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SUFFIELD TECHNICAL NOTE

NO. 236

THE EFFECT OF STARVATION BEFORE AEROSOLIZATION ON THE SURVIVAL OF AIRBORNE BACTERIA (U)

by

J.R. Maltman and G.J. Leahy

DRB PROJECT NO. D-18-20-18

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SUMMARY

Starvation of distilled water suspensions of *Escherichia coli* for periods of time up to 24 hours progressively increases losses of ultraviolet absorbing materials from the cells.

It has also been found that increasing the temperature of the starvation liquid from 4 to 37°C increases the loss of 260 mμ absorbing materials. In addition, aeration of the bacterial suspensions increases losses of these compounds when starvation is conducted at 37°C, but not at 4°C.

In all cases, the greater the loss of ultraviolet absorbing materials induced by starvation, the better was the survival of the airborne bacteria.

It was also noted that the initial pH values (4 to 10) of the distilled water suspensions of *E. coli* which were subjected to starvation procedures showed no appreciable relationship to the increased aerosol survival values.

It is apparent in these studies that increased survival of airborne bacteria induced by prior starvation is most pronounced when aerosols *E. coli*, *Aerobacter aerogenes*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are stored at intermediate, rather than low humidities. However, when *Serratia marcescens* is stored in distilled water, the aerosol survival increase over that obtained with non-starved preparations is greater when the airborne cells are stored at low humidity.

It is also of interest to note in experiments with aerosols of *E. coli* and *Kl. pneumoniae* that starvation fails to appreciably change the survival values compared with non-starved cells when the aerosols are aged at high humidity.

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INTRODUCTION

It is well established that endogenous respiration under starvation conditions induces catabolism of the ribosomes of bacteria. These events are manifested by substantial release of ultraviolet (UV) absorbing materials from the cells and reflect the degradation of ribonucleic acid (RNA). (1-15)

Degradation of protein has also been noted during starvation. (12)

These losses of intracellular materials do not necessarily cause death during the starvation period. It is also evident that release of intracellular materials induced by desiccation-rehydration procedures does not correlate well with cell death. Certain chemical compounds which penetrate the cells will enhance the survival of the airborne cells although more than 260 mμ absorbing material is released by the cells when they are reconstituted in the collecting fluid. (16)

It was therefore considered likely that reduction of intracellular compounds induced by starvation would also exert a governing influence on the survival of bacteria subjected to aerosolization and air storage.

It is the purpose of this paper to present the preliminary results of the changes in bacterial aerosol survival induced by prior starvation of the cells in distilled water.

MATERIALS AND METHODS

1. Organisms

Escherichia coli ATTC 9723, *Aerobacter aerogenes*, *Serratia marcescens*, Detrick strain 100, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* ATTC 10031 were the species employed in this study.

2. Growth Media and Conditions

All bacterial species were grown with aeration (incubator shaker) for 24 hours at 37°C in Bacto Heart Infusion broth containing one per cent Bacto yeast extract.

3. Starvation Technique

The broth cultures were centrifuged and washed in distilled water three times and suspended in distilled water for further treatment. This suspension is termed "nonstarved". These washed cell suspensions were starved (stored) at 4°C or 37°C with or without aeration for various periods of time. At appropriate times, the starved suspensions were again centrifuged and resuspended in distilled water for aerosolization.

pH Measurement

In some experiments, the pH of nonstarved and starved cell suspensions was measured with a Beckman pH meter.

4. Measurement of the Release of Ultraviolet Absorbing Materials

Ultraviolet absorbing materials released from the same bacterial suspension before and after starvation were measured from filtrates of the samples that were passed through 0.45 μ millipore filters. Measurements were conducted in a Beckman DB spectrophotometer using the filtrate of the nonstarved sample as the reference blank.

5. Aerosol Techniques

All aerosols were generated from distilled water suspensions by Collison spray apparatus into a toroid drum. (17) The storage temperature for all aerosols tested was 27°C and relative humidity (RH) levels from 20 to 80 per cent were investigated. At appropriate times, 12.5 litre samples of the aerosol were collected in one minute in impingers containing 10.0 ml gelatin saline.

6. Viable Count Assessment

Viable count assessment of the bacteria collected in the sampling fluid was conducted by surface plating decimal dilutions on Bacto Heart Infusion agar. Each assessment was carried out in duplicate. The results are expressed as survivors per ml.

RESULTS

1. The Effect of Starvation Time on the Aerosol Survival of *Escherichia coli*

When *E. coli* is starved with aeration in distilled water at 37°C for 2, 4, 16 and 24 hours, there is a progressive increase in the ability of the cells to survive the stresses associated with storage in the airborne state at 27°C and 30 per cent RH (Figure 1). There is no appreciable loss of viability in the distilled water bacterial suspension during the starvation period of 24 hours. In a typical experiment, the viable count per ml was 2.02×10^9 and 1.95×10^9 before and after starvation respectively.

2. The Effect of Starvation Conditions on Survival of Airborne Bacteria

(A) Temperature

E. coli was starved with aeration for 24 hours at 4 and 37°C. Results in Figure 2 indicate that the higher temperature is required to induce an appreciable increase in the survival of the aerosol held at 27°C and 30 per cent RH.

(B) Aeration

Figure 3 shows that distilled water suspensions of *E. coli* starved 24 hours without aeration at 37°C do not survive the low humidity storage (30 per cent RH) as well as the preparation starved with aeration at the same temperature.

All subsequent experiments concerned with aerosol survival employed starved suspensions prepared at 37°C with aeration.

(C) pH

E. coli is known to contain ribonucleases that are operative over a pH range of 4.0 to 10.5 with an optimum pH of 7.0 for best activity. In addition, *E. coli* contains enzymes polynucleotide phosphorylase and phosphodiesterase which degrade RNA.

It was therefore considered unlikely that the pH of the fluid in which the bacteria were starved would produce major differences in the increased aerosol stability induced by starvation.

To test this concept, distilled water suspensions of *E. coli* were adjusted to pH 10 with N NaOH or to pH 4.0 with N HCl prior to starvation for 24 hours. After the starvation treatment, the pH had shifted to pH 8.2 and 6.5 respectively, while the release of 260 mμ absorbing material from the cells was recorded as optical densities of 0.16 and 0.17. (See Section 4). Unadjusted distilled water suspensions showed a pH shift from 6.8 to 7.4 under the same conditions and an optical density of 0.19 for released 260 mμ absorbing material. These suspensions were centrifuged and resuspended in distilled water for aerosolization. In all cases, the starvation induced increased survival of aerosols stored at 30 per cent RH and were similar regardless of differences in pH history (Figure 4).

All subsequent experiments were conducted with normal distilled water preparations.

3. The Effect of Relative Humidity

To establish the RH conditions that influenced starvation induced stability, nonstarved and starved E. coli preparations were aerosolized and stored at humidities from 20 to 80 per cent RH. Figure 5 shows that the ratio of the viable count of starved/nonstarved 3-hour aerosols increased from 20 to 50 per cent RH and decreased from 50 to 80 per cent RH. When the aerosols were stored at 80 per cent RH, there was little difference in the survival of airborne E. coli with nonstarved or starved preparations. At all other humidities tested, the starved cell preparations survived aerosol stresses better than nonstarved cells.

4. Influence of Starvation Conditions on the Release of 260 mμ Absorbing Materials

Because temperature and aeration during the starvation period exerted an influence on the ability of aerosols of E. coli to survive low RH conditions, it seemed possible that one of the governing factors might be the magnitude of losses of UV absorbing materials which occur during the starvation period. Figure 6 indicates that the greatest release of 260 mμ absorbing materials takes place when the cells are starved at 37°C with aeration and least when the cells are starved at 4°C with or without aeration. Cells held at 37°C without aeration release intermediate amounts of UV absorbing materials. It is interesting to note that the greater the release of 260 mμ absorbing materials during the starvation in distilled water, the better the aerosol survival. (See Figures 2 and 3).

5. The Effect of Starvation on the Aerosol Survival of Some Gram Negative Bacteria

To ascertain whether the influence of starvation on aerosol survival was restricted to E. coli, some other gram negative species were tested. Figure 7 shows that starvation of Kl. pneumoniae enhances the ability of the aerosols to survive when stored at low humidity. Still greater differences in aerosol survival of starved and nonstarved preparations are found when the airborne bacteria are stored at an intermediate humidity. Under high humidity aerosol storage conditions, there is little difference between survival of starved and nonstarved bacteria. This latter finding is similar to the results obtained with E. coli. Subsequent experiments with other gram negative bacteria were conducted with aerosol storage at 20 and 55 per cent RH.

In Figure 8, it is noted that aerosols of Ps. aeruginosa show enhanced starvation induced survival only at intermediate humidity, while survival of aerosols of A. aerogenes is enhanced to an extent at both low and intermediate humidities (Figure 9).

Starvation of S. marcescens produces appreciable survival benefits in aerosols stored at low humidity, but only marginal influences with aerosols stored at intermediate humidity (Figure 10).

In all of the above experiments with Kl. pneumoniae, Ps. aeruginosa, A. aerogenes or S. marcescens, there was no appreciable cell death during the starvation period.

DISCUSSION

In the present study, bacteria have been subjected to starvation, desiccation and rehydration procedures.

It would appear that increased aerosol stability induced by starvation of the cells in distilled water is not due to selection of the resistant members of the bacterial population, for no appreciable loss of viable numbers occurs during the starvation period.

The correlation obtained between the increases in losses of UV absorbing intracellular materials (governed by increased temperature and aeration) and aerosol survival suggests the possibility that intracellular compounds exert a considerable influence on the ability of the cells to withstand desiccation and rehydration events.

This possibility is supported by evidence that certain compounds known to penetrate bacterial cells enhance both aerosol survival and the release of intracellular compounds. (16, 18).

In addition, it is known that drying Staphylococcus aureus and Kl. pneumoniae at high humidity values enhances leakage of 260 $m\mu$ absorbing material on reconstitution, although survival values are higher than those obtained by drying at lower humidities. (19)

Therefore, it seems reasonable to infer that the losses of these intracellular compounds confer survival advantage to cells stressed by aerosolization and air storage procedures.

The question naturally arises whether or not all classes of compounds that are released from the cells during starvation contribute to the increased aerosol survival. Only compounds and macromolecules remaining in the cells should govern the aerosol survival response. Information regarding events occurring during starvation has been obtained with Ps. aeruginosa. (10) These studies indicate that during the endogenous respiration of Ps. aeruginosa, the "free amino acid pool" decreases, whereas the "nucleic acid precursor pool" increases. Ammonia derived from deamination of amino acids has been found to be the end product of endogenous respiration of bacteria. (4, 20) Similar findings were also noted during starvation of A. aerogenes. (12) These studies show that ribonucleoprotein degradation which occurred during prolonged starvation was not accompanied by an accumulation of amino acids. The main portion of the protein nitrogen appeared in the suspending fluid as ammonia.

Since these events appear to be common in bacteria and occur during processes that also enhance the survival of aerosols, it should be possible to inhibit starvation induced aerosol stability by reincorporation of some of the classes of compounds released during starvation in distilled water. In studies to be reported later, some support for the above possibility has been found. (21) E. coli starved in distilled water was treated with single amino acids and thoroughly washed with distilled water prior to aerosolization from distilled water suspension. Decreased aerosol survival of the water starved amino acid treated cells compared to survival of water starved cells has been noted.

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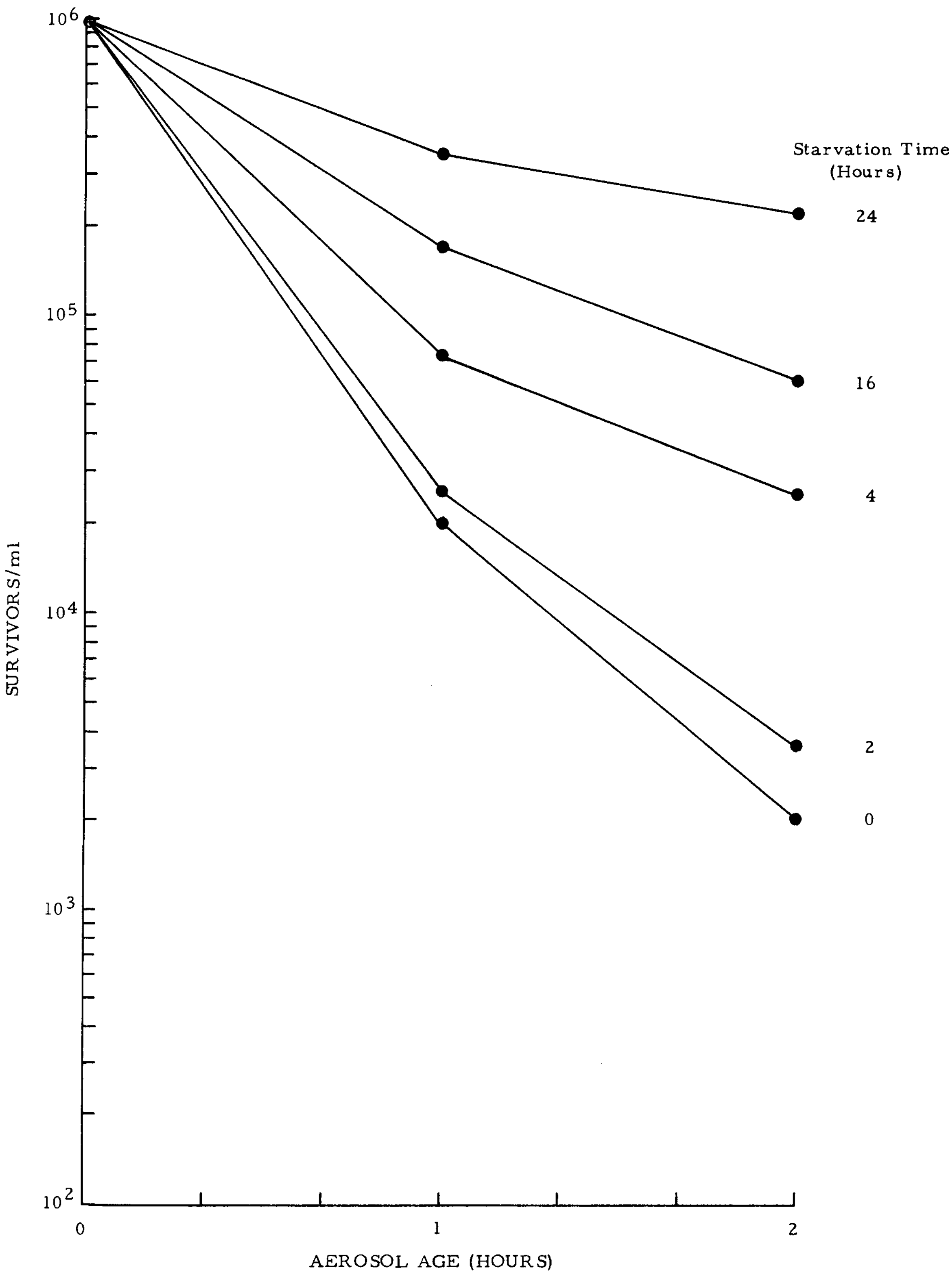

FIG. 1 - The effect of starvation time on the survival of airborne Escherichia coli

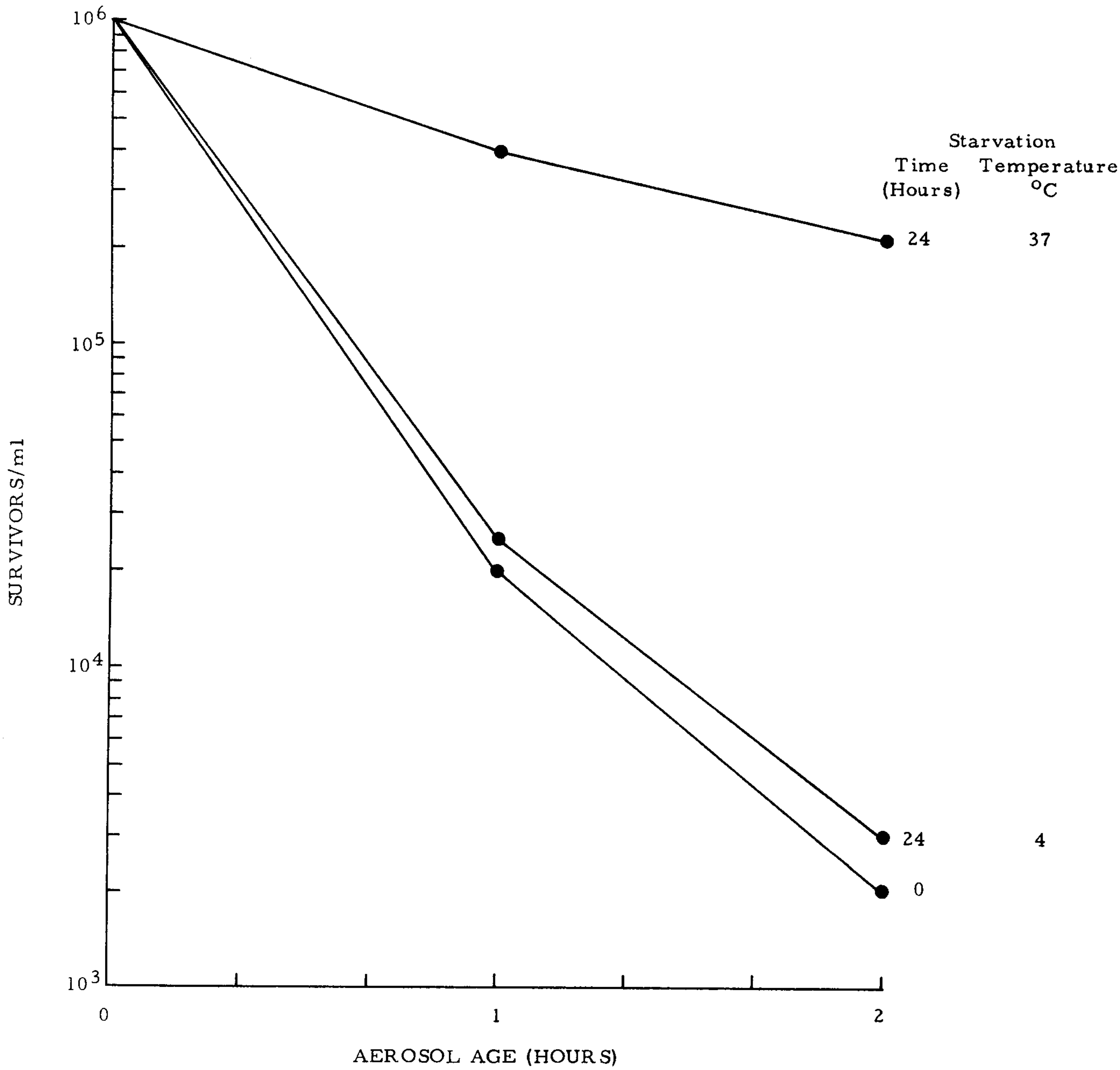

FIG. 2 - The effect of starvation temperature on the survival of airborne Escherichia coli

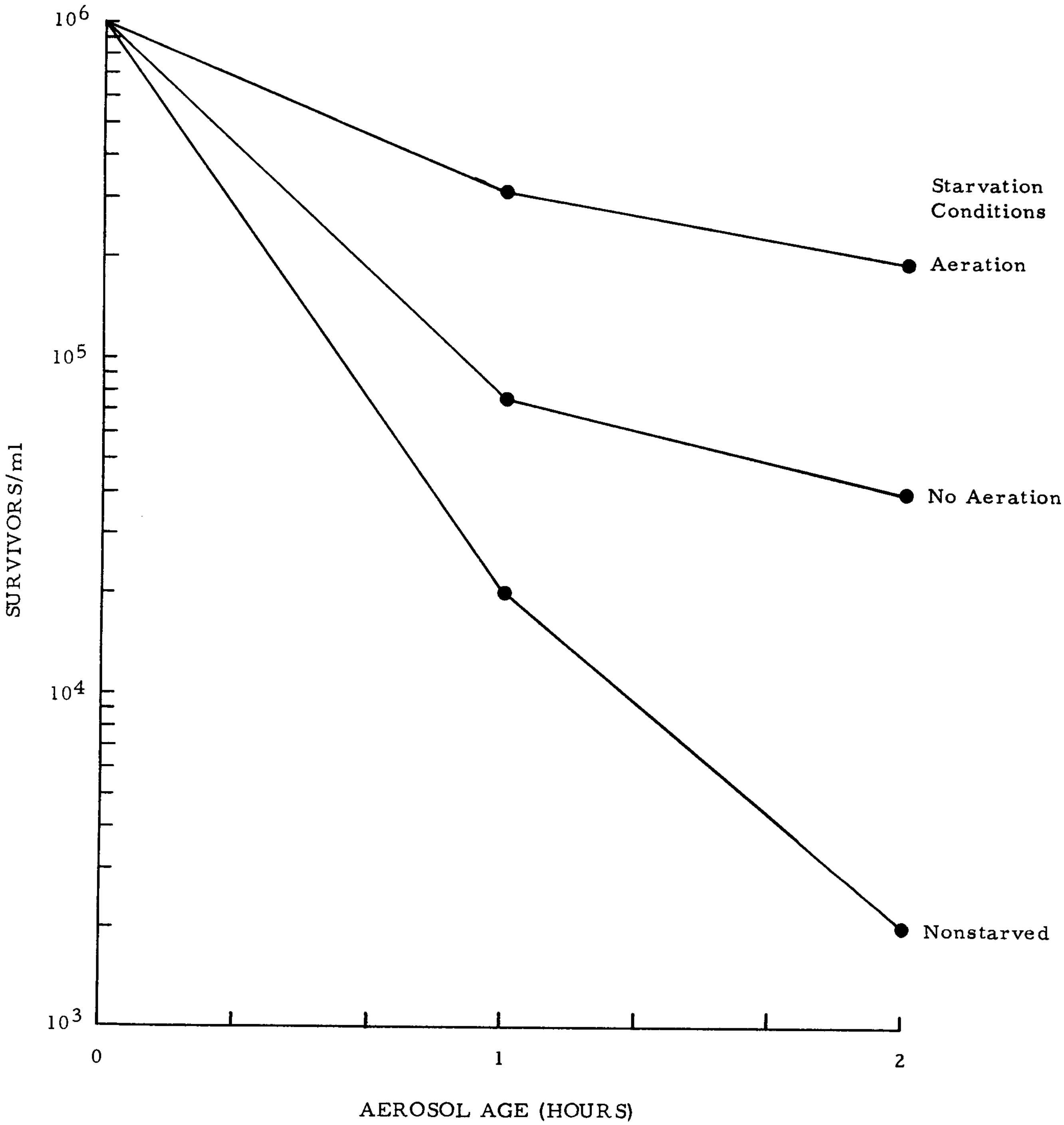

FIG. 3 - The effect of aeration during the starvation period on the survival of airborne Escherichia coli

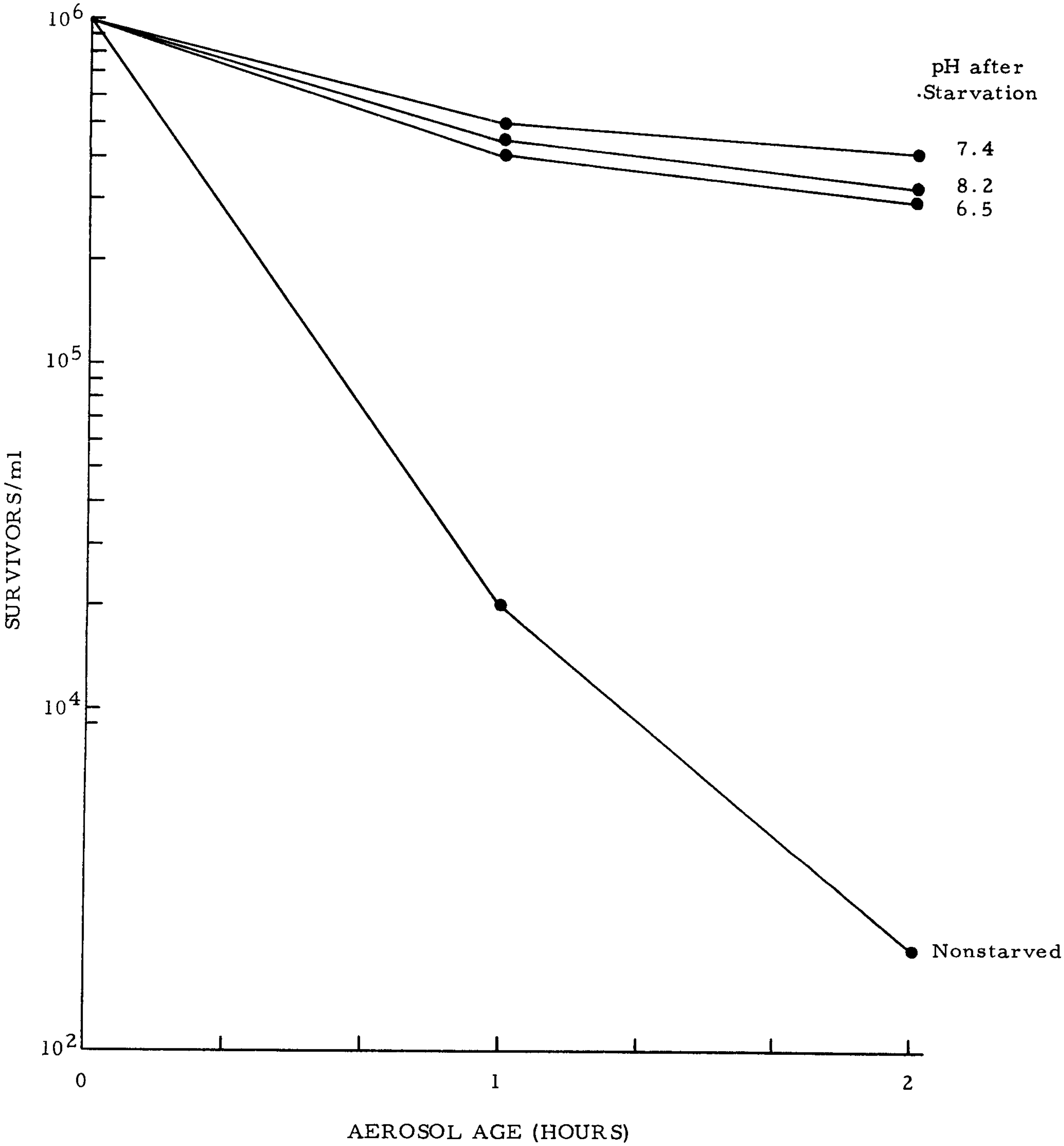

FIG. 4 - The effect of starvation pH on the survival of airborne Escherichia coli

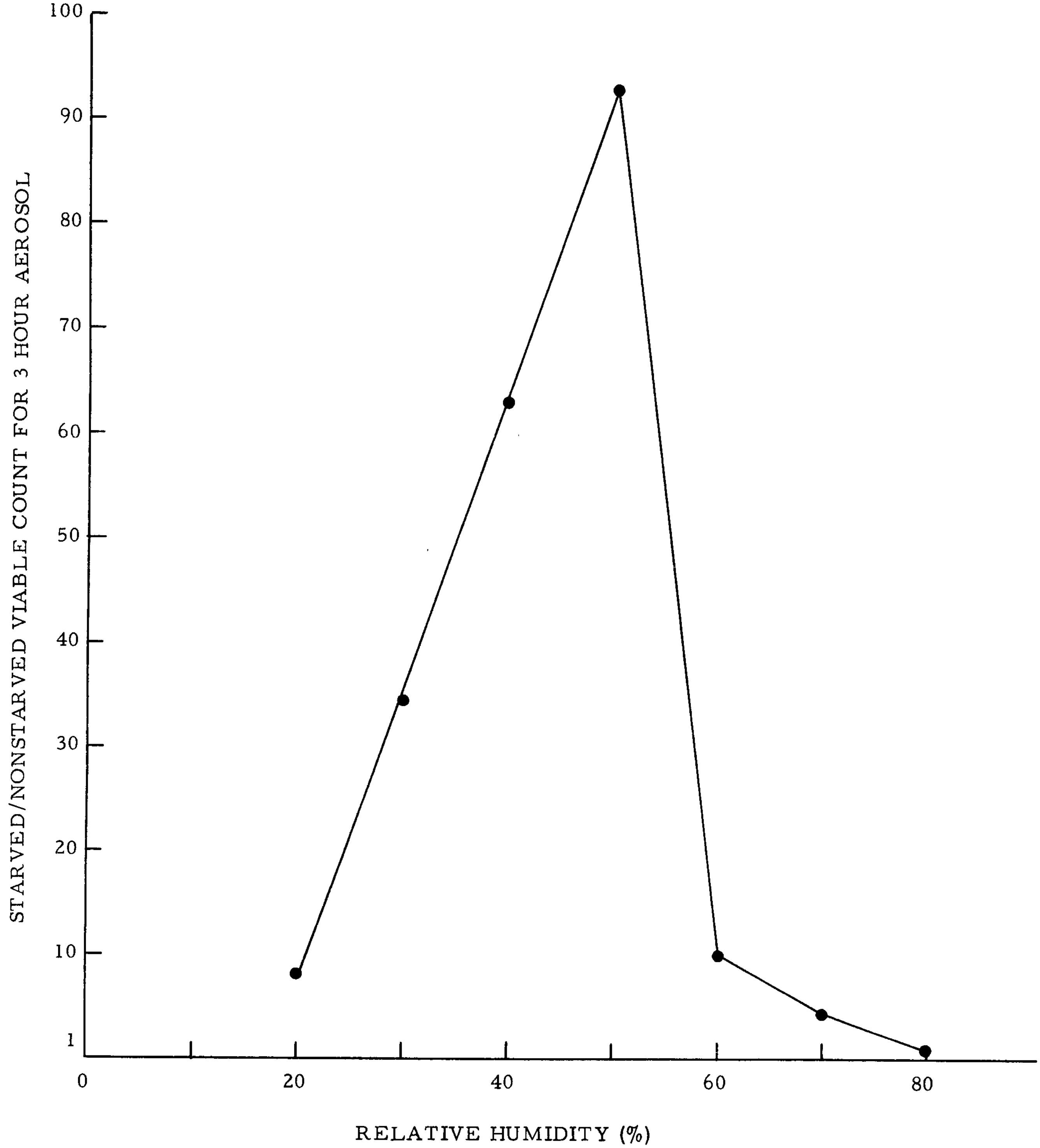

FIG. 5 - The effect of relative humidity on the survival of starved and nonstarved aerosols of Escherichia coli

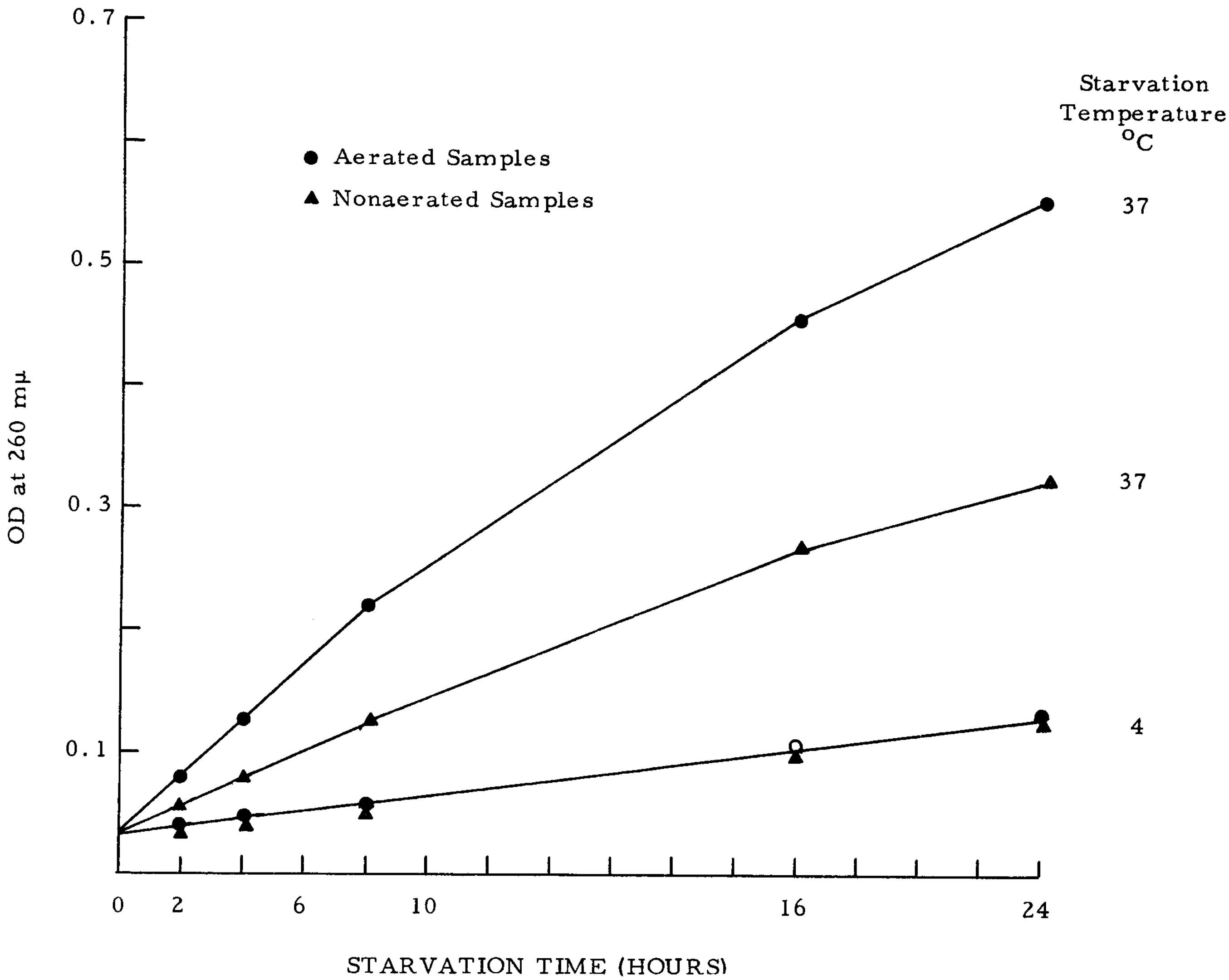

FIG. 6 - The effect of starvation conditions on the release of 260 mμ materials from Escherichia coli

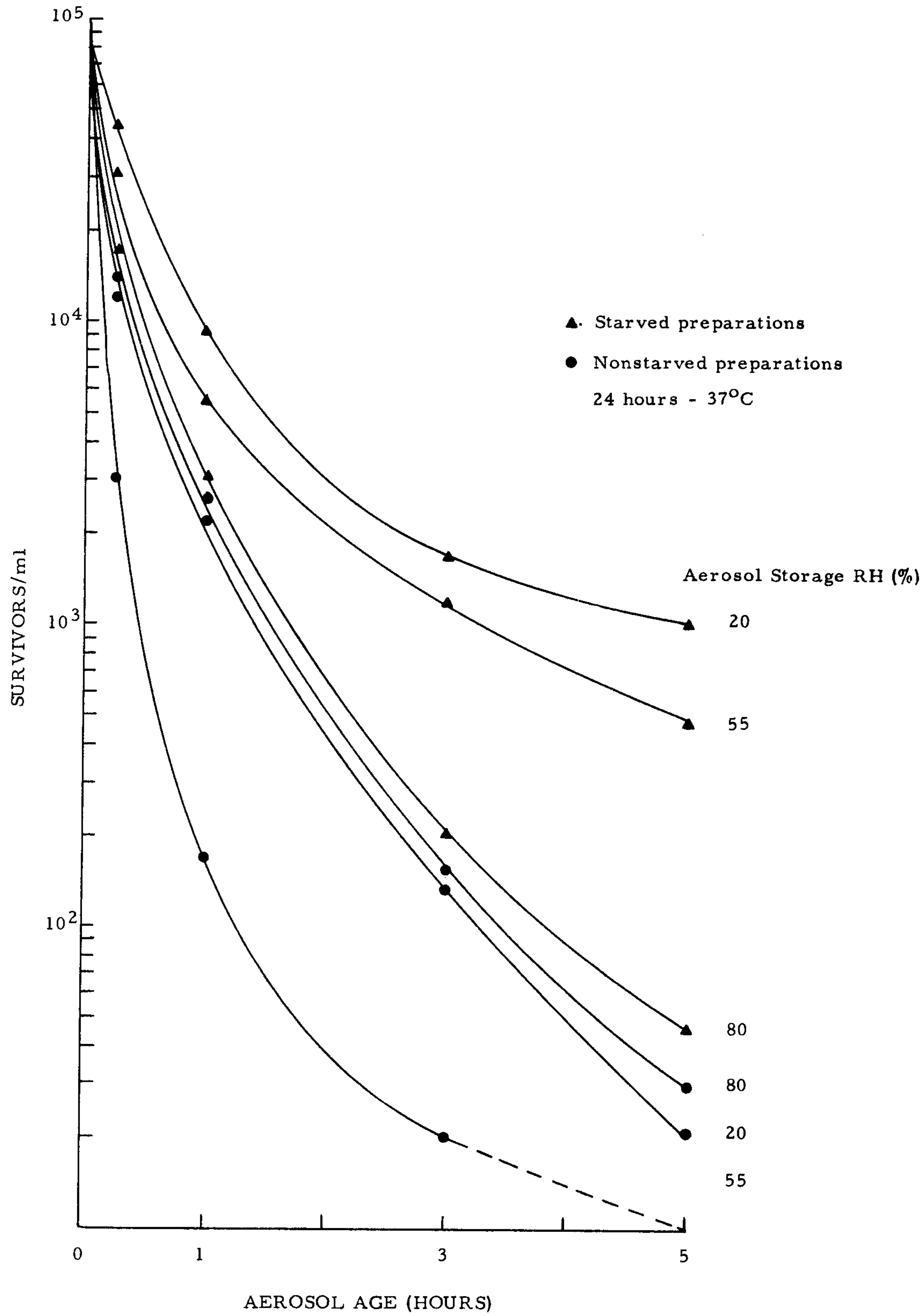

FIG. 7 - The effect of starvation on the survival of airborne Klebsieila pneumoniae

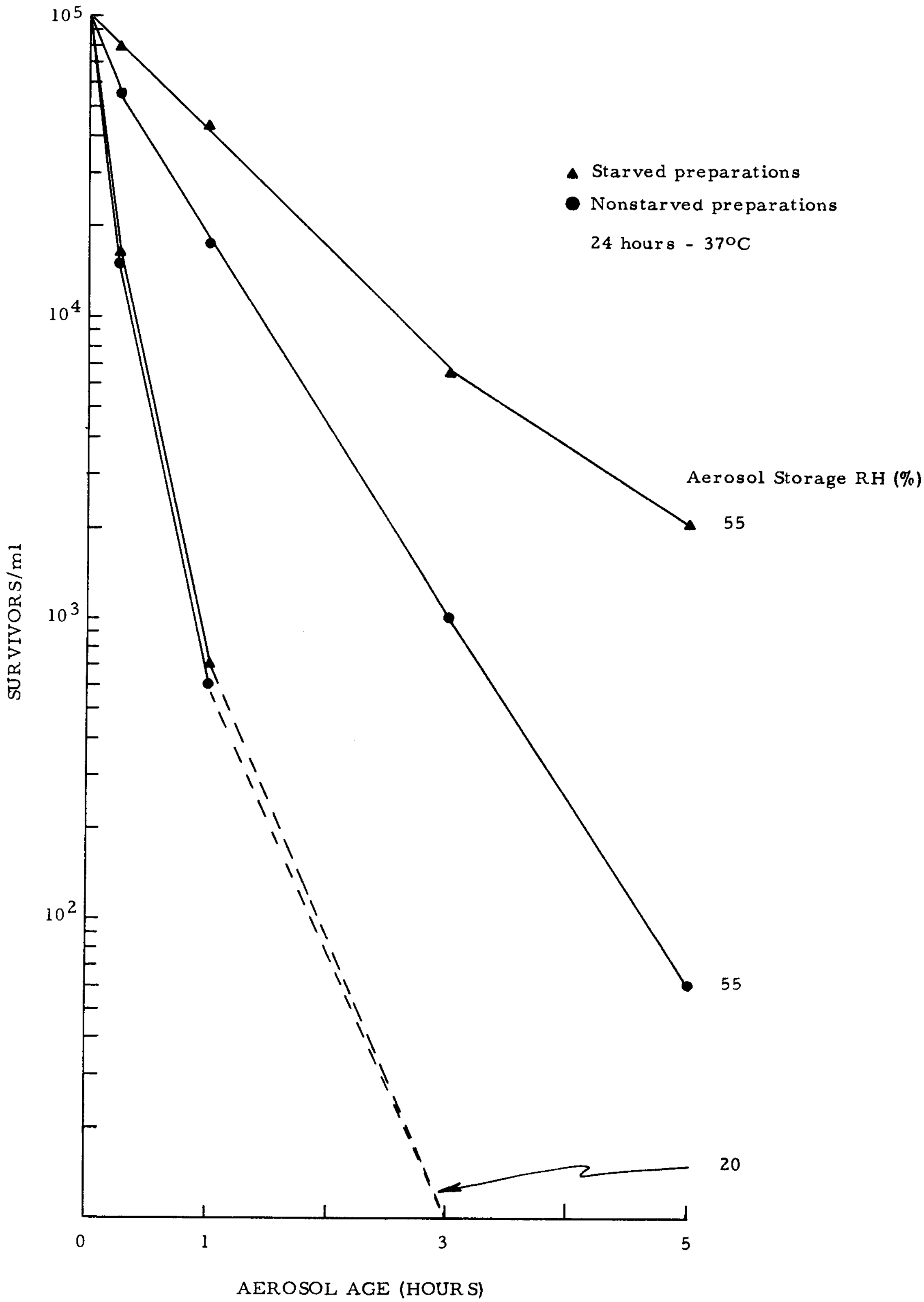

FIG. 8 - The effect of starvation on the survival of airborne Pseudomonas aeruginosa

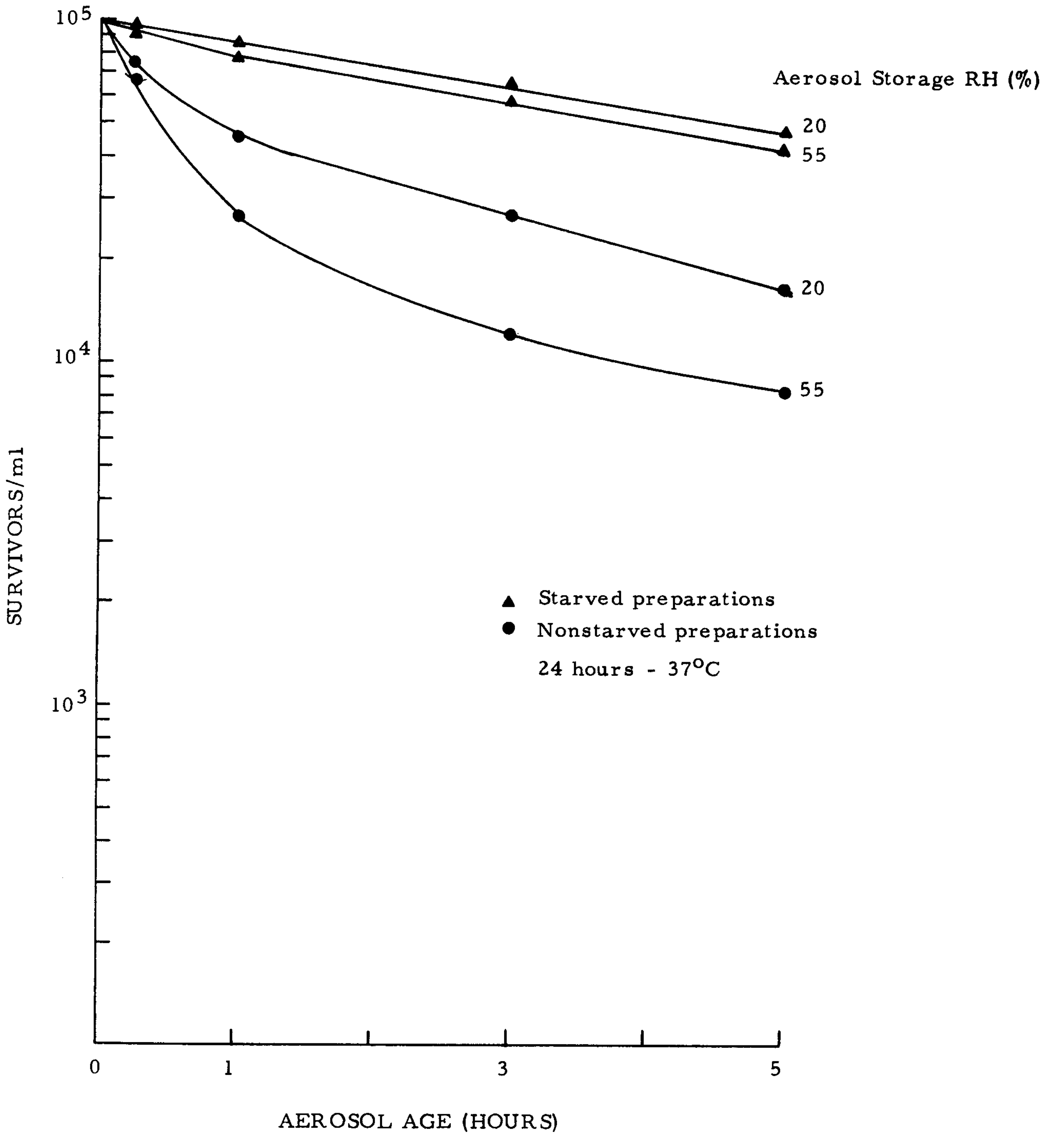

FIG. 9 - The effect of starvation on the survival of airborne Aerobacter aerogenes

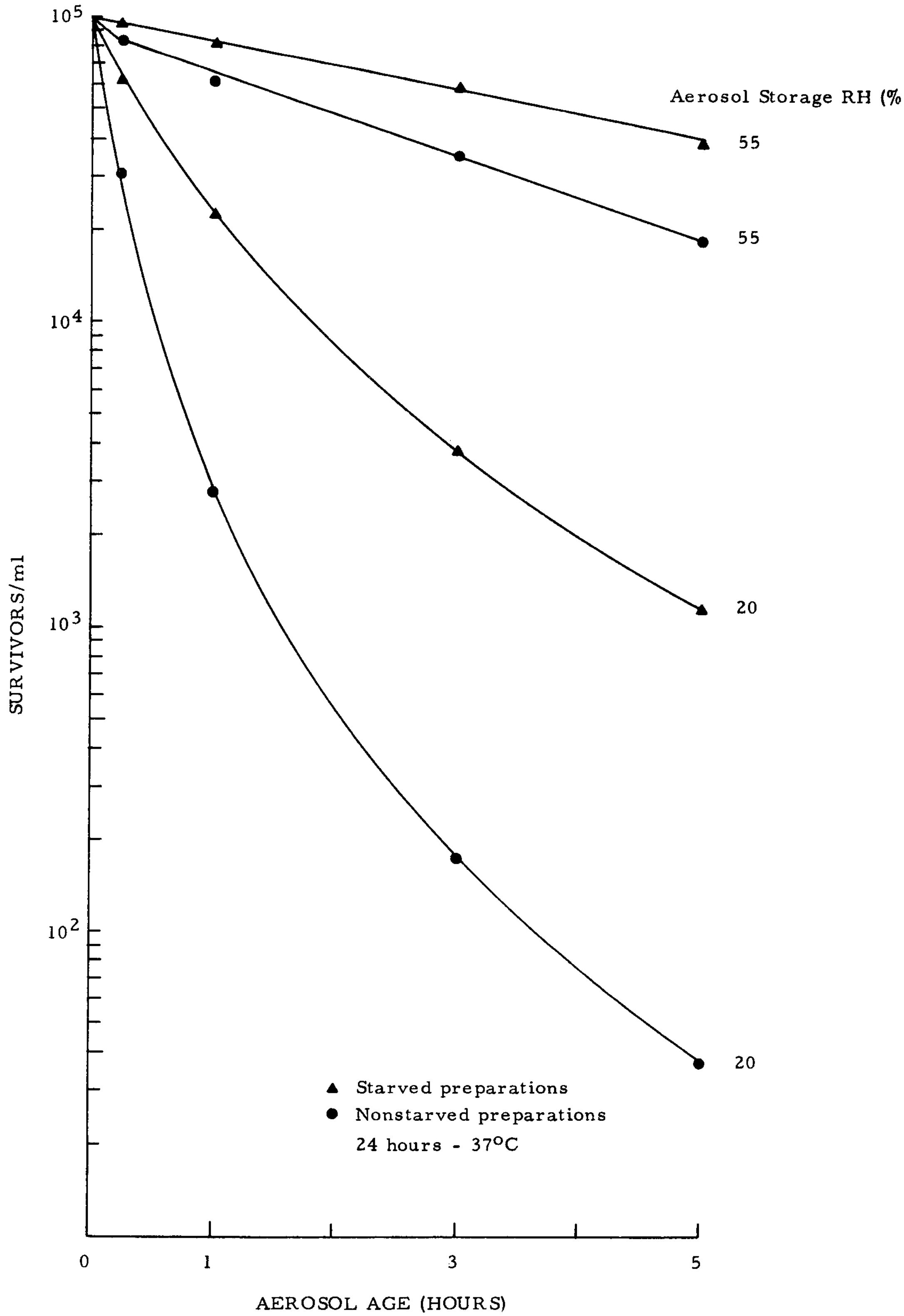

FIG. 10 - The effect of starvation on the survival of airborne *Serratia marcescens*

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7 pp 10 figs. By J.R. Maltman and G.J. Leahy

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Preaerosol starvation of distilled water suspensions of some gram negative bacteria induce losses of ultraviolet absorbing materials from the cells and increase aerosol survival.

Possible relationships of starvation induced degredation of intracellular compounds to aerosol survival are discussed.

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