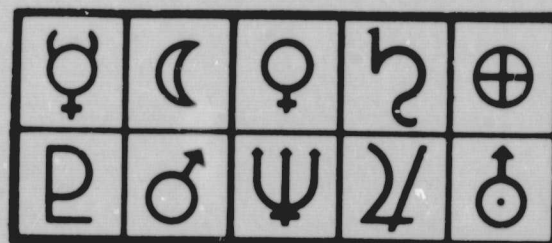


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QR 16
March 1970



PLANETARY QUARANTINE

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Sandia Laboratories Quarterly Report - Planetary Quarantine Program

Sixteenth Quarterly Report of Progress

for

Period Ending March 31, 1970

Planetary Quarantine Department

Sandia Laboratories, Albuquerque, New Mexico

March 1970

Project No. 0064010

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SUMMARY OF ACTIVITIES

Bacterial Spore Inactivation Modeling. The effect of pressure upon dry heat sterilization has been further investigated. Additional data still suggests that the activation volume of the inactivating reaction lies between 10 and 100 liters/mole, but precise data is not available to make a confident determination. Nevertheless, choosing a very conservative value of 1000 liters/mole leads to the conclusion that D-values at 10^{-6} torr and those at 10^{-17} torr should be experimentally indistinguishable if only first order kinetics are involved in dry heat inactivation. This conclusion does not necessarily apply to combinations of heat and radiation.

Modeling of Thermo-Radiation Synergism. The empirical model whose development was reported on in the previous quarterly report had indicated that free-radical reactions may be involved in the thermo-radiation inactivation of spores. This hint was pursued this quarter through the independent development of a rational model based upon the assumption of a free-radical inactivation mechanism. The rational model has the following properties:

- i) it reduced, in form, to the empirical model under circumstances associated with the derivation of the latter,
- ii) it extended the empirical model to cases not previously investigated experimentally, and made predictions of further synergistic gain which were subsequently verified experimentally.

Computerized Identification Scheme. A very versatile code based on the PHS identification scheme has been written. The general nature of the code

should allow for efficient incorporation of changes to be made in future schemes. The code is currently being checked out with past data and keys.

Bioburden Modeling. The general approach to estimating and predicting bioburdens presented in the previous quarterly has been further developed and applied to actual environmental data taken at the ETR. While several areas require further effort, it is now the case that there exist compatible bioburden estimation and prediction models; both physically motivated, capable of rigorous verification and capable of statistical analysis. It is now possible, on a rational basis, to determine "how often" and "how many" samples should be taken to achieve prescribed confidence in estimates and predictions. Furthermore, the final model is relatively simple computationally.

Thermoradiation Sterilization. Activities this quarter were directed toward continuation of the B. subtilis surface studies at the lower temperatures. Experiments were run at 95°C, 90°C, 85°C and 60°C. In addition, we started to evaluate the effectiveness of thermoradiation on buried contamination using methyl methacrylate as a matrix.

Fine Particle Physics. During this quarter a new microbiological air sampler was fabricated and tested. The sampler sizes particles and can sample for a period of one day or longer. In the tests conducted so far, the instrument has performed as expected. The instrument is now being used by the USPHS at Cape Kennedy to measure the mean number of microbes per particle and to examine the frequency distribution of the number of microbes per particle.

Bacterial Spore Inactivation Studies. Further studies on the inactivation of spores by ultraviolet (u.v.) irradiation were undertaken this quarter. Such studies emphasized the dependence of spore death on total u.v. dose. This finding coupled with the observation that the spore survival level ("tailing") became constant, at high dose rates, seems to reinforce the proposition that u.v. damage to a biological system is site specific for the cell nucleic acids. The survival curves for dormant spores and spores in the germinated state were found to be essentially identical. This finding again suggests that u.v. damage is site specific (DNA) and not dependent on the physiological state of the irradiated species. The "tailing" in the survival curves for spores exposed to u.v. radiation was shown not to be an experimental artifact caused by the aggregation of spores in clumps. Such spore aggregates presumably could have protected some spores from the u.v. radiation.

A synergism was observed on simultaneous exposure of spores to heat and u.v. irradiation. This result being reminiscent of that observed in spore inactivation using heat and ionizing radiation.

Finally, N-bromo-succinamide (NBS) was found to be a highly effective chemical in causing spore inactivation. N.B.S. inactivates both the spores germinating capacity and the spores capacity for outgrowth.

BACTERIAL SPORE INACTIVATION MODELING

- A. Description. The objective of this activity is to develop a physically based model of bacterial spore inactivation capable of exhibiting behavior paralleling all known forms of bacterial spore survival data and consistent with known facts about spores. A secondary objective has been to rationally relate environmental parameters to spore inactivation through the physical parameters occurring in the model.
- B. Progress. During this past quarter, further progress has been made in relating pressure to bacterial spore survival by using the more complete formulation

$$\Delta F^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger + p\Delta V^\ddagger$$

of the dependence of the free energy of activation, $\Delta F^\ddagger$, upon the entropy $\Delta S^\ddagger$, enthalpy $\Delta H^\ddagger$ and the volume $\Delta V^\ddagger$ of activation. Here p represents the pressure.

Further evidence obtained this past quarter suggests that $\Delta V^\ddagger$ for spore inactivation lies between 10 and 100 liters/mole. This is consistent with our previous estimates, but the lack of precise data still leaves these numbers suspect.

Nevertheless, it appears possible to make some statements about the confidence that may be placed in experiments at 10^{-6} torr to represent the effects of a deep space environment (about 10^{-17} torr). In particular it is possible to examine the relationship between D values obtained in the laboratory and what one would expect in outer space.

If we suppose first order kinetics, then the expected number of survivors at time t , $E(n(t))$ takes the form

$$E(n(t)) = n(0)e^{-k_p t}.$$

Here, the notation k_p is used to show the dependence of the reaction rate on pressure

$$k_p = \frac{KT}{h} \exp[-(\Delta H^\ddagger - T\Delta S^\ddagger + p\Delta V^\ddagger)].$$

The D-value at pressure p is then

$$D_p = \frac{\ln 10}{k_p} \approx \frac{2.3}{k_p}.$$

Hence, the relation between D values at pressures p_1 and p_2 and reaction rate constants is given by

$$\ln(k_{p_1}/k_{p_2}) = \ln(D_{p_2}/D_{p_1}) \quad (1)$$

where

$$\ln(k_{p_1}/k_{p_2}) = \Delta V^\ddagger(p_2 - p_1)/RT, \text{ from above.} \quad (2)$$

Observe that if $\Delta V^\ddagger$ is positive, then as pressure decreases, the rate constant increases so the D value decreases.

These two relationships permit the change in D-values as a function of pressure change to be theoretically investigated. The change is greatest when $\Delta V^\ddagger$ is maximum (all else being equal), and as noted above precise values of $\Delta V^\ddagger$ are not available. Thus, to be safe, we have chosen what appears to be a very conservative value of 1000 liters/mole for $\Delta V^\ddagger$. This value is an order of magnitude greater than any estimates of $\Delta V^\ddagger$ obtained to date. Specifically, we shall assume that $\Delta V^\ddagger$ is as large as 1000 l/mole and that T is 60°C. Then from (1) and (2) above

$$\ln(D_{p_2}/D_{p_1}) = \frac{(10^6 \text{ cc/mole})(1/7.6)(10^{-19} - 10^{-8}) \text{ atm}}{(333 \text{ deg})(86 \text{ cc atm/deg.mole})}$$

when $p_1 = 10^{-6}$ torr and $p_2 = 10^{-17}$ torr. Thus, we see that

$$\ln(D_{p_2}/D_{p_1}) = -6 \times 10^{-8}$$

so that

$$D_{p_2} = e^{-6 \times 10^{-8}} D_{p_1}$$

$e^{-6 \times 10^{-8}}$ is so very nearly equal to one, that for all practical purposes

$$D_{p_1} = D_{p_2}$$

when $p_1 = 10^{-6}$ torr and $p_2 = 10^{-17}$ torr.

Thus, despite the conservative magnitude of the $\Delta V^\ddagger$ chosen, we find that an experimenter could not distinguish between a D value taken at 10^{-6} torr and one taken at 10^{-17} torr if only first order kinetics are involved in dry heat sterilization. Since there is some reason to believe that inactivation in environments other than dry heat alone is from other causes, this conclusion may not apply in combination environments.

MODELING OF THERMO-RADIATION SYNERGISM

- A. Description. The objective of this activity is to develop a physically based model representing bacterial cell inactivation in lethal environments combining both heat and ionizing radiation. This modeling is based upon experimental activity of the same nature described elsewhere in this report.
- B. Progress. The semi-empirical model

$$E[n(t)] = n(0)e^{-kt}, \quad (1)$$

$$k = k_T + k_S = \frac{kT}{h} e^{14.55 - 16890/T} + r_d^{218/T} e^{6.15 - 2775/T}, \quad (2)$$

which was described in the last quarterly report has continued to provide comparatively accurate predictions of synergistic results during the past quarter for other values of the parameters T , temperature in $^{\circ}\text{K}$, and r_d , radiation dose rate in kilorads per hour. The following table gives a comparison of the most important of this quarter's results based on one experimental run for each temperature listed.

Temp. $^{\circ}\text{C}$	Dose Rate Kilorads/Hour	k-Model Prediction Hour^{-1}	k-Experimental Hour^{-1}
95	10	1.60	1.44
90	10	0.99	.78
85	10	0.87	.88

TABLE I

A Comparison of Model Predictions and Experimental Results

Based on our previous observations that the empirically derived form of the spore inactivation reaction given above is very similar to a free radical mediated polymerization reaction and that free radical inactivation is noticed in microbiological systems, a chemical kinetic model of free radical inactivation has been developed. In this development the assumption is made that each bacterium contains a critical substrate A which is inactivated at a rate k_T due to the elevated temperature and at a rate k_I due to free radical interference in a composite heat and radiation environment. The descriptive reaction equations are



and



where R is the normalized free radical concentration as a function of time. D and X represent inactivated states of the critical substrate A and infer inactivation of the bacterium. Let R(t) be represented by the equation

$$R(t) = C_R(r_d, T)(1 - e^{-k_3 t}) + C_2(r_d, T)e^{-k_2 t} \quad (5)$$

where $C_R(r_d, T)$ represents an equilibrium concentration of free radicals for a constant r_d and T and where $C_2(r_d, T)$ represents a "pool" or bulk

of free radicals formed by a pre-irradiation treatment - that is, formed prior to the introduction of the spores to the elevated temperature. The description of $R(t)$ in Equation (5) has the general form of a "birth and death" process with the exception of k_2 and k_3 being different from one another. To simplify the formulation here k_3 will be assumed to be very large; that is to say that the introduction of the pre-irradiated samples into the composite environment very quickly establishes the equilibrium concentration of free radicals. This allows $R(t)$ to be approximated by

$$R(t) = C_R(r_d, T) + C_2(r_d, T)e^{-k_2 t}. \quad (6)$$

Here k_2 is the rate at which the free radical concentration due to pre-irradiation is utilized.

The differential equation which describes the schematics of Equations (3) and (4) is

$$\frac{dA}{dt} = -k_T A - k_1 A R. \quad (7)$$

Substituting $R(t)$ from Equation (6) gives

$$\frac{dA}{dt} = -(k_T + k_1 [C_R(r_d, T) + C_2(r_d, T)e^{-k_2 t}])A. \quad (8)$$

The solution to this first order equation is:

$$A(t) = A_0 e^{-K(t)} \quad (9)$$

where

$$K(t) = k_T + C_R(r_d, T)k_1 t + \frac{k_1}{k_2} [C_2(r_d, T)](1 - e^{-k_2 t}). \quad (10)$$

Recalling the assumed one to one correspondence between substrate A and bacterial cell, Equation (9) may be written

$$E[n(t)] = n(0)e^{-K(t)} \quad (11)$$

where $E[n(t)]$ is the expected population as a function of time, and $n(0)$ is the initial population at time $t = 0$. The reference starting point $t = 0$ is located just after the pre-irradiation period and just before the beginning of the composite lethal environment.

Now consider the case for which no pre-irradiation is used. With $C_2(r_d, T) = 0$,

$$k(t) = [k_T + C_R(r_d, T)k_1]t = kt. \quad (12)$$

Therefore, comparison with Equation (2) provides

$$k_s = C_R(r_d, T)k_1. \quad (13)$$

Drawing from the fact that free radical concentration in polymers is a function of a fractional power of radiation dose rate and once again comparing k_s of Equation (13) with k_s of Equation (2) provides

$$C_R(r_d, T) = \epsilon r_d^{218/T} \quad (14)$$

where ξ is an undetermined proportionality constant. Continuing the comparison gives

$$k_1 = \xi' e^{-5460/RT} \quad (15)$$

which is intuitively pleasing since k_1 has the general form of an Arrhenius equation and is only a function of temperature. The resulting requirement on the constants which were used is

$$\xi \xi' = e^{6.15}. \quad (16)$$

If the sample is pre-irradiated and then heated without any simultaneous radiation, then $C_R(r_d, T)$ of Equation (10) is zero,

$$K(t) = k_T t + \frac{k_1}{k_2} [C_2(r_d, T)] (1 - e^{-k_2 t}), \quad (17)$$

and the rate of the process is

$$\frac{dE[N(t)]}{dt} = -(k_T + k_1 C_2(r_d, T) e^{-k_2 t}) N(t). \quad (18)$$

Inspection of Equation (18) shows that the inactivation proceeds faster at the initiation of the heating cycle than would the inactivation without pre-irradiation. Also the rate of the pre-irradiated sample inactivation approaches that of the unirradiated sample after a sufficient period of time. Investigation of Equations (11) and (17) for $t \gg 1/k_2$ provides

$$E[N(t)] = N_0 e^{-k_T t} \cdot e^{-\frac{k_1}{k_2} C_2(r_d, T)} \quad (19)$$

and shows that the additional inactivated fraction when pre-irradiation is used is

$$\exp\left[-\frac{k_1}{k_2} C_2(r_d, T)\right] \quad (20)$$

This is consistent with the experimental data for bacterial spore death and with radiation initiated polymerization.

If the spore sample is both pre-irradiated and then exposed to a composite environment, then both C_R and C_2 are non-zero. For this case the increased rate of the reaction is given by the quantity in braces in Equation (8). Also the inactivated fraction is less than that provided solely by lethal temperature by the factor

$$\exp\{-[C_R(r_d, T)k_1 t + \frac{k_1}{k_2} C_2(1 - e^{-k_2 t})]\} \quad (21)$$

and is less than that provided by the composite environment by the factor

$$\exp\left\{-\left[\frac{k_1}{k_2} C_2(1 - e^{-k_2 t})\right]\right\} \quad (22)$$

for any time t .

To verify the preceding kinetic model prediction, a population of dry B. subtilis var. niger spores was pre-irradiated at a dose rate of approximately 12 kilorads per minute for three minutes. Immediately

following the pre-irradiation, the spores were subjected to a composite environment of 19 kilorads per hour and 105°C . The results are shown in Figure 1. Time $t = 0$ again refers to the beginning of the composite cycle. Notice that the pre-irradiation did provide an increased initial rate of death and a larger inactivated fraction at all times. Therefore, the generalized assumptions upon which the model were based do seem to be substantiated. The single set of data does not provide enough information to calculate values for k_1 , k_2 , C_R , and C_2 at 105°C , but good initial approximations for k_1 and k_2 at 105°C on this base are $k_1 = 2.05 \text{ Hr.}^{-1}$ and $k_2 = 2.40 \text{ Hr.}^{-1}$.

The rational kinetic model derived here must be regarded as being at least as valid as the earlier empirical model, since the two have precisely the same form when $C_2(r_d, T) = 0$. In other words, when there is no pre-irradiation both models provide the same prediction. On the other hand the rational kinetic model has a capability for predicting the results when pre-irradiation is used in addition to the combination environment, and the experimental verification of this prediction in a situation where data had not previously been taken lends additional support for the models validity.

A report on this work is nearing completion.

EFFECT OF PRE-IRRADIATION ON INACTIVATION KINETICS

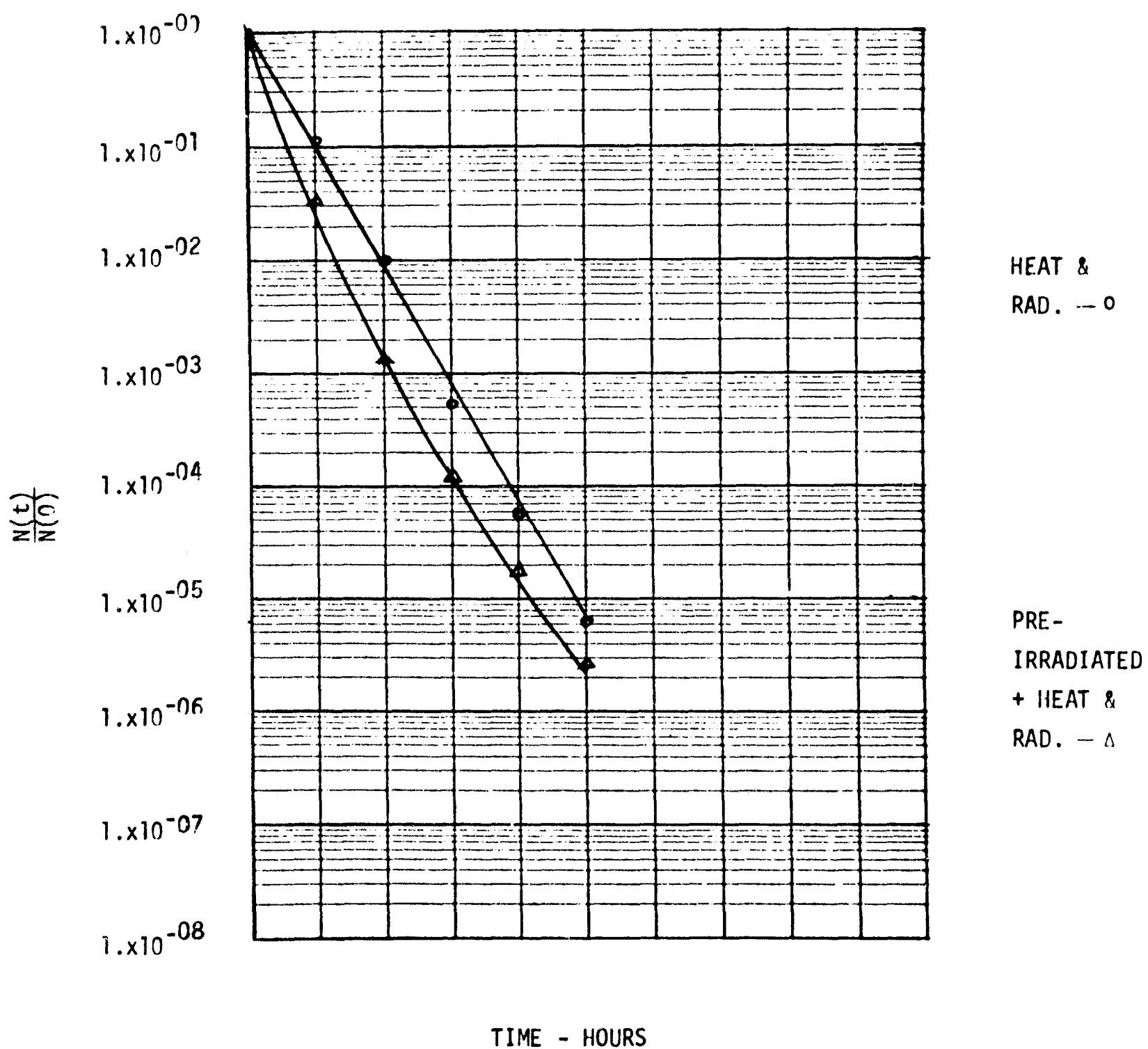


FIGURE 1

COMPUTERIZED IDENTIFICATION SCHEME

- A. Description. The objective of this activity is the development of a computerized version of the PHS microbiological identification scheme now being used at Cape Kennedy. Feasibility of such a scheme was discussed in the previous quarterly report.
- B. Progress. During this past quarter, a very general program for bacterial identification has been written. The program operates in two parts. The first part places an organism in a particular "scheme" (a general grouping of organisms) and the second part then "identifies" (categorizes) within this scheme. A detailed description of the current schemes and organism categories therein will be available from the PHS shortly.

The program is "general" in the sense that it will conveniently permit considerable change in almost all parts of the identification format including: definitions of schemes, definitions of categories within schemes, identification tests and their results, and restructuring of actual identification forms used in colony identification. Thus, not only will this program be directly applicable to future spacecraft data, but because of its versatility, it will, hopefully, provide a reasonable research tool as well.

Ultimately, this program will be part of the Lunar Information System and use the identification keys provided by the PHS. Currently, the program is being checked out against old data with old keys. It will be compared against the Apollo 13 data and the new keys being used by PHS as soon as these are available.

BIOBURDEN MODELING

- A. Description. Compatible models for the estimation and prediction of spacecraft bioburdens are needed for use in spacecraft assembly and test environments. Ideally, these models should have bioburden outputs about which some statistical confidence statement can be made, and, of course, they should be capable of rigorous verification.
- B. Progress. The approach to a predictive bioburden model described in the previous quarterly report has been further developed and analyzed to facilitate its implementation.

The basis of the prediction model is the set of Kolomogorov Forward Equations for a Markov process determined by functions $P_{ij}(\tau, t)$, the probability of having j organisms on a surface at time t given that there were i organisms present at time τ . The equations take the form:

$$\begin{aligned} \frac{\partial P_{ik}(\tau, t)}{\partial t} = & -[\lambda(t) + \mu(t)E_k(t)](1 - q_0)P_{ik}(\tau, t) \\ & + \sum_{j=k+1}^{\infty} \mu(t)E_j(t)P_{ij}(\tau, t)q_{j-k} \\ & + \sum_{j=0}^{k-1} \lambda(t)P_{ij}(\tau, t)q_{k-j}. \end{aligned} \quad (1)$$

where generally:

- i) organisms are assumed attached to particles (clumped)
- ii) $\lambda(t)$ is the particle deposition rate (from all causes) at time t ,
- iii) $\mu(t)$ represents the fraction of particles removed (by any means) from the surface at time t

iv) $E_j(t)$ is the expected number of particles given j organisms on the surface, and

v) q_s represents the probability that there will be s organisms on a particle, $s = 0, 1, 2, \dots$.

It is the probabilities $P_{ij}(\tau, t)$ that are of interest in predicting a spacecraft bioburden since, given an estimate at time τ , these functions yield bioburden predictions at any future time t as a function of the particle deposition rate $\lambda(t)$, the particle removal fraction $\mu(t)$ and the distribution of the number of organisms per particle $q_0, q_1, \dots$. Using this approach, there is no need to separate the bioburden into that from "fallout" and that from "contact" - both are treated the same mathematically while physically both types of burden are reflected in the above parameters.

Generally, a system of equations such as (1) admits no convenient solution (i.e., collection of $P_{ij}(\tau, t)$'s as a known function of the parameters $\lambda(t)$, $\mu(t)$, q_0 , q_1 , etc.). In the case at hand, we have been able to devise a simple numerical procedure for solving (1) for functions of the form $P_{0j}(0, t)$. Specifically, one may show that

$$P_{0j}(0, t) = \sum_{s=0}^{\infty} \frac{e^{-H(t)/\gamma} [H(t)/\gamma]^s}{s!} Q(j, s), \quad (2)$$

where γ (not zero) is the mean number of organisms per particle, $H(t)$ is the mean number of organisms on the surface at time t , and $H(0)$ is assumed to be zero, and finally $Q(j, s)$ represents the probability that s particles carry precisely j organisms.

If the distribution of the number of organisms per particle is known (and is used to determine $Q(j,s)$ and γ), then the above is indeed a solution of (1) for $i = 0$ at $\tau = 0$, provided one can obtain values of $H(t)$, the mean number of organisms at time t .

Happily, $H(t)$ satisfies a simple first order differential equation

$$H'(t) = \gamma \cdot \lambda(t) - \mu(t)H(t) \quad (3)$$

which, while it cannot always be solved in simple form for $H(t)$ (as a function of $\lambda(t)$, $\mu(t)$ and γ), it can most readily be solved numerically. Thus for all practical purposes if γ , $\lambda(t)$ and $\mu(t)$ are known, (3) can be solved and the burden at time t can be predicted using the functions

$$P_{0j}(0,t).$$

Additionally, since we may consider $P_{0j}(0,t)$ known, it is quite straightforward to compute mean burdens and confidence intervals for these means to compare with actual surface data estimating the mean burden. Before giving some examples of this procedure, it is interesting to note that if the distribution of the number of microorganisms is Poisson, that is

$$q_i = \frac{\gamma^i e^{-\gamma}}{i!},$$

then Equation (2) represents a Neyman Contagious Distribution of Type A. This same distribution is being used both as an estimation model, and

to determine the sampling requirements for the Apollo missions (cf. "The Determination of Quantitative Microbial Sampling Requirements for Apollo Modules", SC-RR-69-23, January 1969). If the distribution is not Poisson, (2) is then a generalization of the Neyman Contagious Distribution of Type A, but in any case this will yield an estimation model and prediction model which estimate and predict the same distribution (viz., $P_{0j}(0,t)$) - that is, they are compatible.

Finally, the above comments are dependent upon knowledge of the distribution of organisms per particle. Research aimed at obtaining such information is described in this report under the section on Fine Particle Physics.

Turning to implementation of the model, let us make the following assumptions

- i) Surface samples are taken at time $t_1 < t_2 < t_3 < \dots < t_m$.
- ii) The predicted burden is desired at $t_f > t_m$.
- iii) At time t_k , n_k square inches are sampled from a total surface area of A square inches.
- iv) A uniform sampling error, $\epsilon (0 \leq \epsilon \leq 1)$ is made in taking surface samples.
- v) The initial expected burden $H(0)$ is zero.
- vi) $q_0, q_1, \dots$ are known.

Under these circumstances, if $x_i(t_k)$ is the number of microorganisms recovered in the i^{th} square inch at time t_k , then

$$\bar{X}(t_k) = \frac{(1+\epsilon)A}{n_k} \sum_{i=1}^{n_k} x_i(t_k) \quad (4)$$

is an unbiased, consistent estimator of $H(t_k)$, the expected number of organisms on the surface at time t_k . Furthermore $H(t)$ is a function (in principle, as discussed above), of $\lambda(t)$, the particle deposition rate and $\mu(t)$ the particle removal fraction. If $\lambda(t)$ and $\mu(t)$ are restricted to specified classes of functions, then a $\hat{\lambda}_m(t)$ and $\hat{\mu}_m(t)$ may be chosen which minimize

$$D(\lambda, \mu) = \sum_{i=1}^m [\bar{X}(t_i) - H(t_i)]^2. \quad (5)$$

These functions $\hat{\lambda}_m(t)$ and $\hat{\mu}_m(t)$ represent approximations to the physical $\lambda(t)$ and $\mu(t)$ gained solely on the basis of periodic surface sampling. Additional independent measurements of $\lambda(t)$ and $\mu(t)$ would serve to help define the classes of functions from which $\hat{\lambda}_m(t)$ and $\hat{\mu}_m(t)$ are chosen.

Given $\hat{\lambda}_m(t)$ and $\hat{\mu}_m(t)$ - the deposition and removal functions estimated from data from m sampling periods, the mean number, $H(t)$ can be obtained for any time t essentially by solving Equation (3) numerically with $\lambda(t) = \hat{\lambda}_m(t)$ and $\mu(t) = \hat{\mu}_m(t)$.

This prediction is denoted

$$\hat{H}_m(t).$$

Given $\hat{H}_m(t)$, one can predict the complete distribution at a future time t ,

$$P_{0j}(0, t),$$

using $\hat{H}_m(t)$ in Equation (2). This yields the expected form of the distribution at future sampling times, for example, and allows one to estimate the number of samples needed then to achieve certain confidence intervals about the sample estimated mean at that point in time using the compatible estimation model in a fashion similar to that referenced above.

In order to demonstrate the applicability of the model, we shall make use of data gathered from stainless steel strips in Hangar A0 at the ETR by the PHS during the period January 17, 1967 through March 30, 1967. (We are indebted to R. Puleo of the Spacecraft Bioassay Unit of the PHS for this data). The data is summarized in the following table.

<u>Weeks of Exposure</u>	<u>Mean Number of Organisms per Strip (Six Strips)</u>
3	16.0
4	82.5
5	33.3
6	41.6
7	22.7
8	34.1
9	36.7
10	33.3

Here, 48 strips were used and six retrieved per week beginning at the end of week three. The surface we are concerned about estimating the bioburden of will be a stainless steel strip of the same size. We will assume:

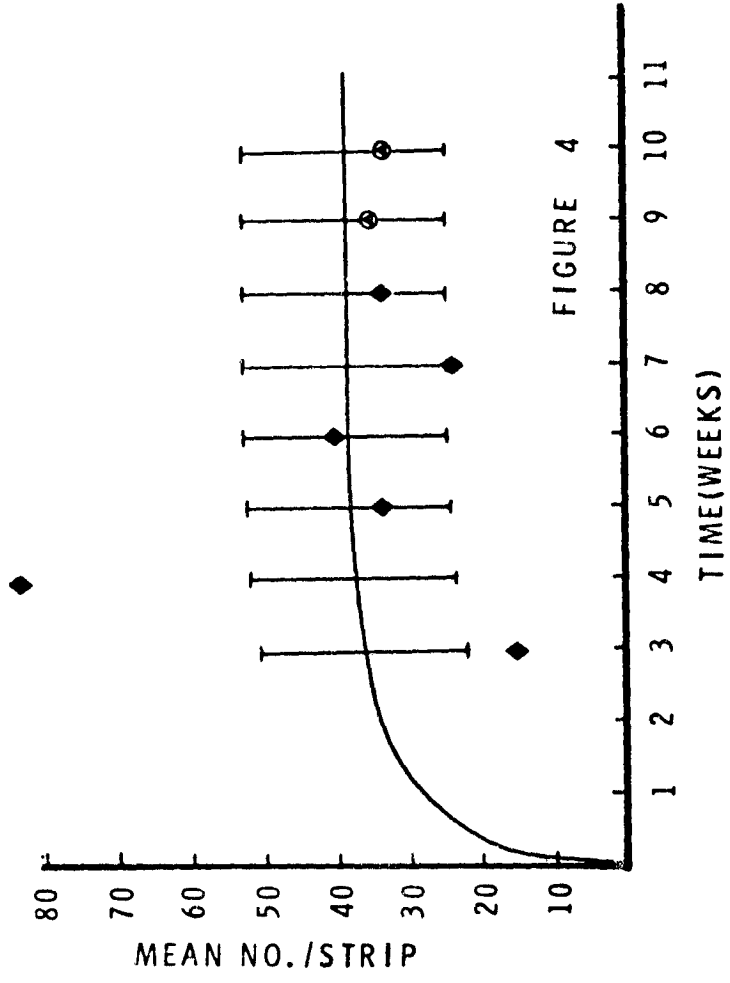
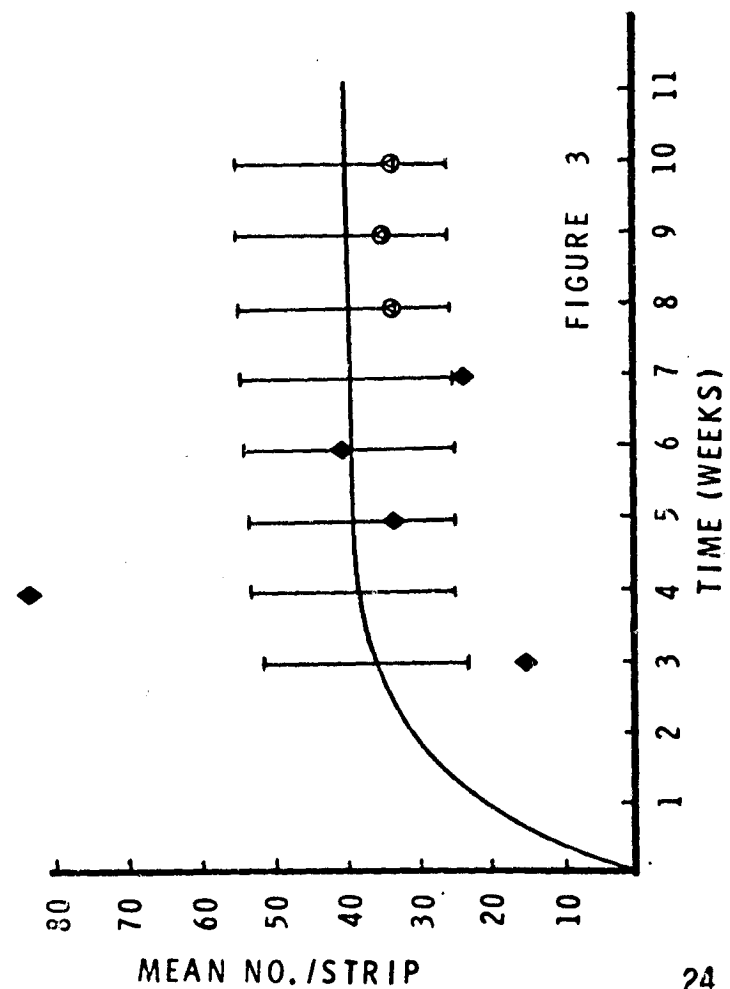
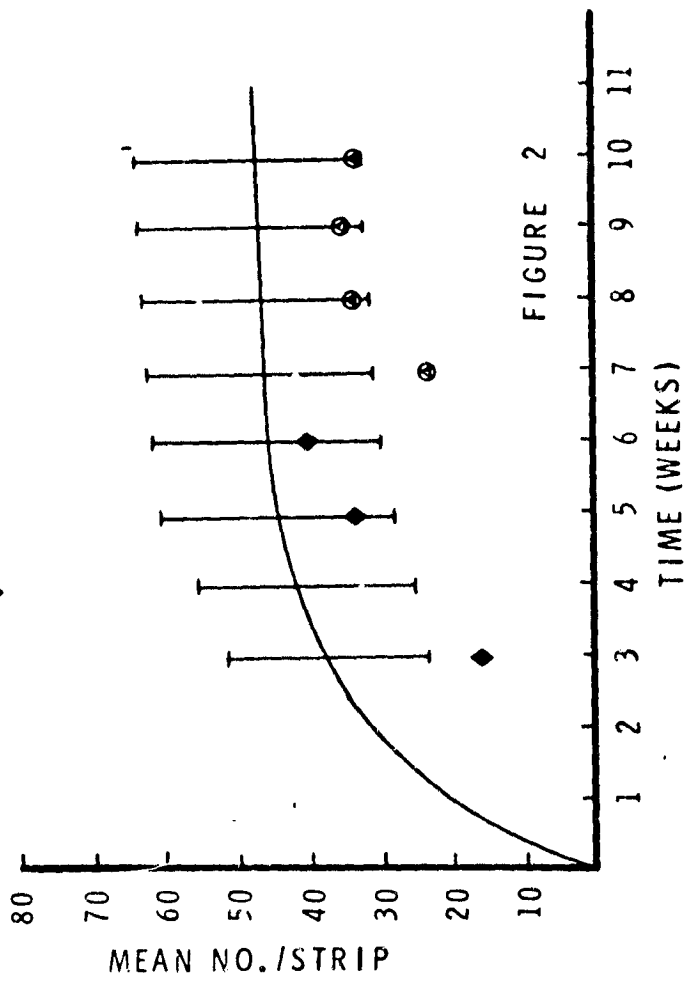
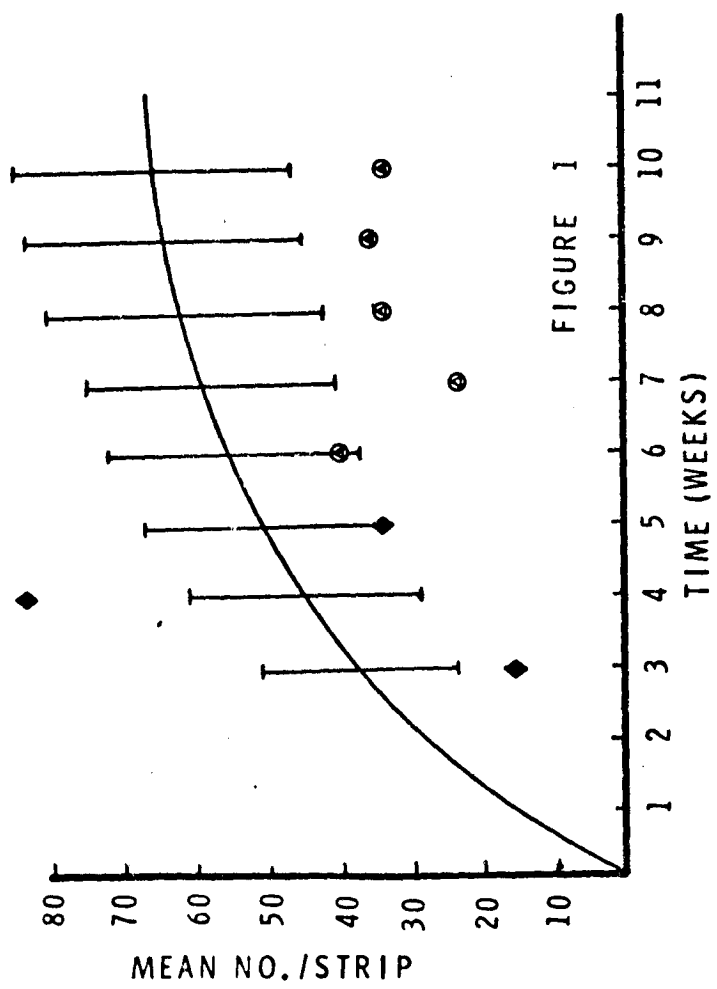
- i) The mean number of organisms per organism-bearing particle is four based upon sampling at Cape Kennedy, and the variance is equal to the mean (as is the case when the distribution is Poisson, for example), and

- ii) The deposition rate and removal fraction in the total period $t = 3$ to $t = 10$ weeks are constant. Thus, each estimate $\hat{\lambda}_m(t) = \hat{\lambda}_m$ and $\hat{\mu}_m(t) = \hat{\mu}_m$ for $m = 3, 4, 5$ and so forth. (This is not physically true, but is used here as exemplary of the more general case).

Figures 1, 2, 3, 4 and 5 are our predictions to time $t = 10$ weeks beginning with data given at $t = 3, 4$ and 5 weeks and increasing by one data point for each figure until data from all weeks, excluding the 10^{th} , are used in predicting the expected burden at the end of the 10^{th} week. The $\blacklozenge$'s represent data being used in the predictions and the $\oplus$'s are data points with which predictions are to be compared. At each time where data are given, the procedures of SC-RR-69-23 referenced above are used to compute the 99% confidence interval for the mean of six samples. These intervals are shown as bars centered about the predicted value of the model. It is interesting to note that the predictions at time $t = 10$ weeks steadily improve and that the estimates $\hat{\lambda}_m$ and $\hat{\mu}_m$ appear to converge. This is shown in the following table.

Figure	Weeks Included In Estimation	Estimated $\lambda(t)$ (assumed constant)	Estimated $\mu(t)$ (assumed constant)
1	3,4,5	4.4	0.25
2	3,4,5,6	6.1	0.50
3	3,4,5,6,7	8.8	0.88
4	3,4,5,6,7,8	9.4	0.96
5	3,4,5,6,7,8,9	9.5	0.99

The data at $t = 4$ weeks of an average of 82.5 organisms/strip seems anomalous when compared with the rest of the data. Figure 6 indicates how the omission of this data and use of data at only 3, 5 and 6 weeks to estimate μ and λ lead to faster convergence of the prediction to the data at $t = 10$



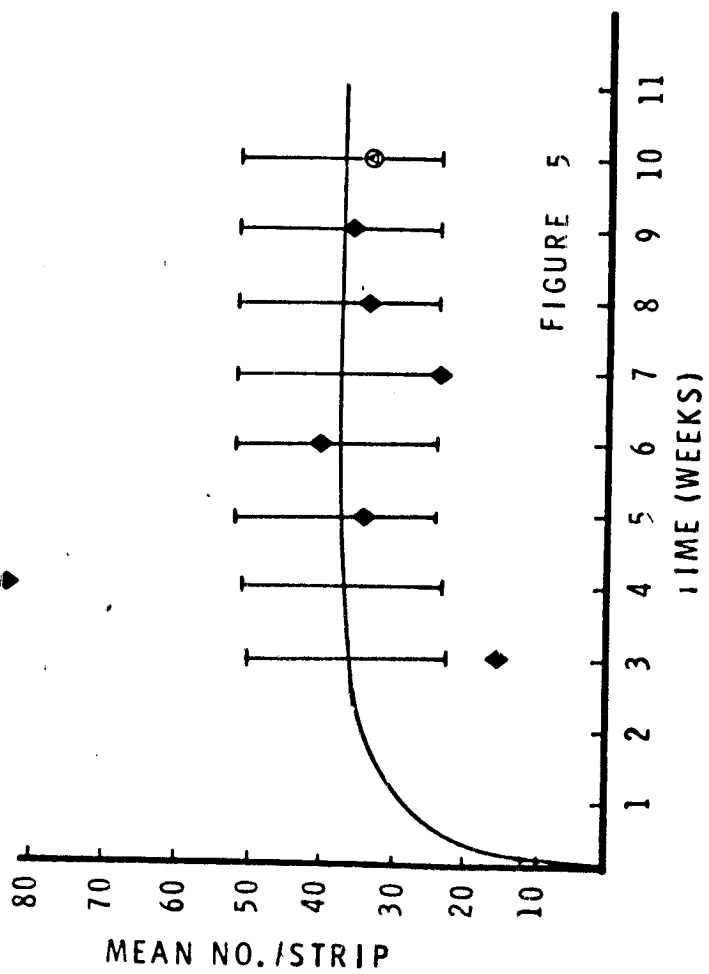


FIGURE 5

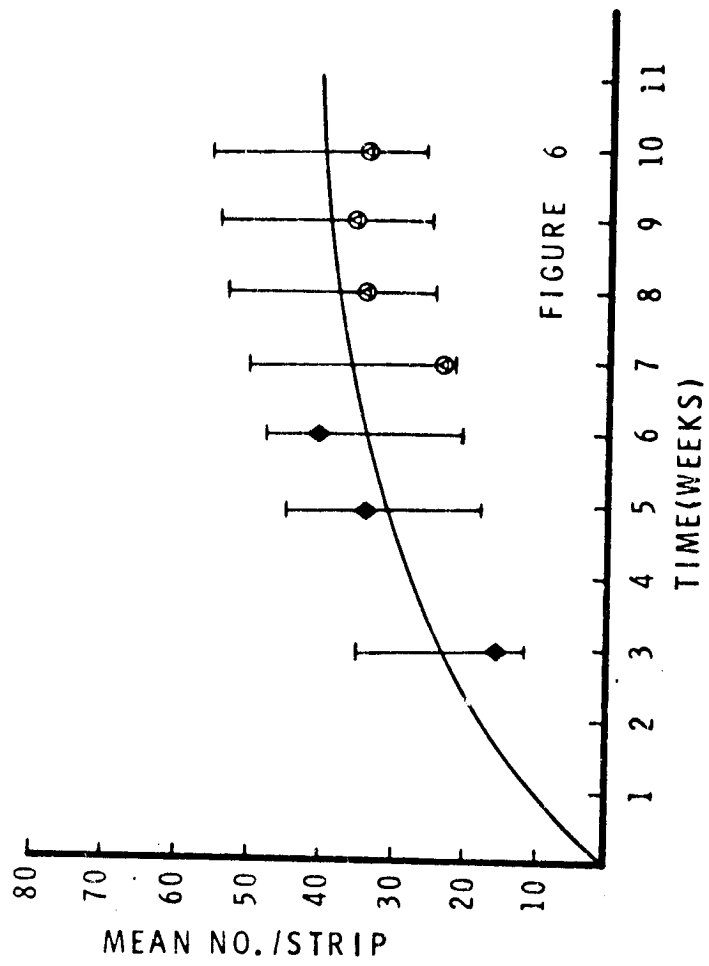


FIGURE 6

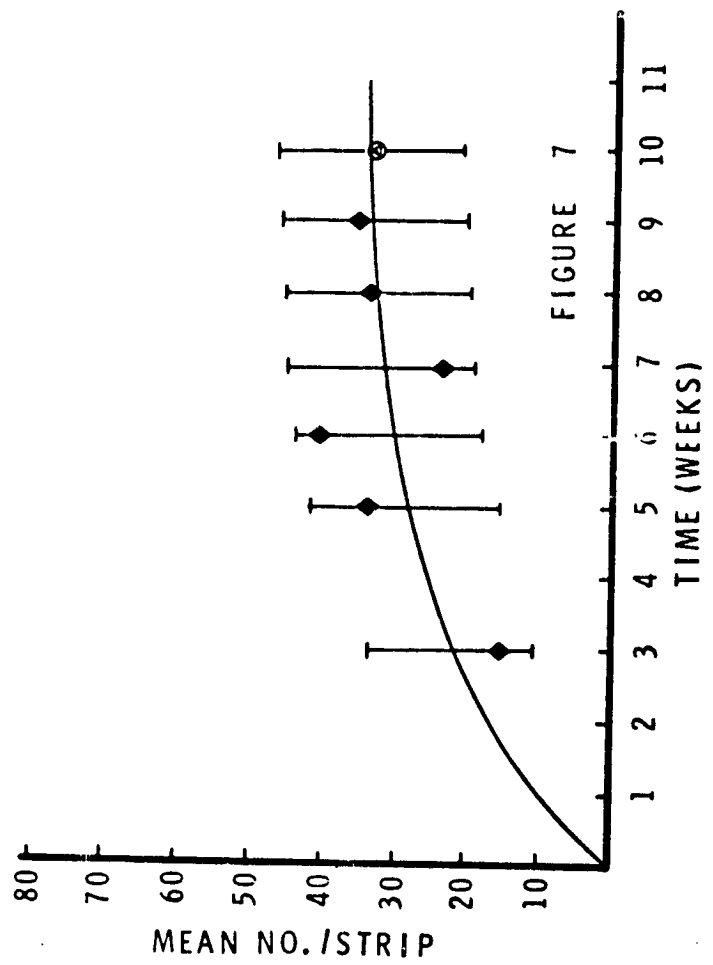


FIGURE 7

weeks (and, indeed, at $t = 7, 8$ and 9 weeks). Figure 7 again omits the data at $t = 4$ weeks but incorporates that at $t = 3, 5, 6, 7, 8$ and 9 weeks to obtain estimates of λ and μ .

Even though the assumption of constant deposition rate and removal fraction is not valid, this does illustrate the use of the model. Several things should be noted about the compatible estimation and prediction models described here. These are:

- a) Prediction of bioburdens depends highly upon their estimation.

The estimation model may be used to determine the number of samples needed for achieving prescribed confidence intervals about estimates of the expected bioburden. This number of samples is then taken and the mean estimate is combined with others previously gotten to obtain future predictions. The estimation model and prediction model both ascribe the same probability distribution to the bioburden at any time t .

- b) The parameters in both models are relatively few in number (assuming $q_0, q_1, \dots$ to be a single "parameter") and all parameters have a well-defined physical interpretation.
- c) Both models are capable of rigorous verification in controlled experimental environments. Experimental effort is underway to do this.
- d) Both models are of a form which can be analyzed to see how "good" their predictions or estimations are.
- e) It has been found that most organisms occur in an environment in clumps. This fact is taken into account in both models.
- f) It has been observed that a bioburden equilibrium or "plateau" is reached if a surface is left in a constant environment over a period of time. The predictive model exhibits this characteristic.

g) The models lend themselves to straightforward computerization.

Both require only minimal computation time due to their relatively simple final form.

h) The models are capable of being used in establishing the sampling protocol ("how often" and "how many").

Current effort is directed toward allowing more general estimates $\hat{\lambda}_m(t)$ and $\hat{\mu}_m(t)$ of $\lambda(t)$ and $\mu(t)$ than the constants above, obtaining experimental information about $q_0, q_1, \dots$, and verifying the models. Several reports relating to this activity are being prepared.

THERMORADIATION STERILIZATION

A. Description. The thermal degradation of spacecraft as a result of present dry heat sterilization is a problem still not completely resolved. There is some concern that silver-zinc batteries, high value mylar capacitors, vidicon tubes, tantalum capacitors (10 to 15 μ f), solid propellant (especially PBAA), culture media, photometers, and other scientific instruments will not withstand present dry heat sterilization cycles without significant degradation. The purpose of the thermoradiation study is to explore possible techniques that will reduce the dry heat time and temperature requirements for sterilization of spacecraft hardware. More specifically, this study will explore the feasibility of using combinations of heat and radiation where synergistic effects may be available to achieve effective sterilization of spacecraft.

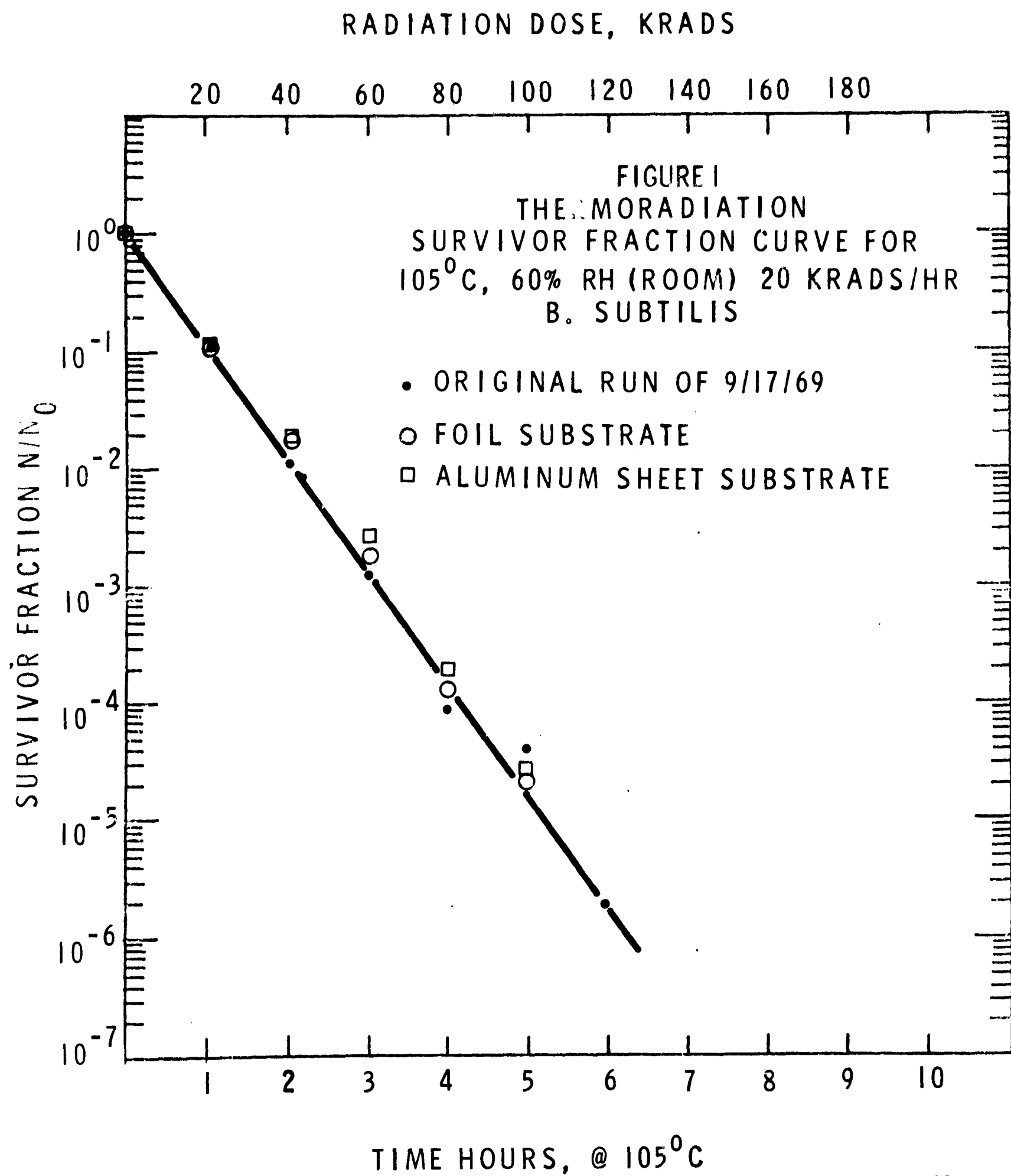
B. Progress.

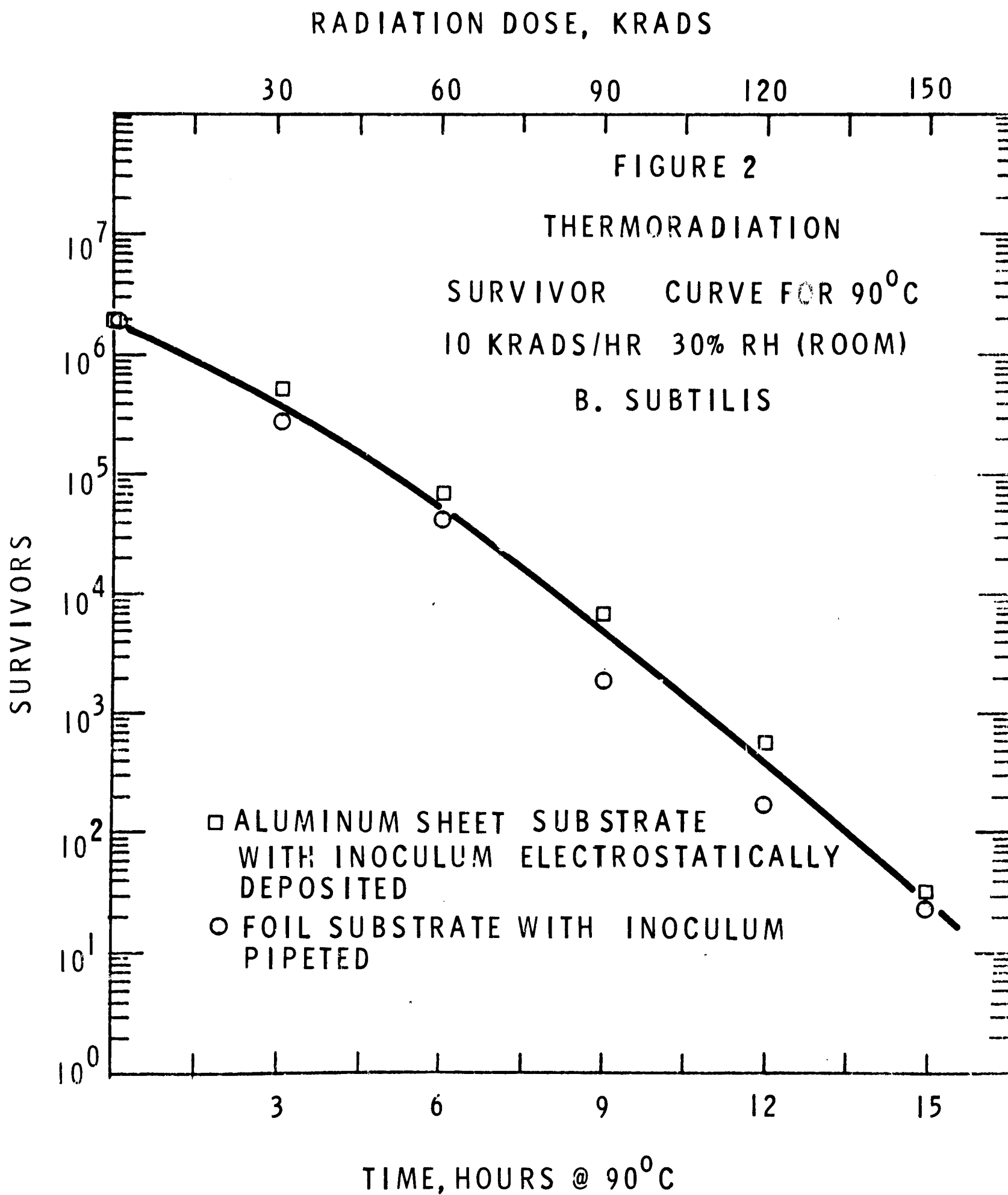
1. Surface Contamination. One thermoradiation experiment was performed at 105°C 60% RH and a radiation dose rate of 20 KRADS/HR. The purpose of this experiment was twofold; first of all, a comparison of foil and .020" aluminum sheet as substrates was desired and, secondly, a confirmation rerun of an old experiment that had been performed several months previously, seemed desirable. The 60% RH experiment was selected since it represented a rather severe variation from normal laboratory conditions. Four replicate samples of inoculated foil substrate and four replicates of aluminum sheet substrate were prepared for each sample period by electrostatically depositing 10^6 spores on each of the substrates.

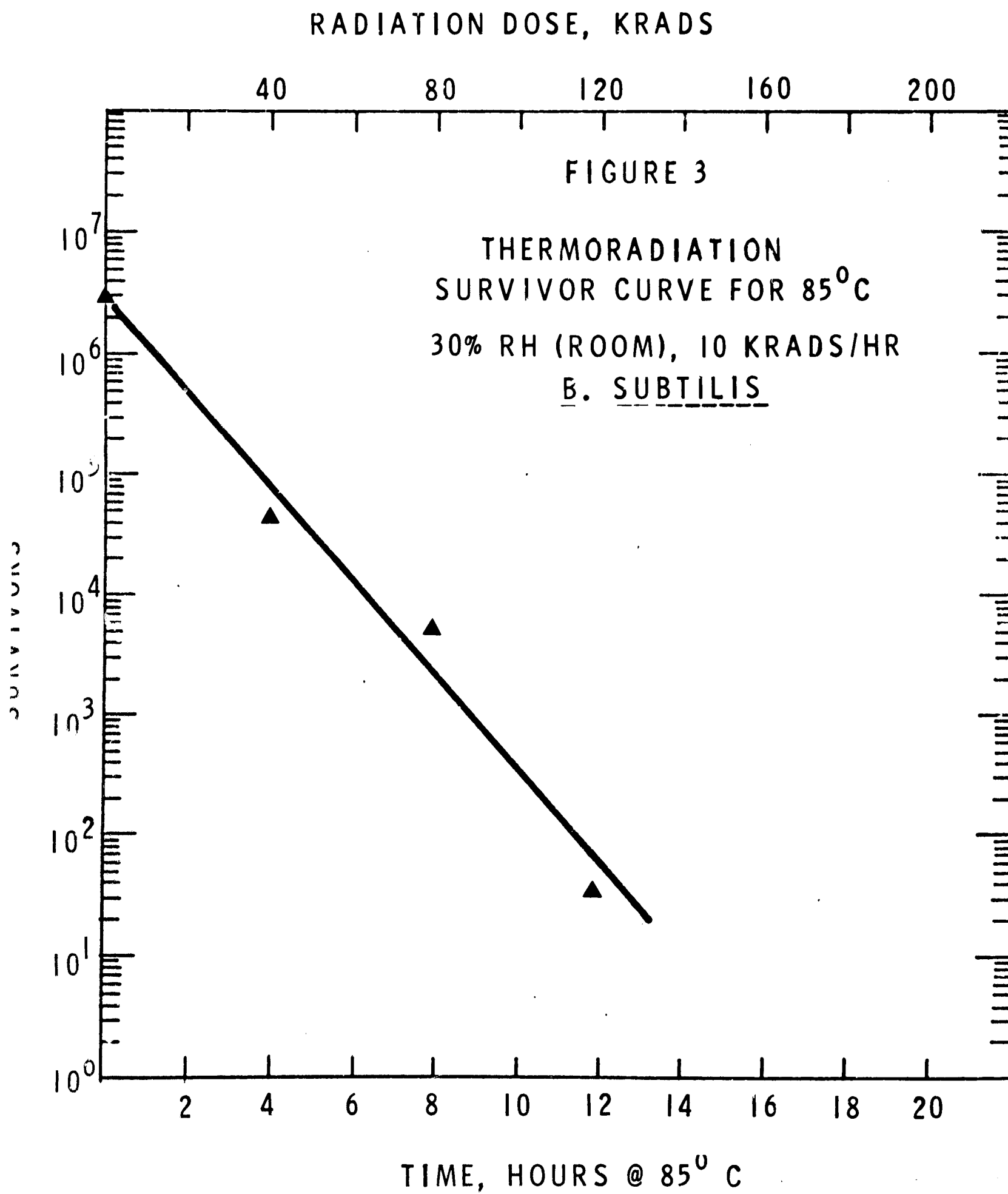
Results are shown in Figure 1 . From the data it would seem there is little effect from the substrates when spores are deposited onto either in the same manner. It does, however, confirm that recovery of spores from foil is satisfactory.

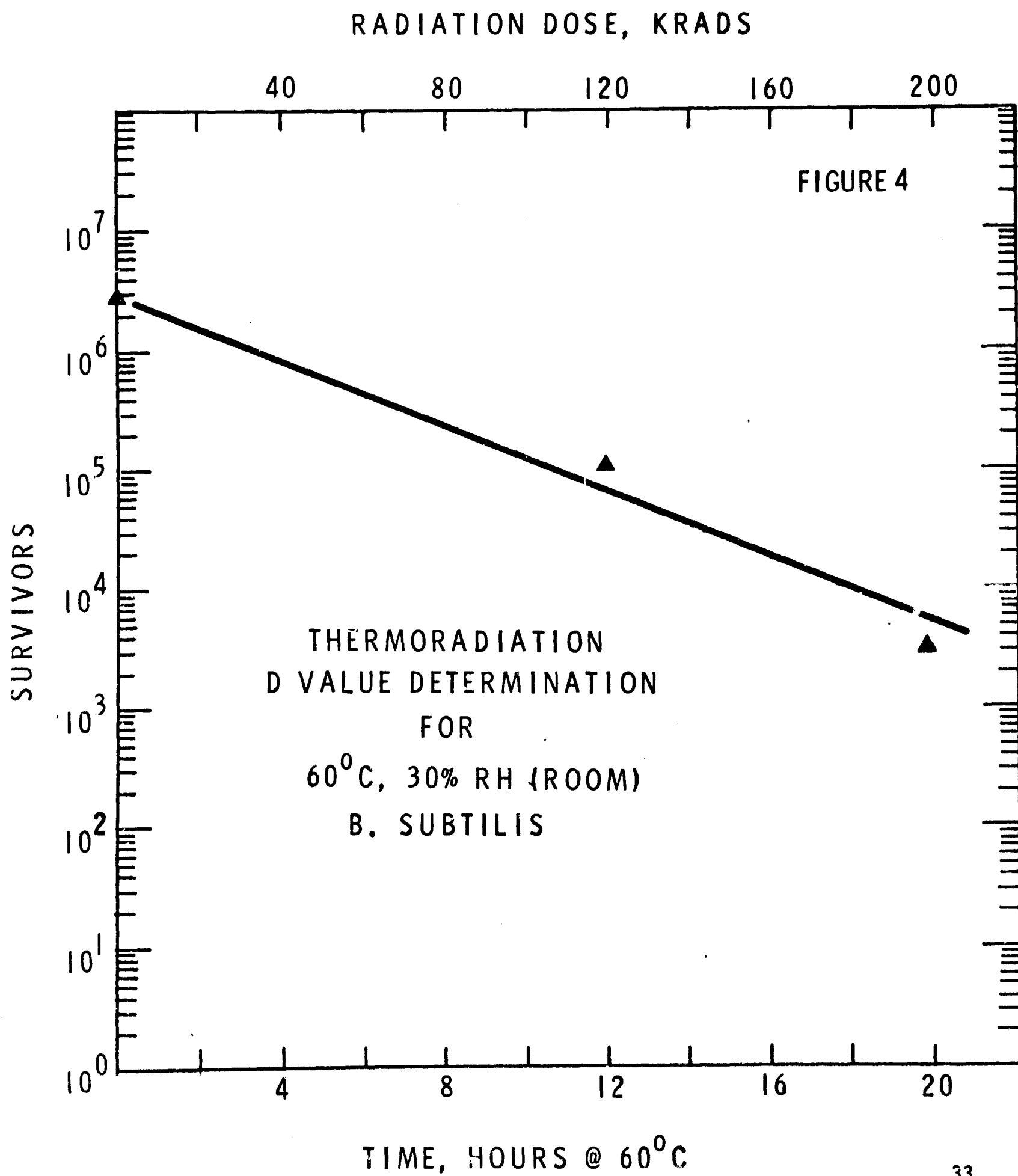
A second comparison of electrostatically deposited spores on aluminum sheet versus pipetted spores on foil was accomplished by running dual sets of samples for each time period. This comparison was performed at 90°C in order to obtain new survival data for B. subtilis at this lower temperature. Results are shown in Figure 2 . It is interesting that the electrostatically deposited samples did in all cases yield a slightly higher number of survivors than did the pipetted samples. The difference, however, is so slight that the additional effort in electrostatic deposition for routine sample preparation may not be warranted. From the standpoint of the lethality of thermoradiation at this temperature, the D value was three hours at 90°C with a dose rate of 10 KRADS/HR.

In addition to the previous low temperature experiment, runs have been completed at 85°C and a two point D value determination at 60°C, results are shown in Figure 3 and 4 . In Figure 3 , the D value for 85°C was 2.6 hours at a dose rate of 10 KRADS/HR. Figure 4 is a D value determination for experiment planning. RH was not controlled since only a rough estimate of the D value was needed for setting up a full experiment. From Figure 4 it appears that the D value at 60°C, 10 KRADS/HR is slightly over seven hours. The full 36 hour experiment will be run on April 2, 1970.









2. Buried Contamination. Thermoradiation, radiation and dry heat studies of B. subtilis in plastics was initiated this quarter. The first material to be used was methyl methacrylate since the dry heat studies of Angelotti, et. al.^{*}, demonstrated a thirty-fold increase in D value for B. subtilis when spores were embedded in methacrylate. For example, he determined D values of 30 hours at a temperature of 105°C. Using these studies as a base line, we began to develop the laboratory techniques necessary to handle inoculation, exposure and recovery of spores from methacrylate. Norm Peterson, PHS Phoenix, was able to provide valuable assistance in demonstrating the preparation of methacrylate. We are now able to cast methacrylate sheet .050 ± .001 inch, yielding 50 to 100 samples per sheet with up to 10⁹ spores per 5/8 diameter sample. After exposure these samples are dissolved in dilution bottles containing 100 ml of acetone. Dilutions are plated out on filters and counted after incubation. Before polymerization, spores were equilibrated for several days in the desired relative humidity atmosphere. A number of short experiments have been performed to determine approximate D values for planning experiments. Spores equilibrated at 10% RH yielded dry heat only D values of about 100 hours at 60°C and 20 hours at 105°C. Radiation only D values were found to be 200 KRADS at room temperature and thermoradiation D values were five hours at 105°C with a dose rate of 30 KRADS/HR. These values are only rough approximations for planning full experiments and were based on two or three point exposures.

^{*}Robert Angelotti, et.al., "Influence of Spore Moisture Content on the Dry Heat Resistance of Bacillus subtilis var. niger", Appl. Microb., May 1968, pp. 735-745.

FINE PARTICLE PHYSICS

- A. Description. A need has arisen recently for a microbiological air sampler which can size particles and operate for periods of days. The Andersen sampler sizes particles quite well, but is capable of operating only several hours before the agar plates begin to dry and crack. A modification of this sampler was undertaken which would extend the operating time considerably. Two uses of this type of instrument are for microbiological monitoring of clean rooms and for relatively long term sampling of airborne microbes in natural environments. The instrument is designed to measure the mean number of microbes per particle in given size ranges of particles, and to examine the frequency distribution of the number of microbes per particle within these size ranges. These parameters are needed for bioburden modeling being done at Sandia.
- B. Progress. The essential difference between the modified and the original Andersen sampler is that the agar surface in the original sampler is replaced by a membrane filter atop a flat piece of saline solution-saturated open-celled foam. The wet filter produces a cool adhesive surface against which particles can impact and adhere. The moisture keeps viable particles from drying over the sampling period. By the equipment shown in Fig. 1, when water evaporates from the filter, a sensing element detects the drop in water level and opens a valve to add deionized water to replace the lost water. In this manner the filter can be kept wet and the sampler can be operated for indefinitely long periods of time. To the present the sampler has been operated for

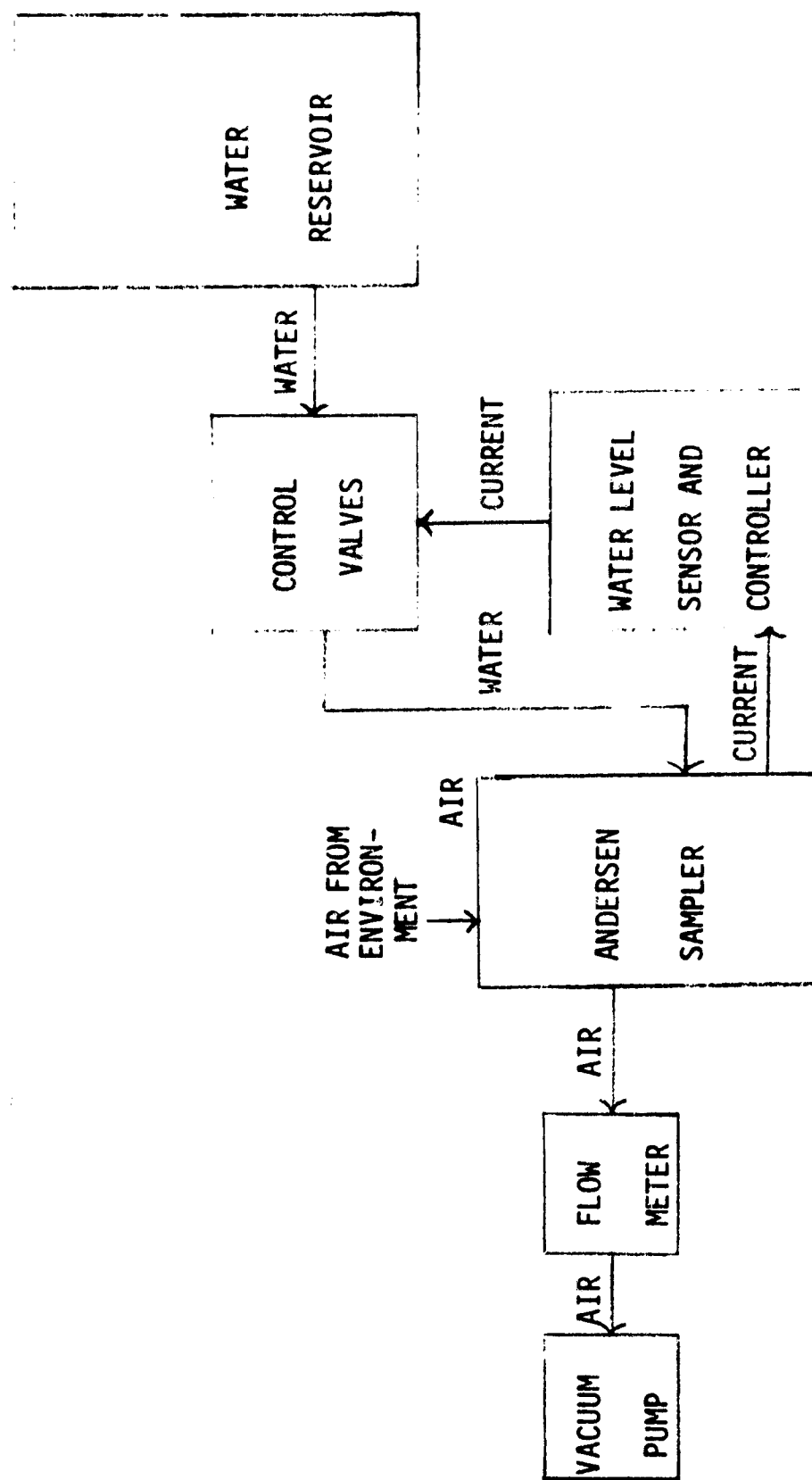


FIGURE 1. FLOW DIAGRAM OF THE MODIFIED ANDERSEN SAMPLER

only 24 hours at a time. There are no technical difficulties, however, in extending the sampling period to five days, or even longer.

Tests indicate that the collection efficiency of an Andersen sampler for single Bacillus subtilis var. niger spores using this collection mechanism is almost identical with the collection efficiency of the sampler using agar plates. Slippage is very small, but detectable, as opposed to the original sampler in which slippage is almost undetectable at normal levels of microbial density. Two variables that remain to be measured are the effect on collection efficiencies for larger particles and the possibility of due off of microbes on the wet membrane filter.

BACTERIAL SPORE INACTIVATION STUDIES

A. Description. Further studies on the inactivation of spores were undertaken this quarter. Ultra-violet radiation offers a unique tool for studying spore inactivation, since the lethality is a result of radiation damage to spore DNA. Thus, by using ultra-violet radiation as an inactivating agent, a defined bio-molecule is affected and one can study the parameters which influence spore survival. Prior work (Sandia Laboratories Quarterly Report No. 15) established that repair phenomena was important in defining spore survival after ultra-violet irradiation. This quarter studies have been initiated which consider chemical means of altering the spore's capacity for repair and/or outgrowth.

B. Progress.

1. The "tailing" observed in the survivor curve after ultra-violet irradiation of spores was shown (Quarterly Report No. 15) not to be due to the presence of a resistant spore sub-population, but was consistent with the existence of a functional spore repair system. Microscopic examination of the spores electro-statically deposited on metal disks showed that approximately one percent of the spores existed as spore aggregates. Since the "tailing" is observed at about the one percent survival level, the possibility existed that the "tailing" was due to a shielding of spores in spore aggregates from the lethal effects of ultra-violet radiation. This possibility was rejected by an experiment in which spores were pipetted onto aluminum disks and subjected to ultra-violet radiation. Microscopic examination of spores pipetted onto disks showed that approximately ninety percent of the spores existed as very large spore aggregates. The survival

curve for the highly aggregated spore system was superimposable on the survival curve for the electro-statically deposited spore system. Therefore, the "tailing" observed in ultra-violet irradiation of spores is not a consequence of protection due to spore clumping.

Figure 1 shows the survival curve for spores at varying dose rates of ultra-violet irradiation. One observes that the spore survival level at an equivalent dose (dose = dose rate X time) is essentially the same for the three dose rates, i.e., a given total dose will define the spore survival level regardless of the dose rate. This observation that spore survival is dose dependent is consistent with knowledge that ultra-violet radiation damage is site specific for DNA. Presumably, a given dose will damage spore DNA to a certain extent and the spore survival level will depend on the extent of DNA damage and the repair of a fraction of this damage. Such considerations suggest that there should be a limit to the amount of DNA damage that can be caused by ultra-violet irradiation. These expectations were realized in the following two experiments. First, spores were irradiated at $40 \text{ ergs/mm}^2/\text{sec.}$ for up to twelve minutes. The resulting survival curve showed that the "tail" flattened out after six minutes and no further decrease in spore survivors was observed. Secondly, on increasing the dose rate to $80 \text{ ergs/mm}^2/\text{sec.}$, it was observed that the initial slope of the survivor curve was greater than at the $40 \text{ ergs/mm}^2/\text{sec.}$ dose rate but the "tailing" occurred at the same survival level for both dose rates.

A series of experiments were undertaken to study the combined effects of heating and ultra-violet irradiation on spore survival.

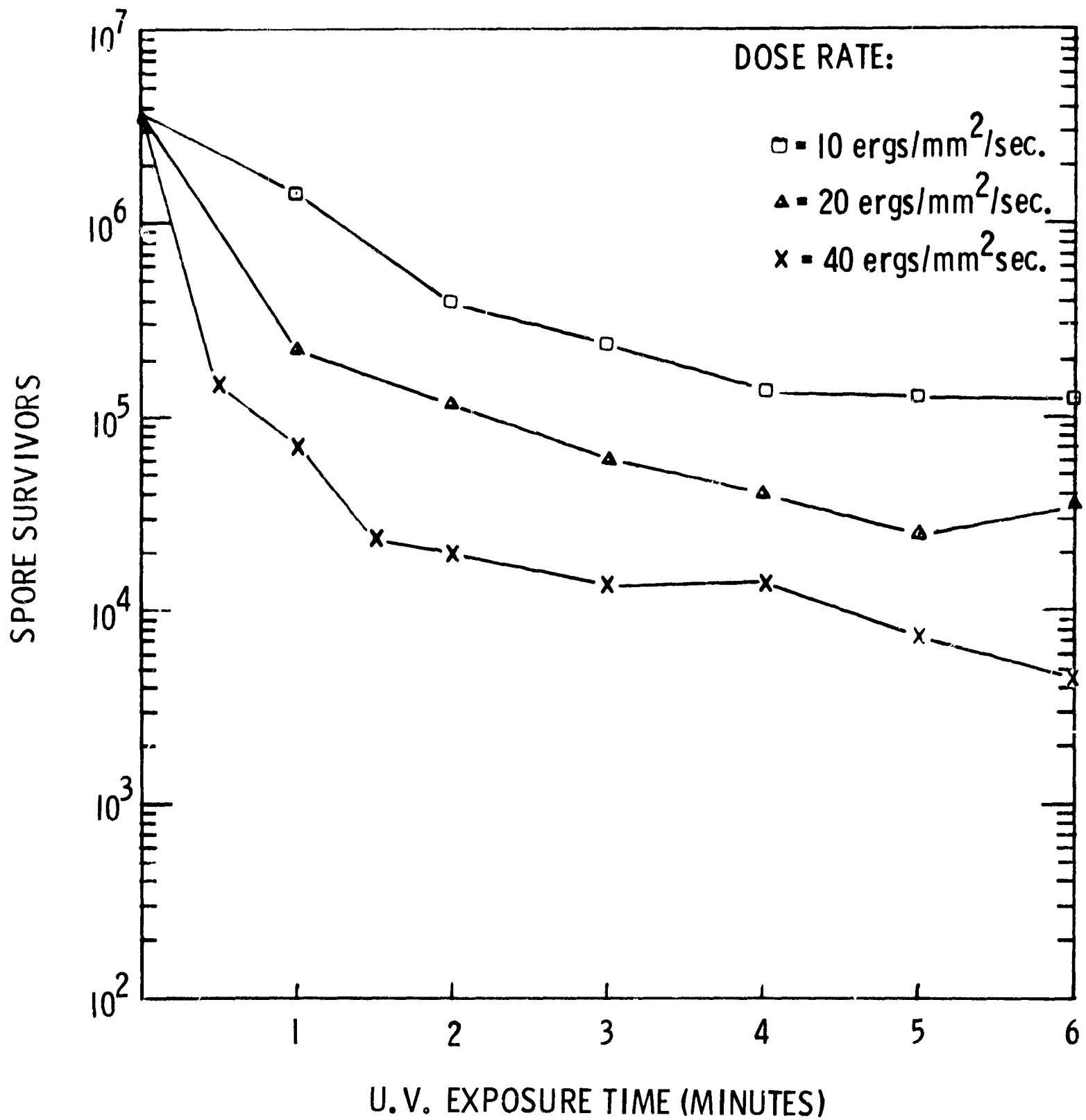


FIGURE I. U.V. SURVIVAL CURVES AT VARYING DOSE RATES

The rationale for such a study is that ultra-violet irradiation would cause a site specific damage to the spore, while heat would cause a non-specific thermal damage to various components of the spore required for survival. If components of the spore repair system are damaged by the thermal treatment, one might expect a synergism in spore death on the simultaneous application of heat and ultra-violet irradiation. Control spores subjected only to heat showed negligible spore death because of the short heating times employed. Figure 2 shows that increasing the heat input with simultaneous ultra-violet irradiation causes an increase in spore death. Since heat alone caused no spore death, a synergism may be said to exist when spores are irradiated and heated simultaneously. This finding is reminiscent of the synergism observed when spores are subjected to the simultaneous inactivating treatments of ionizing radiation and heat.

The transition: dormant spore → germinating spore → vegetative cell involves fundamental changes in the biochemical and physiological properties of the spore. Since heat resistance is lost as dormant spores enter the germinated spore state (Quarterly Report No. 15), it was of interest to see if the response of the germinated spore to ultra-violet radiation was different from that of the dormant spore. This study was realized by allowing spores to germinate, isolating the germinated spores via Millipore filtration, and subjecting these spores to ultra-violet irradiation. It was observed that the dormant spores and the germinated spore had superimposable survivor curves after ultra-violet irradiation. This finding is consistent with the idea

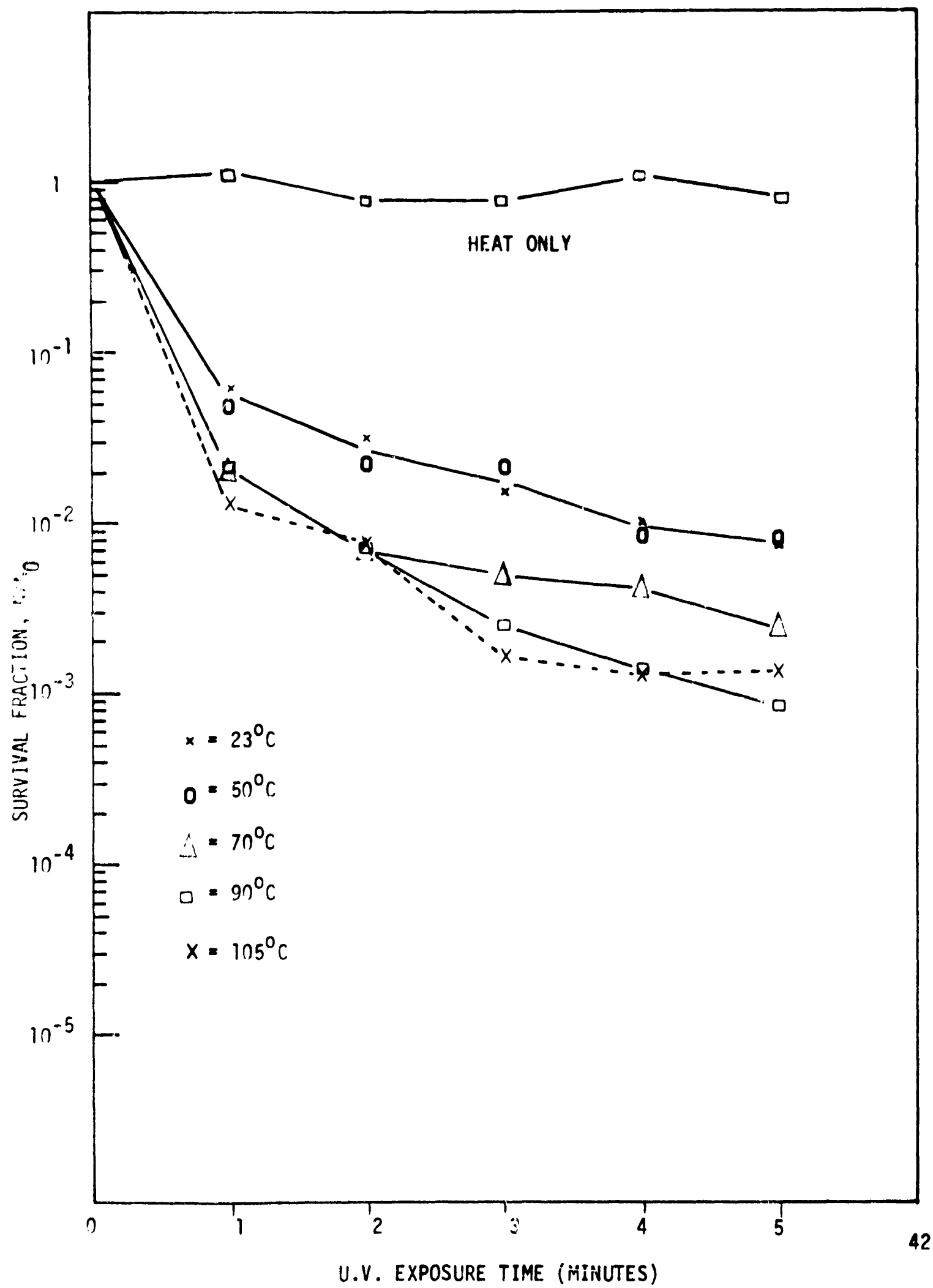


FIGURE 2. SURVIVAL CURVE FOR SIMULTANEOUS U.V. IRRADIATION AND HEATING OF SPORES

that ultra-violet damage is site specific and is independent of the physical state of the spore. This finding also implies that the repair process occurs during the transition from germinated spore to vegetative cell.

2. The idea that repair is an important parameter in defining spore survival suggested that if some method of inactivating this repair capacity could be developed then significant progress towards a sterilization protocol may be realized. Reports in the literature that various chemicals can interfere with cellular repair phenomena suggested that these chemicals may also affect the repair capacity of a spore. Therefore, studies on the effect of various agents in defining spore survival were initiated.

The first of the chemicals studied was N-Bromosuccinimide (N.B.S.). This agent is known to oxidize various amino acid residues in proteins and, therefore, would be expected to act in a non-specific manner on any proteinaceous component of the spore. It was found that N.B.S. can interfere with both spore germination and subsequent spore outgrowth. The effect of N.B.S. on spore germination was studied by monitoring the rate of the optical density changes of a spore suspension containing varying concentrations of N.B.S. This study revealed that as the N.B.S. concentration increased, there was subsequent decrease in the rate of spore germination. When the germination rate became zero, no subsequent outgrowth of the spore occurred and, hence, no colonies were formed when the "non-germinating" spores were plated

cut in culture media, i.e., non-germination is the experimental equivalent of spore "death". The following experiment was undertaken to answer the question of whether N.B.S. affected stages in spore development other than germination. Spores were allowed to germinate, isolated by Millipore filtration, and exposed to an N.B.S. solution for two minutes. The spores exposed to N.B.S. showed approximately two to three log lower survivor level than the non-N.B.S. treated control. It appears that N.B.S. can interfere at both the level of spore germination and subsequent spore outgrowth.

PUBLICATIONS

1. M. E. Morris, "Acoustic Dustfeeder", Journal of the American Association for Contamination Control, Vol. 2, No. 2, January 1970, pg.31.
2. M. E. Morris and J. W. Beakley, "Production of Aerosols of Viable Particles of Different Sizes", Journal of the American Association for Contamination Control, Vol. 2, No. 2, January 1970, pg. 47.

PRESENTATIONS

1. H. D. Sivinski presented a talk on Modeling and Studies of Inactivation Mechanisms to NASA Headquarters, Planetary Quarantine Officer, on January 27, 1970; Division of Biology and Medicine, Washington, D. C., January 28, 1970; Chief, Physical Defense Division, Ft. Detrick, Frederick, Maryland, January 28, 1970; U. S. Army Natick Laboratories, Natick, Massachusetts January 29, 1970.
2. M. C. Reynolds presented talks on Thermal Radiation Sterilization to the above mentioned installations on the same trip.
3. W. J. Whitfield made a presentation as a Member of the Faculty to the Rochester Institute of Technology Contamination Control Seminar entitled, "Principles of Design of Laminar and Non-Laminar Clean Rooms", Rochester, New York, March 5, 1970.
4. H. D. Sivinski presented a talk concerning Thermal Radiation to the Division of Military Applications, AEC Headquarters, Germantown, Maryland, March 24, 1970.
5. H. D. Sivinski, W. J. Whitfield, and C. A. Trauth visited NASA Headquarters, Washington, D. C. on March 24, 1970. for Sandia Laboratories Research Objectives Discussions.
6. C. A. Trauth and W. J. Whitfield held discussions at the Division of Biology and Medicine, Headquarters, AEC, on March 24, 1970, regarding Planetary Quarantine Research.