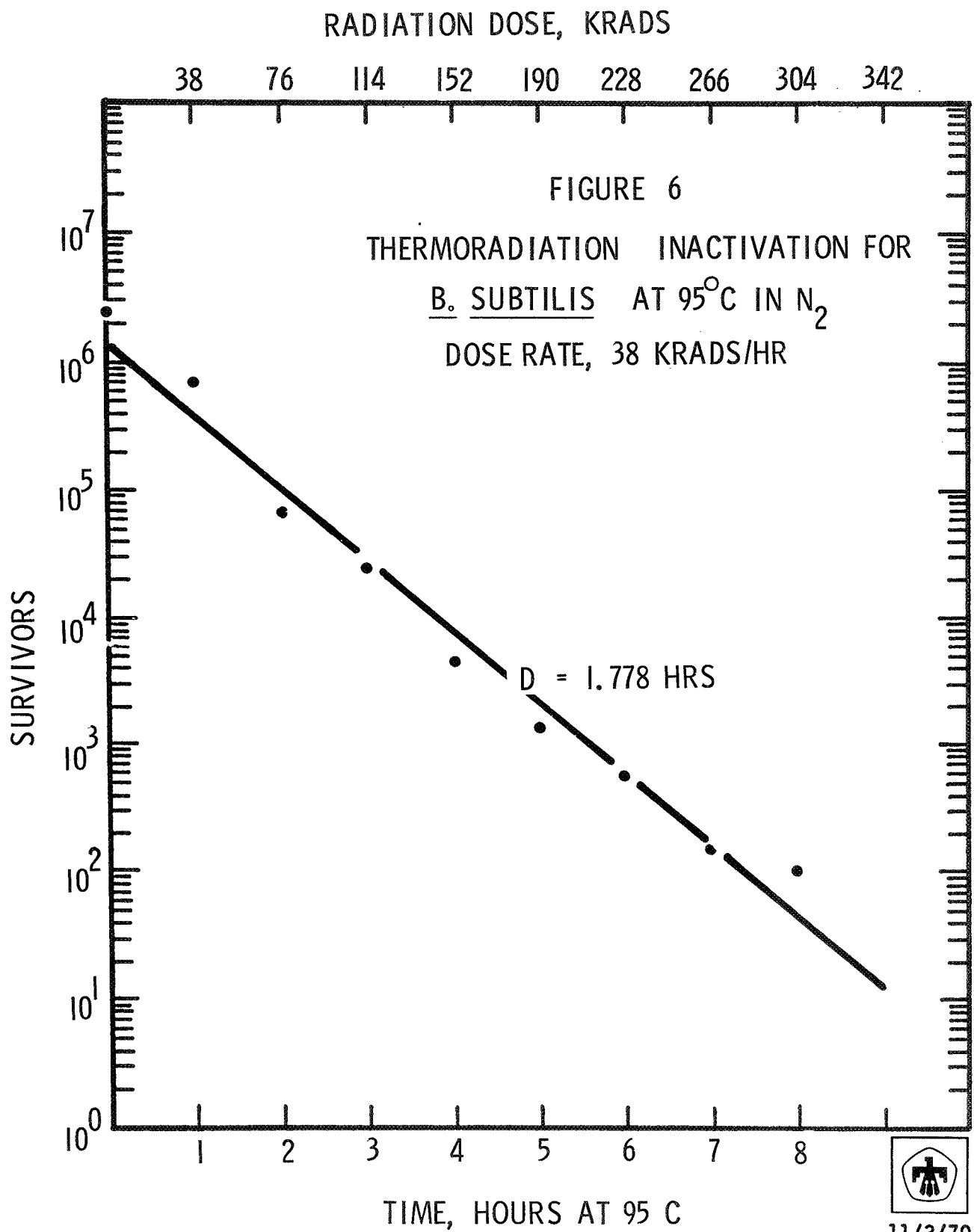
3 6 9 12 15 18 21 24



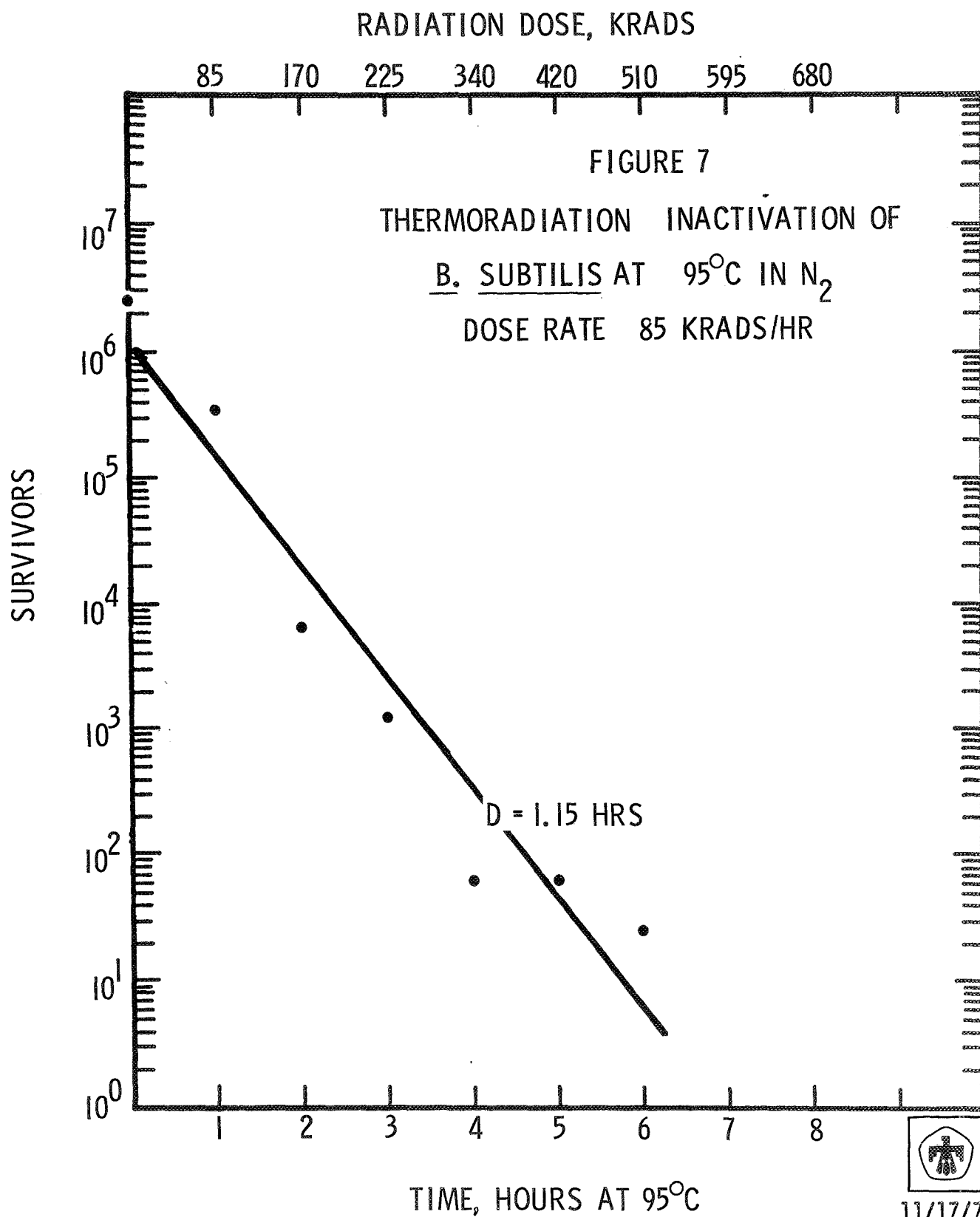
12/10/70

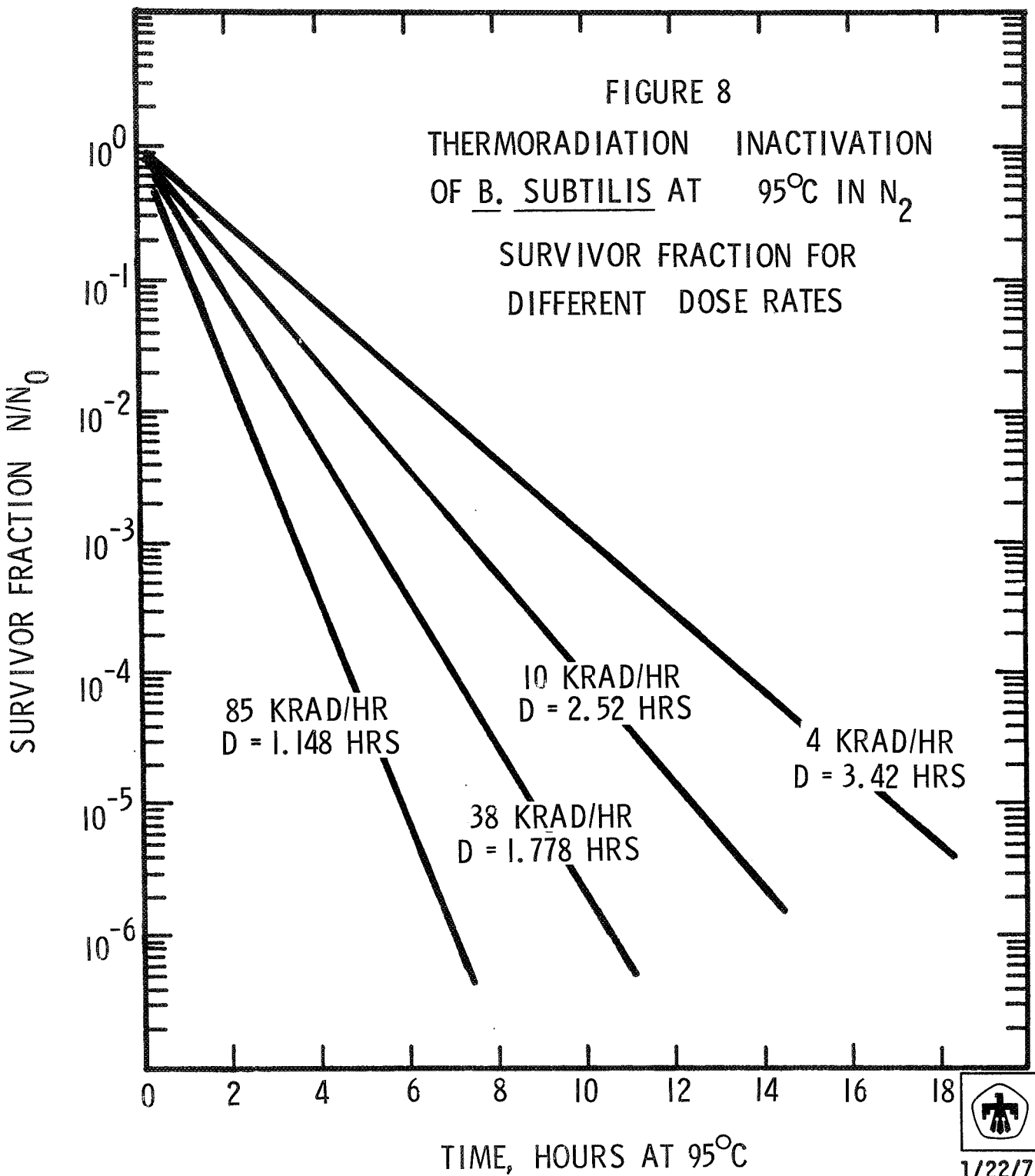


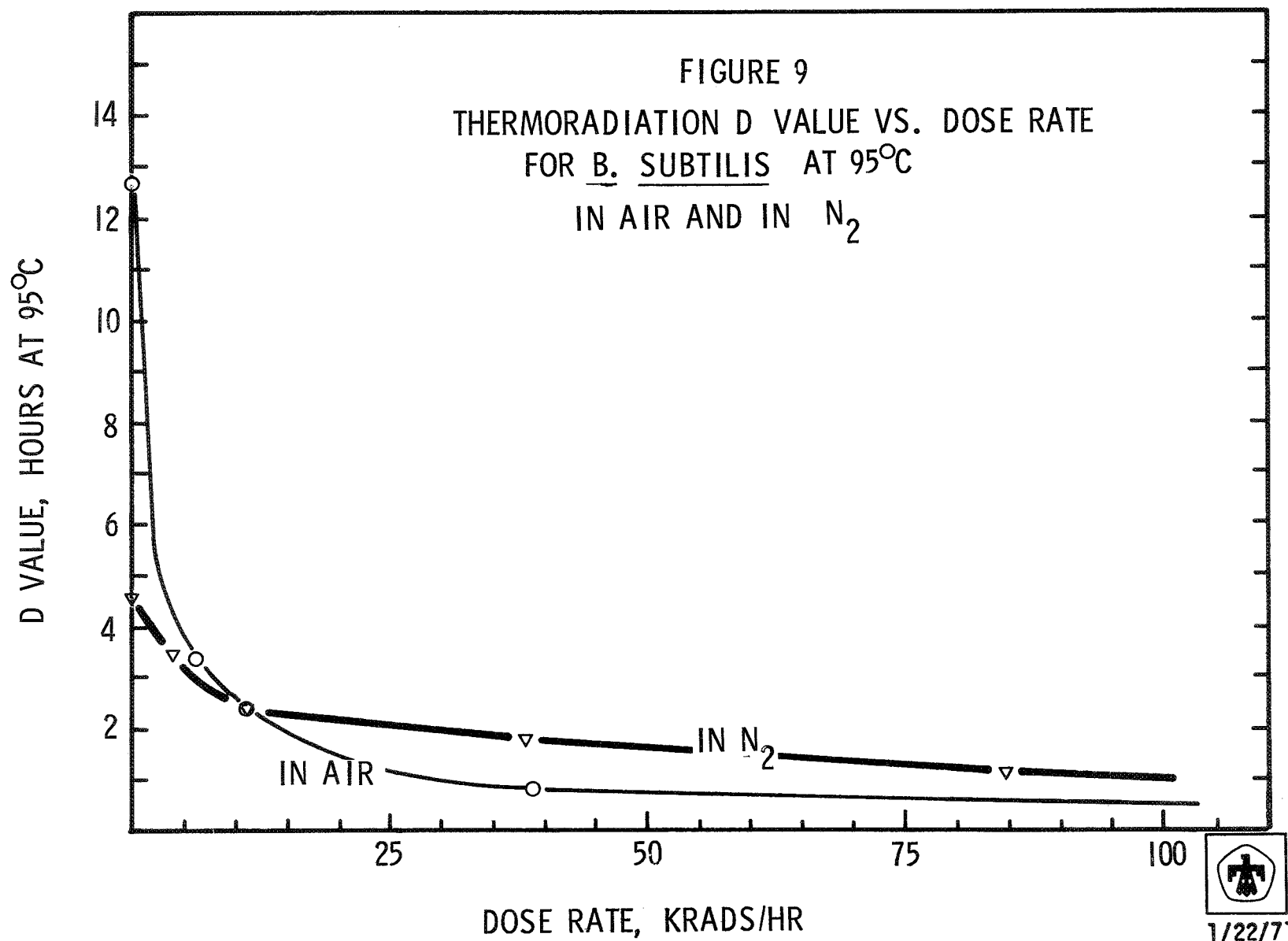
12/3/70



11/3/70







1/22/71

Mechanism Studies in Thermoradiation Inactivation

- A. Description. Analyses of the inactivation of spores by dry heat have been presented in previous reports (SC-RR-70-439 and SC-RR-67-256). These analyses have yielded a mathematical expression (model) which successfully describes and predicts the response of bacterial spores to dry heat inactivation at any given condition of time and temperature. The basic premise of these analyses has been that spore inactivation proceeds by some event or chain of events which have the property(ies) of a chemical reaction and, therefore, will obey the theories of reaction rate kinetics. This premise serves as the conceptual basis for the model. Inactivation is considered to result from physico-chemical transformations occurring within the spore, and such inactivating transformations are held to be governed by the theories of chemical kinetics. Formally, the model attempts to relate a kinetic expression to the physico-chemical changes occurring within the spore. Therefore, although the model is derived from an analysis of survival data, it is not simply an expression obtained by curve fitting of the data. The conceptual basis of the model implies that the terms of the mathematical expression which constitute the model should reflect the various inactivating mechanisms operative in the organisms. An analysis of microbial inactivation with such a conceptual basis should yield a model which predicts not only the response of bacterial spores to a given inactivating treatment, but should also give insights into the mechanism(s) of the inactivating process.

It has been possible to analyze the kinetic data obtained for the synergistic inactivation of spores by thermoradiation (SC-RR-69-857 and SC-RR-70-423) in a similar manner and to develop a model (SC-RR-70-203) which accurately predicts the rate of spore inactivation as a function of radiation dose rate and temperature and the extent of spore survival after exposure to a given dose of combined heat and ionizing radiation. The fact that a one term model often successfully describes the dry heat inactivation of spores, while a two term model is needed to describe the synergistic inactivation of spores by thermoradiation may have implications in terms of the mechanistic processes involved in the two inactivating environments.

This quarter's research efforts were directed towards identifying the biopolymer substrates within the spore which experience the inactivating physico-chemical transformations in a thermoradiation environment. The approach used in this study is to consider a spore as a biological system composed of various classes of biopolymers (nucleic acids, proteins, lipids). The method selected for studying the mechanism of the synergistic inactivation of spores by thermoradiation is to study the individual response of each class of biopolymer to thermoradiation, and to observe if any correlations exist between the inactivation of a given class of biopolymer and the inactivation of spores.

A bacterial virus, T⁴ phage, has been exposed to thermoradiation and the kinetic data has been analyzed in terms of an extension of the thermoradiation inactivation model for spores (SC-RR-70-203).

T4 phage consists essentially of a nucleic-acid molecule enclosed in a protein coat and as such offers the simplest biological entity which exhibits a biologically functional nucleic acid. Therefore, by subjecting T4 phage to thermoradiation and by observing the effect of such exposure on the biological functioning of the virus one can gain additional information on how the biological activity of nucleic acids is affected by thermoradiation. (See Pollard, E., and Setlow, J., Arch. Biochem. Biophys. 43, 136-142 (1953) for indication that it is, indeed, the nucleic acid that is primarily affected.) An understanding of the effect of the combined inactivating environment upon the functional nucleic acid of the T⁴ phage within a spore could provide a base for understanding the role of the nucleic acid in the inactivation of the spore by thermoradiation.

B. Progress

1. Experimental Procedures. Three different series of experiments were run. These are designated A, B and C below. Experimental protocol A consisted of exposing a T4 phage suspension (approximately 5×10^6 phage, 1% trypticase soy broth) to heat alone and to a composite environment of heat and gamma radiation. In each case, samples consisted of 2 ml amounts of the 5×10^6 phage per ml suspension placed in test tubes which were covered with sterile aluminum foil. Heating was accomplished using a Blue M, "Magni-Whirl," constant temperature bath which provides a control of $\pm 0.1^\circ\text{C}$ at the designated temperature. The test temperatures used

for both the heat alone and the thermoradiation experiments were 23, 55, 60, 64 and 66°C. Duplicate sets of experiments were run at each temperature with the exception of the 23°C and 60°C studies. Six experiments were carried out at 23°C and only one experiment was performed at 60°C. For the thermoradiation runs, a dose rate of approximately 30.6 kilorads per hour was used in each case. For all experiments, duplicate dilutions were prepared from each of the duplicate samples which were taken at exposure times of 0, 30 and 60 minutes. The assay of the phage counts was accomplished using standard plaque counting procedures with E. coli as the host bacteria. A detailed description of this assay procedure will be presented in another report now being prepared.

In experimental protocol B, both heat alone and thermoradiation experiments were performed at 23°C and 64°C as explained previously with the exception that the radiation dose rate was lowered to approximately 7.9 kilorads per hour. This was done to determine the dose rate dependency of the rate of phage inactivation in the composite environment. Experimental protocol C consisted of irradiating the phage suspension at a dose rate of 30.6 kilorads per hour at 64°C and 66°C while using an initial viable phage population of approximately 10^8 phage per ml rather than approximately 5×10^6 phage per ml. This was done to determine the initial concentration dependency of the rate of phage inactivation in the composite environment.

2. Experimental Results. In each case, the inactivation of a freshly prepared phage population provided a very straight inactivation curve on a semilog plot. However, it was observed that phage stocks over three weeks old would exhibit a biphasic inactivation curve with the initial rate of inactivation greater than the later rate. This was seen for both the heat alone and the thermoradiation treatments.

Since the semilog plots of the phage inactivation are very linear, the expected population of phage, $E[P(t)]$, as a function of time, t , may be represented by the expression

$$E[P(t)] = P(o)e^{-k_T t} \quad (1)$$

for the thermal inactivation and

$$E[P(t)] = P(o)e^{-kt} \quad (2)$$

for the thermoradiation inactivation where $P(o)$ is the initial viable phage population and k_T and k are the inactivation rate parameters in inverse hours for thermal inactivation and thermoradiation inactivation respectively. For each experiment values for k_T and k have been determined by fitting the natural logarithm of the data with a straight line using a least squares norm. These results for experimental protocol A are presented in Table I.

For experimental protocol B, the value of k at the lower dose rate of 7.9 kilorads per hour was found to be approximately 0.1719 hour^{-1} at 23°C and approximately 2.682 hour^{-1} at a temperature of 64°C . Comparison with analogous values in Table I provides an indication of the dose rate dependency of k .

When the initial phage population was increased from approximately 5×10^6 phage per ml to approximately 10^9 phage per ml, at 30.6 kilorads per hour the value for k was found to be 3.813 hour^{-1} at 64°C and 8.527 hour^{-1} at 66°C . These values are lower than the means of the analogous values in Table I.

A graphic example of the phage inactivation which shows the comparison between thermal and thermoradiation inactivation rates is shown in Figure 1. This graph shows very dramatically that the phage are inactivated synergistically by thermoradiation since the degree of inactivation at any time in the heat and radiation environment is greater than that of the sum of the inactivations of the thermal environment and the radiation environment at ambient temperature.

3. Inactivation Modeling for T4 Phage. With the kinetic model representing the thermoradiation inactivation of the T4 phage,

$$E[P(t)] = P(0)e^{-kt} ,$$

TABLE I
THERMAL AND THERMORADIATION INACTIVATION
RATES OF T4 PHAGE

Temperature °C	Thermal Inactivation Rate k_T Hour ⁻¹	Thermoradiation Inactivation Rate* k Hour ⁻¹
23	≈ 0	0.641
23	≈ 0	0.666
23	≈ 0	0.649
23	≈ 0	0.732
23	≈ 0	0.751
23	≈ 0	0.708
55	0.235	1.019
55	0.150	0.973
60	0.543	2.118
64	2.116	4.554
64	2.3810	4.438
66	6.014	8.887
66	6.595	11.439

*Radiation dose rate = 30.6 kilorads/hour

(Equation 2), an attempt was made to further analyze the parameter k . Since the radiation induced inactivation of the phage is relatively constant for temperatures below approximately 50°C, a three term model for the parameter k has been selected. This expression is

$$k = k_T + k_R + k_{TR} \quad (3)$$

where k_T is the inactivation rate due to heat alone, k_R is the temperature independent radiation inactivation rate associated with bond breakage or other point defects in a critical substrate resulting from the action of gamma photons, and k_{TR} is the temperature dependent inactivation rate of a chemical process sponsored by the action of the radiation. One interpretation of k_{TR} is that it represents the rate at which free radicals formed by the radiation react with a critical substrate such as the DNA of the phage. The forms of the three rate parameters for T4 phage are:

$$k_T = \frac{KT}{h} e^{245.451/R} \cdot e^{-107416/RT}, \quad (4)$$

$$k_R = 0.0227 r_d, \quad (5)$$

and

$$k_{TR} = r_d^{481.65/T} \cdot e^{95.099} \cdot e^{-66564.25/RT} \quad (6)$$

where

$$K = 1.38045 \times 10^{-16} \text{ erg./deg.Kelvin,}$$

$$h = 1.8403 \times 10^{-30} \text{ erg} \cdot \text{hours,}$$

$$R = 1.98726 \text{ calories/deg./mole,}$$

$$T = \text{temperature in degrees Kelvin, and}$$

$$r_d = \text{radiation dose rate in kilorads/hour.}$$

At a dose rate of 30.6 kilorads/hour the parameter k_{TR} may be written

$$k_{TR} = e^{95.099} \cdot e^{-63289.9/RT}. \quad (7)$$

The parameter values in Equations 4, 6 and 7 and the forms of the reaction rate expressions are completely analogous to those reported for spore inactivation in SC-RR-70-203, and in QR-15 and QR-16. The actual parameters values of k_T and k_{TR} were determined by minimizing the sum of the absolute values of the difference between the mean of the data at each temperature and dose rate and the value provided by the model. Data for k_T was readily available from the actual experiments, and data for k_{TR} was obtained by subtracting the sum of k_T and k_R from k at each temperature. The form of k_R in Equation 6 was obtained by fitting the means of the inactivation rate data at 23°C and dose rates of 0, 7.9 and 30.6 kilorads per hour with a straight line using a least squares norm.

Figure 2 provides a comparison of the model predictions of $k = k_T + k_R + k_{TR}$ at a dose rate of 30.6 kilorads per hour with the means of the experimentally determined values of k at each temperature. Figure 3 shows two separate plots of k_T and $k_R + k_{TR}$ obtained from the model definitions of these terms along with the means of the corresponding experimentally determined values. An important point concerning the information presented in Figure 3 has to do with the relative placement of the curve of $k_{TR} + k_R$ and of k_T . People who have investigated the use of combinations of heat and various kinds of radiation for the inactivation of various biological systems in the past have reported seemingly conflicting data on the action of the combined environment and on the existence of synergistic inactivation. An explanation of these conflicting reports may be obtained from Figure 3. In the case of T4 phage, anyone checking the effect of temperature changes in the range of temperatures up to approximately 50°C on the radiation sensitivity of T4 phage inactivation would report no noticeable effect. This is true since the k_{TR} term representing the inactivating action of the radiation induced free radicals is essentially zero for temperatures below 50°C, and the k_R term is constant. On the other hand, for temperatures above approximately 66°C the k_T term in the inactivation expression of k would completely dominate the k_{TR} term since k_T is becoming larger at a much faster rate than is

k_{TR} with increases in temperature. Therefore, anyone investigating thermoradiation inactivation of T4 phage at temperatures above 66°C would probably report that no synergistic inactivation existed since the k_{TR} term would be lost in the experimental noise when trying to determine it from the expression

$$k_{TR} = k - k_T - k_R. \quad (8)$$

In other words, even though synergistic inactivation does exist at the higher temperatures, it is not readily observable on an experimental basis. On the same basis, the scatter in experimentally determined values for k_{TR} becomes much greater at the higher temperature since the scatter in k becomes larger for very fast reactions and since k_{TR} provides only a very small percent of k .

3. Inactivation Modeling for Spores. Since a three term model for the inactivation rate parameter k was found to be required for T4 phage inactivation, the fact was made apparent that the inactivation of Bacillus subtilis var. niger spores in the dry state should depend upon the same general model when exposed to thermoradiation. This had not been observed previous to the phage work since all experiments with spores had been carried out at temperatures greater than 23°C for which range there was an evident temperature sensitivity for the radiation induced inactivation. A preliminary form of the k_R term for spore

inactivation has been determined by fitting the inactivation rates due to radiation at 25°C and several dose rates with a straight line using a least squares norm. This provides an approximate form for the parameter k_R of

$$k_R = 0.02336 r_d. \quad (9)$$

This expression is probably slightly in error and will be refined when radiation inactivation rates at lower temperatures are determined.

Using the expression for k_R defined in Equation 9, values of k_{TR} were determined at various temperatures and dose rates by subtracting the sum of k_R at the dose rate of interest and the value of k_T at the temperature of interest from the experimentally determined value of k . The values of k_{TR} determined in this manner were then used to determine the parameters of the k_{TR} model. This resulting model for the spores is

$$k_{TR} = r_d^{159.122/T} \cdot e^{16.2665} \cdot e^{-12944.08/RT}. \quad (10)$$

The above parameter values were found by minimizing the sum of the absolute values of the difference between the value of k_{TR} determined from the experimental data and the value predicted by the model using a numerical search routine on a CDC 6600 computer. The expression for k_{TR} at the dose rate of 30.6 kilorads/hour is

$$k_{TR} = e^{16.2665} e^{-11863.0/RT}. \quad (11)$$

Also, the unchanged form of the rate parameter k_T is

$$k_T = \frac{KT}{h} e^{12.63/R} e^{-33590.0/RT}. \quad (12)$$

The form of k resulting from the sum of the expressions in Equations 9, 10 and 12 do in fact provide better prediction of thermoradiation inactivation rates for spores in the temperature range from 30°C to 100°C than does the two term model for k . Therefore, the use of the three term model not only improves the model rationale by including the effect of temperature independent bond breakage, but also provides better predictions of experimental results.

5. Comparing Spore and Phage Inactivation Parameters. The fact that the phage were inactivated in a wet system whereas the spores were inactivated in a dry system provides some problems when comparing the two sets of inactivation parameters. However, one similarity between the two models does become readily apparent. This similarity exists in the k_R terms of Equations 5 and 9. The dose rate dependency of the temperature independent bond breakage appears to be practically the same for both the T4 phage and for the B. subtilis var. niger spores. Since both the T4 phage and the spores possess double stranded DNA and since the bond

breakage in the phage does probably occur within its nucleic acid structure, the similarity of the k_R terms could indicate that the spore's nucleic acid is also the site of the inactivating bond breakage. This point is emphasized by the fact that the breakage is seemingly independent of the suspending medium of the microorganisms, i.e., the phage were suspended in 1% T.S.B., whereas the spores were deposited dry on an aluminum foil.

The differences of the parameters in the two k_{TR} terms of Equations 6 and 10 are only reasonable since the generation, lifetime, mobility and action of any radiation induced free radicals would be very different in the wet and dry systems.

FIGURE 1
SYNERGISTIC INACTIVATION
OF T4 PHAGE

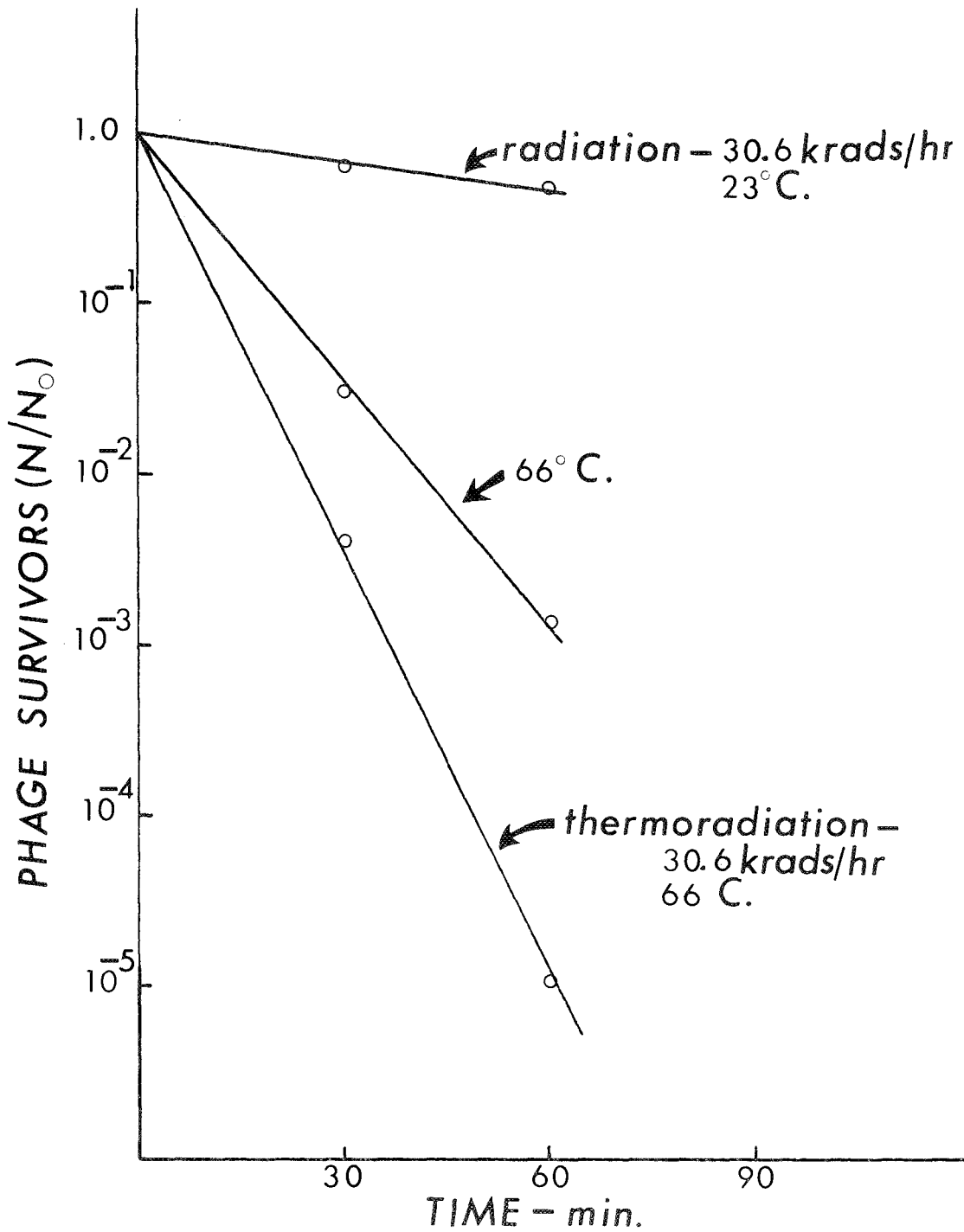


FIGURE 2

THERMORADIATION
INACTIVATION OF T4 PHAGE

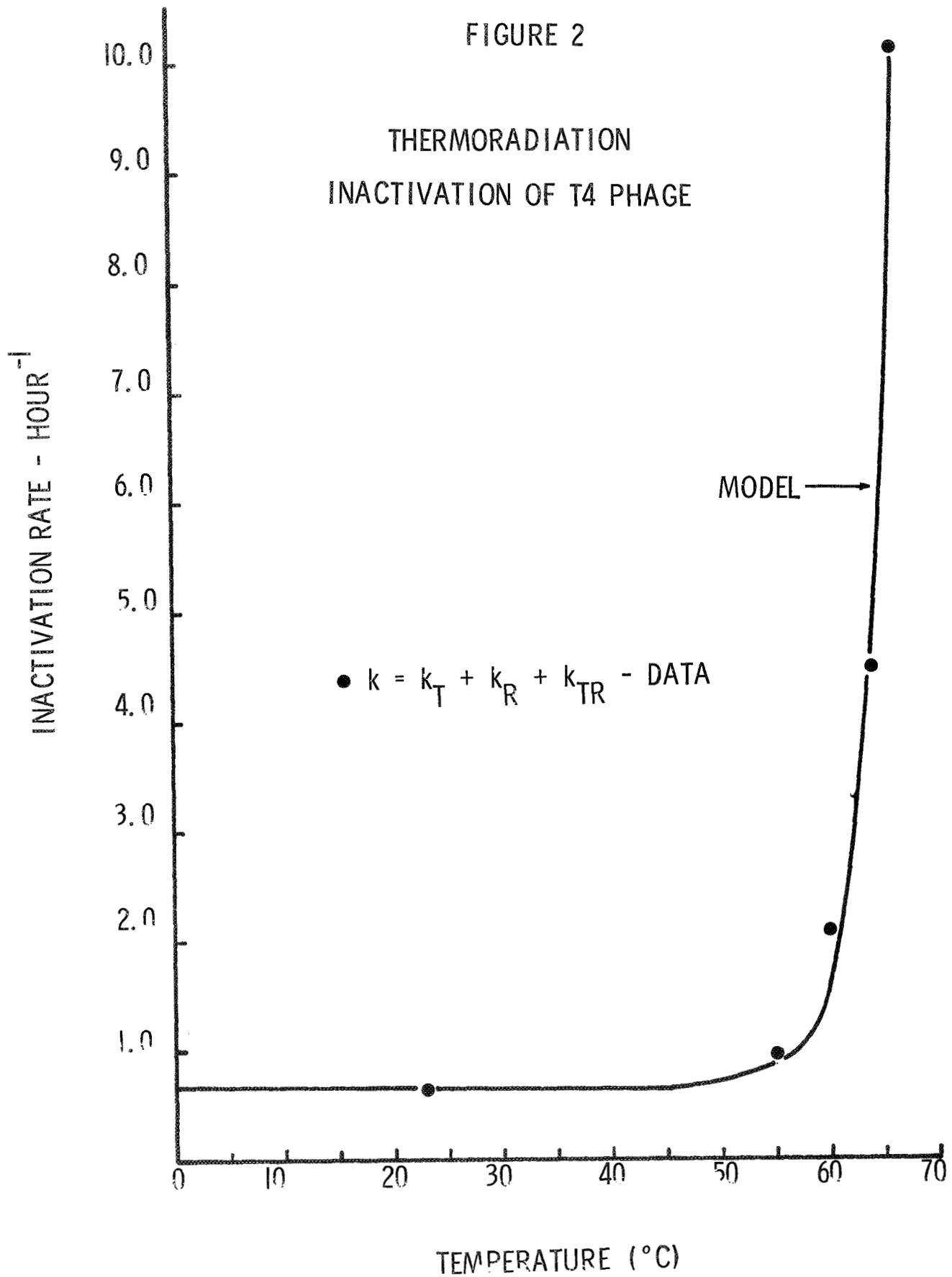
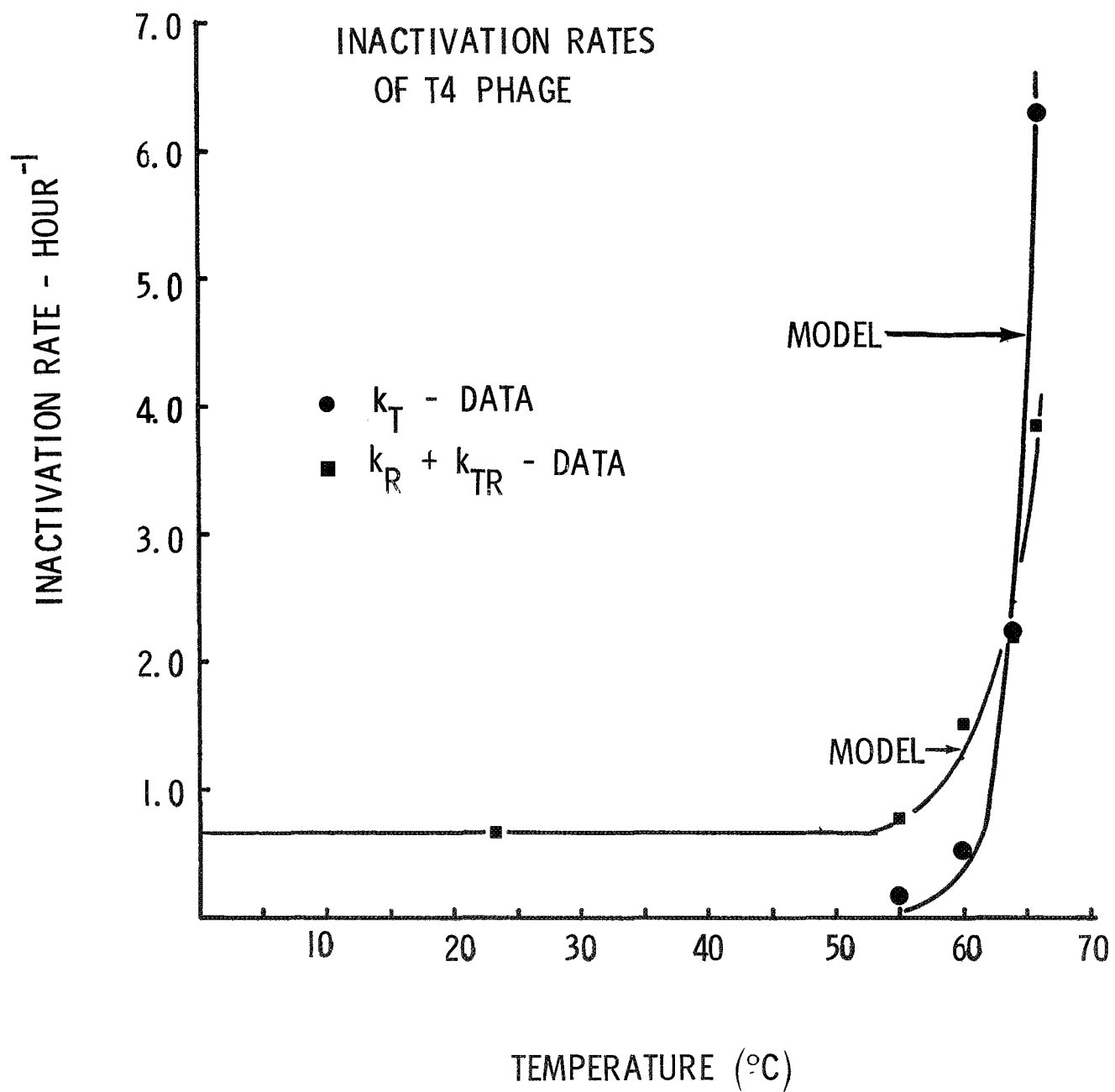


FIGURE 3



Water Activity Experiments

- A. Description. The desirability of closely controlled RH flow through systems in water activity experimentation was discussed in QR's 13, 14 and 15, and in SC-RR-70-439. Briefly, this desirability stems from the unknown effect of pressure on dry heat sterilization, QR's 15 and 16, SC-RR-70-439; and Brannen, Biophysik, 1, (1970) 55-59. In addition it has been hypothesized, SC-RR-70-439, that the water effect is primarily evident through changes in the activation entropy of chemical reactions.

Relative humidity control apparatus was designed and constructed by Whitfield, Garst and Lindell. Descriptions of these apparatus are available in SC-RR-70-409, SC-RR-70-775 and QR's 17 and 18.

- B. Progress. A series of A_w experiments, Table 1, were carried out from July through December 1970. Experiments 1 through 6 were performed utilizing the equipment described in SC-RR-70-409. With this equipment, we were able to cover relative humidities from .66% to 1.6% at 105°C, the exposure temperature for this series. In order to investigate sterilization at lower relative humidities, the humidity control system described in SC-RR-70-775 was used for experiments 7 through 26. With the increased capability of this equipment we were able to determine the effect of relative humidities at 105°C from .66% down to .0035%.

For all experiments other than 19, 21, 23 and 25, approximately

10^6 spores of B. subtilis were pipetted on aluminum foil discs. These foils were covered by another foil and replicates were loosely wired to a sample strip. The sample strips were then placed in a dessicator jar over Drierite and under vacuum of 22" Hg for 15 hours.

Although we had previously determined that samples prepared this way gave essentially the same results as spores inoculated on planchetts and directly exposed to the oven environment, we again verified this similarity in experiment 19. In this experiment samples prepared as above and sample strips carrying spores on open planchetts were simultaneously exposed to the oven environment. The results shown in Figure 1 assured us that covering the foils did not appreciably alter the results.

Experiments 21, 23 and 25 were preconditioning experiments. In experiment 21, one set of samples was preconditioned over P_2O_5 for one week while the other was preconditioned over P_2O_5 for 28 hours, both under 22" Hg vacuum. In experiment 23, samples were exposed to 30% relative humidity at ambient pressure for 15 hours. In experiment 25, the spores were lyophilized and held over P_2O_5 at a pressure of less than 1 mm Hg for a week. The results of these preconditioning experiments are shown in Figure 2. The results indicate to us that significantly different preconditioning environments do not materially effect the results.

Results for the relative humidities from .0125% and 1.6% are indicated in Figure 3. The abscissa gives relative humidity at 105°C and the ordinate shows the ratio of survivors after four hours

exposure at the indicated relative humidity to the initial population. The data indicate that the hypothesized dip in D value associated with monolayer state of adsorbed water probably exists. Further analysis and experiments are required for more definite conclusions.

Figure 4 shows a more complete set of results of $N(t)/N(0)$ after 4 hours at 105°C for the RH range .0035% to .14%. The RH scale here is considerably expanded.

Our next series will involve different temperatures and the same RH settings so that physico-chemical parameter values can be obtained for use in previously developed modeling of water activity. The modeling technique is described in SC-RR-70-439.

Equipment for controlling both pressure and RH is now being fabricated. The series of experiments to be carried out with this equipment will hopefully answer questions raised and discussed in SC-RR-70-439 concerning pressure effects in dry heat sterilization without unnecessary complications from RH effects.

TABLE I

Experiment No.	Date	% RH	$N(t)/N(0)$ After 4 Hrs. At 105°C	Atmospheric Pressure	RH Measured By
1	7/9/70	.66	4×10^{-2}	30.11 ~~30.23~~ " Hg	Sensor
2	7/13/70	.66	2×10^{-2}	30.15 ~~→~~ "	Sensor
3	7/14/70	.76	2×10^{-2}	30.19	Sensor
4	7/15/70	.99	10^{-2}	30.33 ~~30.39~~ "	Sensor
5	7/16/70	1.3	2×10^{-2}	30.25 ~~30.26~~ "	Sensor
6	7/21/70	1.6	6×10^{-2}	30.26 ~~→~~ "	Computed from Inputs
7	7/28/70	.22	10^{-3}	32.14 ~~30.17~~ "	Sensor
8	7/31/70	.18	10^{-3}	30.11 ~~30.09~~ "	Sensor
9	8/4/70	.25	10^{-3}	30.21 ~~30.17~~ "	Sensor
10	8/5/70	.32	10^{-3}	30.17 ~~30.20~~ "	Sensor
11	8/11/70	.09	5×10^{-5}	838 ~~839~~ m bars	Sensor
12	8/19/70			837 ~~836~~ "	Sensor Failure
13	9/9/70	.14	2×10^{-4}	839 ~~835~~ "	Sensor
14	9/11/70	.05	10^{-4}	844 ~~843~~ "	Dewpoint
15	9/15/70	.03	10^{-4}	834 ~~836~~ "	Dewpoint
16	9/15/70	.075	1.5×10^{-4}	836 ~~834~~ "	Dewpoint
17	9/17/70	.5	6×10^{-3}	838 ~~841~~ "	Dewpoint
18	9/18/70	.135	2×10^{-4}	835 ~~→~~ "	Dewpoint
19	12/14/70	.0035		837 ~~834~~ "	Dewpoint
20	12/18/70	.0035	2×10^{-4}	828 ~~829~~ "	Dewpoint
21	12/18/70	.0035		828 ~~829~~ "	Dewpoint
22	12/22/70	.0225	10^{-3}	828 ~~830~~ "	Dewpoint
23	12/22/70	.0225		828 ~~830~~ "	Dewpoint
24	12/23/70	.0125	10^{-3}	830	Dewpoint
25	12/29/70	.0035		838	Dewpoint
26	1/1/71	.005	10^{-3}	837 ~~835~~ "	Dewpoint

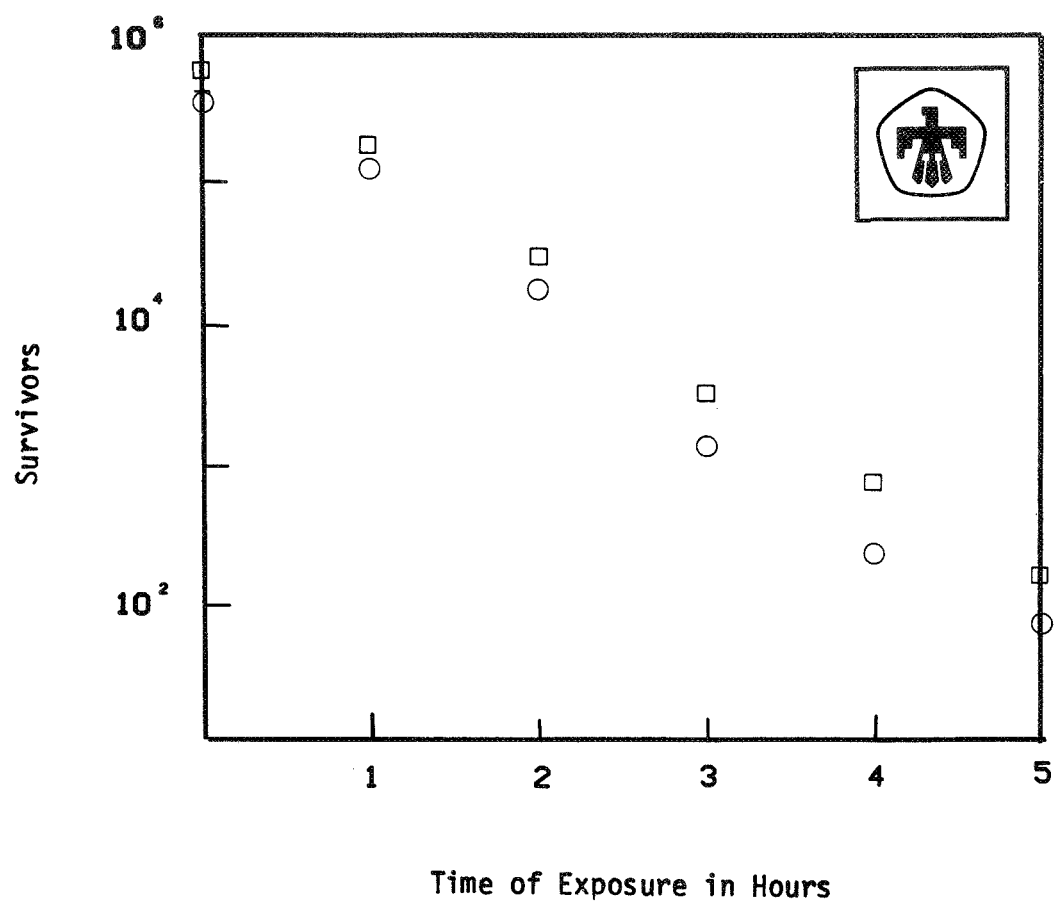


Figure 1. Comparison of spore survival for spores exposed on planchets, $\square$, to spores in aluminum foil, $\circ$, wafers. Exposure is at 105°C at a relative humidity of .0035%.

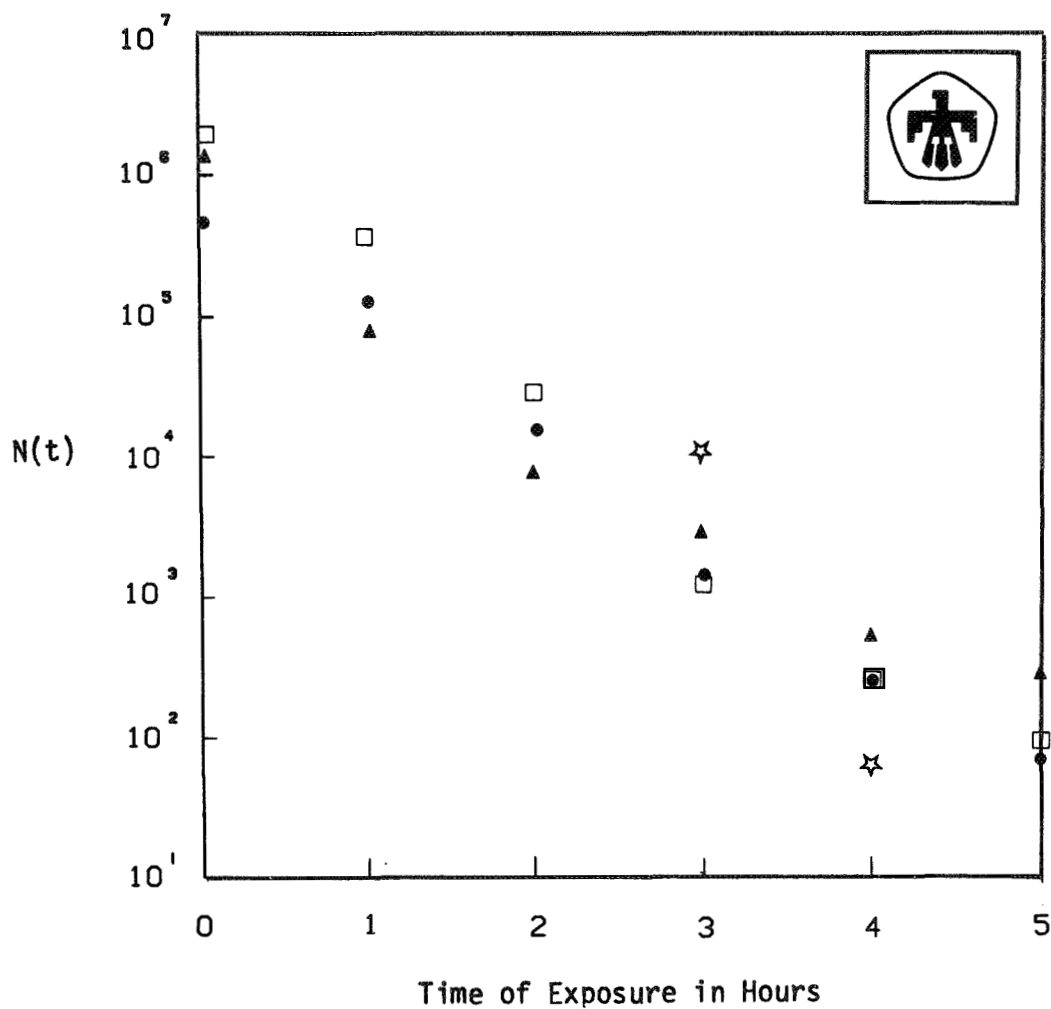


Figure 2. Preconditioning Experiments

Preconditioning Treatment

- 15 Hours over drierite @ 22" Hg vac.
- ☆ 15 Hours at ambient pressure 30% ambient RH
- 48 Hours over P₂O₅ @ 22" Hg Vac.
- ▲ Lyophilized - 1 week over P₂O₅

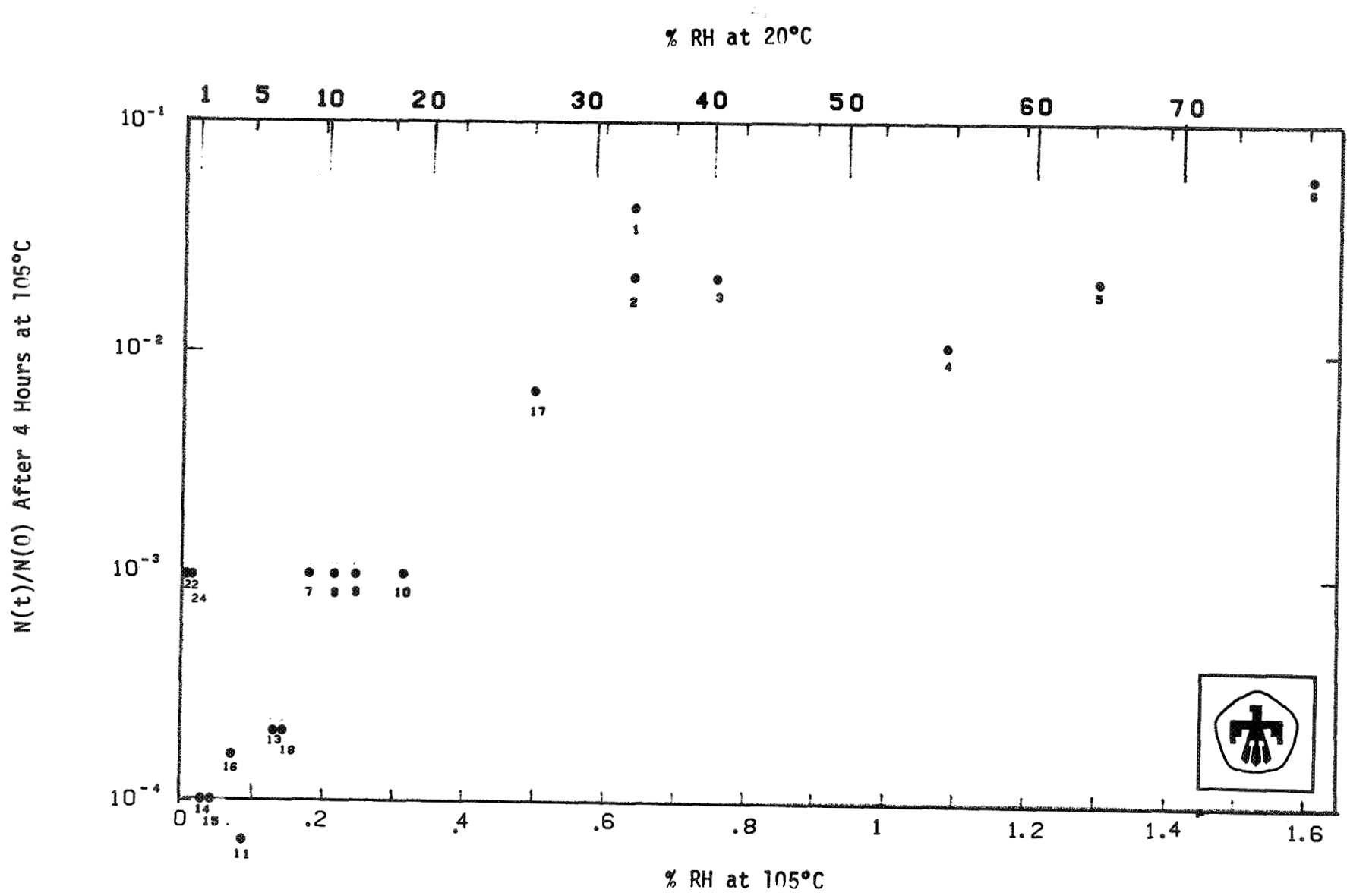


Figure 3. Water Activity Experiments

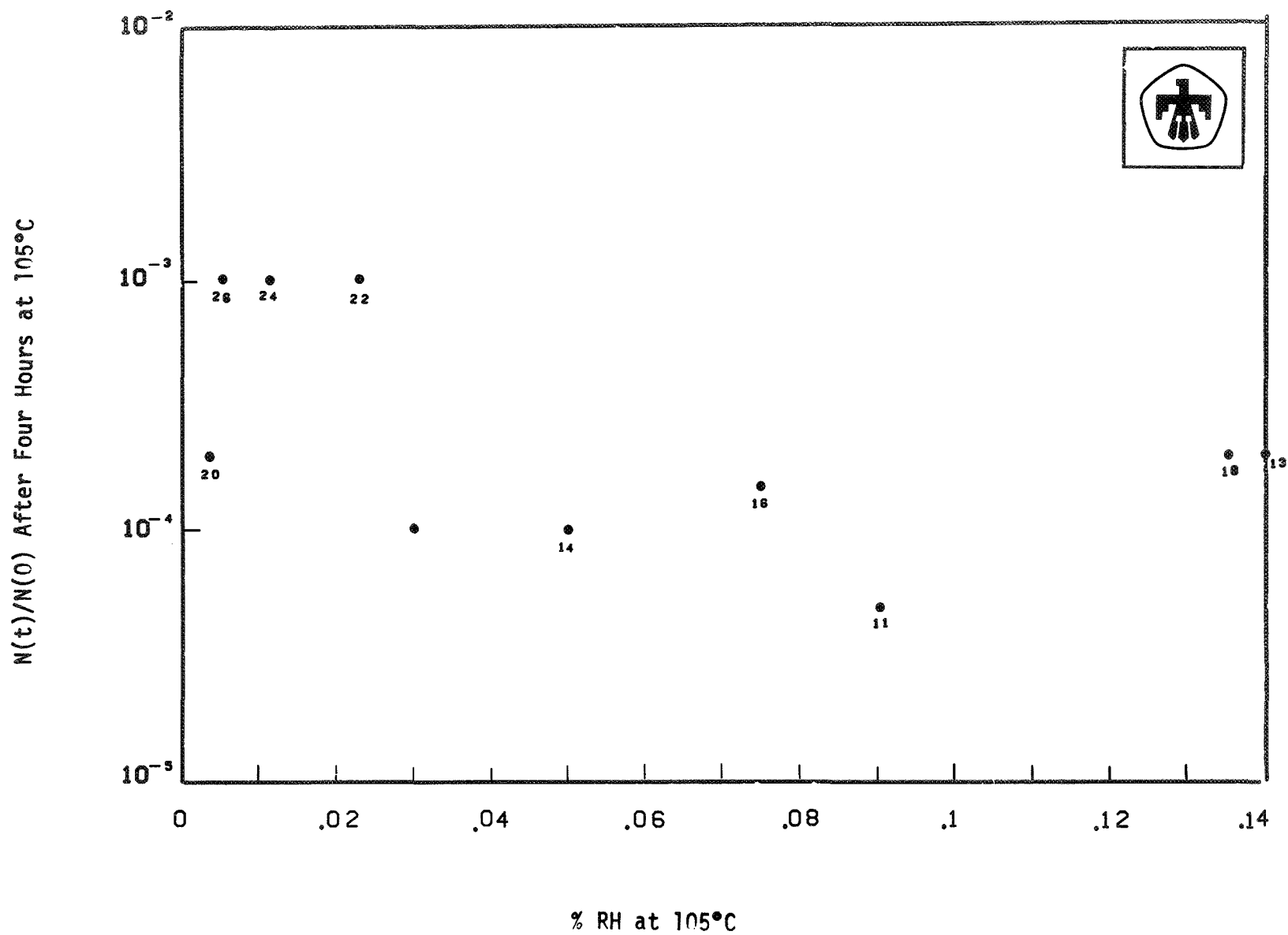


Figure 4. Water Activity Experiments

Computerized Identification System

- A. Description. The objective of this activity has been the development of a computer simulation of the PHS microbiological identification scheme being used at Cape Kennedy. Feasibility of such a simulation was reported in QR-15. The computer program accomplishing this simulation had to be written so that it could easily be modified to include the PHS improvements in their identification system. To accomplish this, a very general approach was adopted so that a program could be written which performed the identification in a manner similar to that used in the feasibility previously reported, but contained almost none of the PHS identification scheme. Hence, the scheme itself could be read in as an input and thus easily modified at any time with little or no change to the program itself. As a result of this abstract approach, almost any identification system could be simulated in addition to the one in use by the PHS. Thus it could be used for other applications in addition to the one for which it was developed, namely, incorporation into the Lunar Information System. This incorporation is planned to take place in time for use on Apollo 14.
- B. Progress. As indicated in the previous quarterly report, the program for the computerized identification scheme has been written and checked out. During this quarter, a Research Report documenting this has been written. Also, the program has been prepared for inclusion into QUAL in the Lunar Information System at Cape Kennedy.

The program has been used with available data on Apollos 10, 11, 12 and 13 to further check the program and obtain estimates of its accuracy and efficiency in simulating PHS identifications.

The PHS identification system was first used for Apollo 10 and although it has been used for all succeeding flights, improvements have been added. The biggest change occurred just prior to Apollo 13. As a result of the data already accumulated, large portions were completely rewritten and new identification schemes added to the system. The organism categories were redefined and the data reporting form was rewritten. There were no changes that affected that portion of the system which identified organisms as Staphylococcus or Micrococcus (Scheme A), so the program could be used with this data on a comparative basis for all Apollo missions sampled.

As discussed in the QR-15 where the program used to establish feasibility was reported, the PHS identification does not always lead to a unique identification because of occasional test variability. As a result, the PHS adopted a set of conventions to determine when an identification could be made. If all of the data is available and agrees with that specified for an organism category, there is no difficulty: this is termed an exact identification. It is possible for the data to disagree with a single organism category by a single test result, in which case an identification is made and referred to as one single disagreement. Similarly, disagreement of the data with that specified by the identification system can occur

between two organism categories by a single (perhaps different for each category) test result. Here one of the two is chosen as the identification. This is referred to as a single disagreement. Similarly, three single disagreements are allowed and one of the categories is chosen for the identification. Occasionally, biological variation makes necessary an identification even though two tests disagree with those specified by the identification system for an organism category. This is permitted if only one organism is involved and is denoted as one double disagreement. The computerized identification system program records by which of the above PHS conventions an identification was made. As incorporated in QUAL, the program will further simulate PHS practice in choosing between the multiple possibilities for identification, that is: which of the two or three possibilities are to be used as an identification will be incorporated in a uniform manner. During the checking of the program, all multiple identification possibilities were listed and compared with PHS identification. It was considered that agreement occurred if the PHS identification was among those listed by the computer.

The program (with the PHS identification system input data specified for Apollo 13) was run with the available data from all Apollo flights, that is, 10 through 13. As previously mentioned, that part of the system involving the Baird-Parker scheme (Scheme A) was not changed throughout these four missions. The results for

Scheme A are presented in Figure 1. In Figure 1 the entries are the number of times agreement, in the above sense, was obtained with the PHS identification. The column headed "Total" gives the total number of agreements obtained for each organism category in Scheme A. The column headed "PHS Total" lists the number of PHS identifications as obtained from QUALSUM. The difference between the two "total" columns represents the number of disagreements between the computerized scheme and the PHS identification. The percentages of agreement arising from each type of agreement and for the whole of Scheme A for each flight are listed below the body of the table. For convenient comparison, these are reproduced here:

	<u>Agreement</u>					
	Exact	1 Single	2 Single	3 Single	1 Double	Total
Apollo 10	38	25	19	9	0.6	91.6
Apollo 11	40	23	16	8	1.0	88.0
Apollo 12	46	25	15	7	0.1	93.1
Apollo 13	51	17	14	10	0.1	92.1

As can be seen, the total agreement runs somewhat in excess of 90%. In addition to evaluating the identification program, the above table illustrates a number of interesting points. For example, it would appear that with increasing experience the percentage of exact identification increases while the frequency with which the conventions dealing with one or two disagreements between the test data and those specified for the identified organism decrease. There is

an exception to this trend in the case of 3 single disagreements.

Since the program now operates in a mode that is compatible with the identification scheme for the whole of Apollo 13, a comparison for all categories on this mission can be obtained. In this case, for the 1020 samples taken for Apollo 13 there was agreement in 935 cases, giving 91.6% agreement on all categories on this mission. It should be noted that some keypunch errors still remain on the available data cards. Furthermore, inspection reveals that some of the disagreements are a result of incomplete data. Hence the 8.4% disagreements would in fact be smaller if these difficulties were corrected. Many of the disagreements arise from the computer's inability to make an identification within the aforementioned set of rules (e.g., it may find two double disagreements) while persons at the bench do assign an identification. This is a type of disagreement about which little can be done without further analytical effort.

This activity is essentially completed. An approach to simulating the PHS microbiological identification system has been developed which permitted the writing of a program which simulates the identification process with more than 91% accuracy. A document detailing this approach and program has been written. The program will be incorporated into QUAL as part of the Lunar Information System in time for use with the Apollo 14 data.

APOLLO 10

Org.	Exact	1 Single	2 Single	3 Single	1 Double	Total	PHS Total
1	44	52	36	0	0	132	142
2	184	20	46	0	0	250	261
3	13	3	11	7	0	34	38
4	31	73	8	8	2	122	131
5	50	97	1	0	4	152	163
6	43	40	31	5	2	121	134
7	19	8	0	90	0	117	142
8	6	4	53	0	0	63	85
9	16	6	39	0	0	61	68
10	0	0	0	0	0	0	0
11	12	2	3	0	0	17	20
12	0	0	0	0	0	0	0
13	51	10	17	2	0	80	94
14	0	2	0	0	0	2	4
Total	438	317	245	112	8	1151	1282
%	38	25	19	9	0.6	90	

APOLLO 12

Org.	Exact	1 Single	2 Single	3 Single	1 Double	Total	PHS Total
1	0	1	3	0	0	1	1
2	268	1	56	0	0	325	334
3	8	11	7	2	0	28	29
4	53	126	19	12	0	210	228
5	48	92	7	1	0	148	152
6	77	108	19	0	1	205	216
7	28	1	3	99	0	131	160
8	17	0	54	0	0	71	82
9	19	5	37	0	0	61	77
10	1	0	0	0	0	1	1
11	11	3	4	0	0	18	20
12	1	1	0	0	0	2	3
13	196	44	35	0	1	276	283
14	0	0	0	0	0	0	0
Total	727	393	241	114	2	1477	1566
%	46	25	15	7	0.1	94	

APOLLO 11

Org.	Exact	1 Single	2 Single	3 Single	1 Double	Total	PHS Total
1	11	4	3	5	0	23	28
2	257	0	81	1	0	339	355
3	4	0	0	1	0	5	6
4	55	134	18	3	2	212	242
5	76	123	0	0	0	199	215
6	71	39	30	1	0	141	154
7	32	14	3	100	2	151	207
8	11	1	67	0	2	81	103
9	11	1	11	0	1	24	36
10	0	0	0	0	0	0	0
11	13	1	9	0	0	23	26
12	0	0	0	0	0	0	0
13	56	21	15	0	8	100	104
14	1	0	0	0	0	1	1
Total	598	338	237	111	15	1299	1477
%	40	23	16	8	1	88	

APOLLO 13

Org.	Exact	1 Single	2 Single	3 Single	1 Double	Total	PHS Total
1	0	0	0	0	0	0	0
2	142	0	7	0	0	149	152
3	0	0	1	1	0	2	2
4	8	20	17	3	0	48	51
5	17	17	0	0	0	34	34
6	21	24	2	0	0	47	48
7	31	12	0	71	0	114	124
8	20	2	50	0	1	73	80
9	45	2	12	8	0	67	75
10	0	0	0	0	0	0	0
11	3	0	0	0	0	3	4
12	0	0	0	0	0	0	0
13	116	58	21	0	0	195	220
14	0	0	0	0	0	0	0
Total	403	135	110	83	1	732	790
%	51	17	14	10	0.1	93	

FIGURE 1

Publications

1. A. L. Roark, "A Stochastic Approach to Bioburden Estimation and Prediction - A Preliminary Report," SC-RR-70-561, August 1970.
2. J. P. Brannen, "Microbial Sterilization in Ultra-High Vacuum and Outer Space: A Kinetic Comparison," Space Life Sciences, 2 (1970) 219-220.
3. R. E. Trujillo and Nancy Laible, "The Reversible Inhibition of Spore Germination by Alcohols," Applied Microbiology, 2(4) October 1970.
4. A. L. Roark and W. R. Gavin, "A Users Manual for the Planetary Quarantine Lunar Information System," SC-M-70-604, November 1970.
5. D. M. Garst and K. F. Lindell, "A Precisely Controlled Low Range Humidity System," SC-RR-70-775, November 1970.
6. V. L. Dugan, "A Preliminary Analysis of the Radiation Burden of a Typical Mars Lander Mission," SC-RR-70-769, December 1970.

Presentations and Briefings

1. H. D. Sivinski, V. L. Dugan and W. J. Whitfield attended a conference at JPL, Pasadena, California, September 23, 1970 on Radiation Sterilization Experimentation.
2. J. P. Brannen attended the 1970 Fall SIAM Meeting, Boston, Massachusetts, October 12, 13 and 14, 1970 and presented a

paper entitled "Digital Simulation of Two-Point Boundary Value Problems and Digital Simulation of Implicit Differential Equations."

3. W. J. Whitfield presented a briefing at the Industrial Clean Room Contamination Control Conference at the American Association for Contamination Control and the Rochester Institute of Technology, Rochester, New York on October 13, 1970.
4. W. J. Whitfield attended a conference on Clean Room Facilities at Medtronics, Inc., Minneapolis, Minnesota on October 14, 1970.
5. H. D. Sivinski spoke to the Logistics Management Class, 4153rd USAR School, Albuquerque, New Mexico on November 5, 1970 on "Planetary Quarantine."
6. H. D. Sivinski, W. J. Whitfield and C. A. Trauth, Jr. attended the semi-annual Spacecraft Sterilization Technology Seminar in Williamsburg, Virginia, on December 1 and 2, 1970. Papers presented were "Thermoradiation Modeling," and "Open and Closed Systems in Thermoradiation Sterilization," by C. A. Trauth, Jr. and "Thermoradiation Experimentation," and "Open and Closed Systems in Dry Heat Sterilization," by W. J. Whitfield.
7. H. D. Sivinski and J. P. Brannen presented a series of invited biomathematical seminars and a colloquium at Iowa State University on December 8 and 9, 1970. The topics were "Use and Misuse of Kinetic Models in Biomathematics," "Computerized Biomodeling," "Some Consequences of Computerized Biomodeling," and "Some Applications of Computerized Biomodeling to Planetary Quarantine Problems."

Committee Activities

1. D. M. Garst conducted a committee meeting and made a report to the National Council, Midyear Meeting of AACC, at Scottsdale, Arizona on December 2 and 3, 1970.

Distribution:

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L. B. Hall, NASA, Code SL
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Washington, D. C. 20546 (25)

B. W. Colston, Director
Space & Special Programs Division
Office of Operations
U. S. Atomic Energy Commission
Albuquerque, New Mexico 87115

L. P. Daspit, Jr.
Viking Project Quarantine Officer
Viking Project Office, NASA
Langley Research Center
Hampton, Virginia 23365

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Richard G. Bond
School of Public Health
College of Medical Science
University of Minnesota
Minneapolis, Minnesota 55455

John H. Brewer
Mountain View Road
Star Route 2
Brownwood, Texas 76801

Edward B. Kasner
Director of Research Services
Graduate College
University of New Mexico
Albuquerque, New Mexico 87106

Frank B. Engley, Jr., Chairman
Department of Microbiology
School of Medicine
University of Missouri
Columbia, Missouri 65201

Gilbert V. Levin
Biospherics, Inc.
4928 Wyaconda Road
Rockville, Maryland 20853

Irving J. Pflug
Professor of Environmental Health
545 Space Science Center
University of Minnesota
Minneapolis, Minnesota 55455

Gerald J. Silverman
Department of the Army
U. S. Army Natick Laboratories
Natick, Massachusetts 01760

John A. Ulrich
Department of Microbiology
School of Medicine
University of New Mexico
Albuquerque, New Mexico 87106

Samuel Schalkowsky
Exotech Systems, Inc.
525 School Street S. W.
Washington, D. C. 20024

Mark A. Chatigny
Research Engineer
Naval Biological Laboratory
Naval Supply Center
University of California, Berkeley
Oakland, California 94625

Richard G. Cornell
Associate Professor of Statistics
Department of Statistics
Florida State University
Tallahassee, Florida 32306

Dr. Richard C. Corlett
Department of Mechanical Engineering
University of Washington
Seattle, Washington 98105

Lawrence P. Chambers
NASA Headquarters
Office of Manned Space Flight
Code MLR
Washington, D. C. 20546

Arthur H. Neill
Code SL
400 Maryland Avenue S. W.
Washington, D. C. 20546

Richard H. Green
Sterilization Group
Jet Propulsion Laboratory
4800 Oak Grove Drive
Pasadena, California 91103

Rudy Puleo
Public Health Service
Spacecraft Bioassay Laboratory
Drawer Y
Cape Canaveral, Florida 32900

USAEC, Division of Technical
Information
P. O. Box 62
Oak Ridge, Tennessee 37830
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P. E. Postell

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Research
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Martin S. Tierney
Group J-10
Los Alamos Scientific Laboratory
Los Alamos, New Mexico 87544

Jack Kaye
11607 Georgetowne Court
Potomac, Maryland 20854

Robert Angelotti
Deputy Director
Division of Microbiology
Food and Drug Administration
Health, Education and Welfare
200 C Street S. W.
Washington, D. C. 20546

Vance I. Oyama, Chief
Life Detection Systems Branch
NASA, Ames Research Center
Moffett Field, California 94035

Byron W. Brown, Jr.
Department of Preventive Medicine
Stanford University School of Medicine
Stanford University Medical Center
Palo Alto, California 94304

Don G. Fox
Sterility Control Officer
NASA Headquarters, Code SL
400 Maryland Avenue S. W.
Washington, D. C. 20546

A. A. Rothstein
Manager, Planetary Quarantine
Viking Program
Martin Marietta Corporation
P. O. Box 179
Denver, Colorado 80201

Hillel S. Levinson
U. S. Army Natick Laboratory
Natick, Massachusetts 01760

B. S. Schweigert, Chairman
Department of Food Science
College of Agriculture
Michigan State University
East Lansing, Michigan 48823

H. O. Halvorson
Biochemistry Department
St. Paul Campus
University of Minnesota
St. Paul, Minnesota 55414

A. Anellis
U. S. Army Natick Laboratories
Natick, Massachusetts 01760

Martin S. Favero
Department of Health, Education,
and Welfare
CDC-Phoenix Field Station
4402 North 7th Street
Phoenix, Arizona 85014

Mr. James Martin
Viking Project Engineer
Langley Research Center, NASA
Langley Station
Hampton, Virginia 23365

Q. Ussery
Code NC3, Quality Assurance Branch
Manned Spacecraft Center, NASA
Houston, Texas

F. J. Beyerle
George C. Marshall Space Flight
Center
Manufacturing Engineering Laboratory
Code R-ME-MMC
Huntsville, Alabama 35812

J. Gayle
Code SOP
Kennedy Space Center, NASA
Cape Canaveral, Florida

Murray Schulman
Division of Biology and Medicine
Headquarters, AEC
Washington, D. C. 20545

N. H. MacLeod
Space Biology Branch
Code 624, Bldg. 21, Room 161
Goddard Space Flight Center
Greenbelt, Maryland 20771

Jeptha E. Campbell, Ph.D., Chief
Division of Microbiology
Food and Drug Administration
DHEW, Public Health Service
1090 Tusculum Avenue
Cincinnati, Ohio 54226

G. Rotariu
Process Radiation Staff
Division of Isotopes Development
Headquarters, AEC
Washington, D. C. 20545

Martin G. Koesterer, Microbiologist
Bioscience Operation
Valley Forge Space Technology Center
P. O. Box 8555
Philadelphia, Pennsylvania 19101

Carl W. Bruch
Chief, Bacteriology Branch
Division of Microbiology
Food and Drug Administration
Third and C, S. W., Room 3876
Washington, D. C. 20204

John W. Beakley
Department of Biology
University of New Mexico
Albuquerque, New Mexico 87106

Loren D. Potter, Chairman
Department of Biology
University of New Mexico
Albuquerque, New Mexico 87106

Loris W. Hughes
Department of Biology
New Mexico State University
University Park, New Mexico

Richard W. Porter
Corporate Engineering Staff
General Electric Company
570 Lexington Avenue
New York, New York 10022

Fred L. Whipple
Smithsonian Astrophysical Observatory
Cambridge, Massachusetts 02138

J. J. McDade
Environmental Research Laboratory
Building 1710
Dow Chemical Company
Midland, Michigan 48640

Otto Hamberg
Aerospace Corporation
Building A2, Room 2019
2350 East El Segundo Blvd.
El Segundo, California

H. W. Johnson, LTC
U. S. Army Medical Research and
Development Command
Washington, D. C. 20314

Donald A. Kautter
Department of HEW
Food and Drug Administration
Division of Microbiology
BF-135
200 C Street S. W.
Washington, D. C. 20204

Lt. Keith C. Hopkins (SAH)
Biomedical Group
Air Force Weapons Laboratory
Kirtland Air Force Base
Albuquerque, New Mexico 87115

Briggs Phillips
Becton, Dickinson Research Center
P. O. Box 11276
Raleigh, North Carolina 27604

Dr. Wolf Vishniac
Department of Biology
University of Rochester
Rochester, New York 14627

J. A. Hornbeck - 1
J. M. Wiesen - 100
W. J. Howard - 1000
D. B. Shuster - 1200
W. A. Gardner - 1500
H. E. Lenander - 1600
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