A STUDY OF THE MICROBIOLOGY OF SELECTED DEHYDRATED FOOD PRODUCTS

by

F. E. Wells

PROGRESS REPORT NO. 1
15 October 1963 - 14 February 1964

Contract No. DA19-129-AMC-206(N)
Project No. 1K643303D548

MRI Project No. 2738-B

For

U. S. Army Natick Laboratories
Natick, Massachusetts
Attn: Program Coordination Officer
Food Division

PREFACE

Progress Report No. 1 on Midwest Research Project No. 2738-B presents the pertinent technical information obtained under Contract No. DA19-129-AMC-206(N) for the U. S. Army Quartermaster Research and Engineering Command, Natick, Massachusetts. The period covered in this report is 15 October 1963 to 14 February 1964.

Dr. F. E. Wells has served as project leader. The work has been done in the Food Technology and Nutrition Section, which is the responsibility of Mr. O. B. Gerrish. The Food Technology and Nutrition Section is a part of the Division of Biological Sciences, which is under the direction of Dr. W.B. House.

Approved for:

MIDWEST RESEARCH INSTITUTE

W. B. House, Director
Biological Sciences Division

18 March 1964

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SUMMARY

A bacteriological survey was made of 35 Quartermaster rations and ration components. This survey indicated that the highest numbers of aerobes were isolated from cabbage slaw and from meat-vegetable combinations.

With the exception of carrots and cabbage slaw, coliform were not found.

Anaerobic spore counts were highest on meat and vegetable combinations. Salmonella were not found on any of the items.

Additional preliminary work with intentionally contaminated vegetables suggests that initial numbers of cells may influence the percentage of survivors on freeze-dried products within certain limits of bacterial species and food types; i.e., Salmonella and Staphylococci on green beans.

A method for determining survival of bacteria in the absence of food substrate (on nonnutrient surfaces) has been worked out. Assessment of the lethality of the pre-freezing, the freezing, and the drying phases of the freeze-dry process has been determined. A comparison of the results of cells freeze-dried on nonnutrient surfaces and on vegetables indicates the greatest influence of the food in controlling survival of bacteria comes into play during the freezing phase.

Reduction in counts for Salmonella oranienburg, Staphylococcus aureus, Aerobacter aerogenes, Alkaligenes faecalis and Escherichia coli-B was much greater with fruits than with vegetables.

I. INTRODUCTION

The microbiology of dehydrated foods is not well defined. In the past, too much emphasis has been placed upon quality factors only, i.e., the meeting of a standard specification which was related to total bacterial numbers. Freeze-dehydration of foods has revived interest in the assessment of survival of microbes on dehydrated foods. The following studies were initiated to investigate the degree of contamination by certain microbial groups. In addition, the effects of the various phases of freeze-dehydration on survival of bacteria on selected foods are being studied.

This report covers the preliminary steps taken to achieve the foregoing goals.

II. EXPERIMENTAL

A. Total Aerobic Counts

Sampling of dehydrated mixed-food items, such as stews, hashes or meats with gravy mix, is the most critical part of a bacteriological survey of these products. Most procedures call for "homogenizing" the samples in water in some type of mechanical blender. While such practice works well with single dehydrated foods, errors crop up with mixtures of foods, a soup, stew or hash mixture. The components of the mixtures are usually not in equal proportion. Some may be freeze-dried, others may be vacuum-dried and some may be air-dried. Furthermore, particle sizes are not the same, nor is shape. Such diverse characteristics lead to sampling errors.

In order to reduce sampling errors with mixtures, we dry-ground the entire sample in a sterile Waring blender. The ground material was tumble-mixed and samples of this "powder" used for preparing dilutions in the usual manner for standard examinations.[1] Exceptions to this procedure will be indicated later.

Sterile distilled water was used for the initial dilution; but as sample became less with greater dilutions, phosphate buffered water was used.

Initially, both plate count agar and Trypticase soya agar were used as pour plate media. However, the results of initial comparisons on a variety of dehydrated foods indicated no advantage for one over the other. Subsequently, the plate count agar (PCA) has been used exclusively. This selection was based upon the presence of glucose in the PCA as being a more readily available energy source for cells which were injured or were slow growing.

All plates for total aerobic counts were routinely incubated at 33 ±2°C for two days. An exception to the dry-grind pre-sampling technique used for most samples was made with single food items which were vacuum- or air-dried. These included dried carrots, diced potatoes, and dried apricots. For the most part, these foods were too hard or too tough to grind in the Waring blender. As single item foods, they were weighed directly into sterile dilution bottles. They were allowed to stand 30 min., were then vigorously shaken and other dilutions prepared from them. These counts were also made using PCA in a pour plate and by using a 33°C incubation temperature for two days.

B. Coliform Counts

A standard test using a solid medium (violet red bile agar) was used for all samples and tests. This test was performed by transferring 1 ml. of an appropriate dilution (in duplicate) to petri dishes. The medium cooled to 48°C, was then poured into the dish and the medium and sample quickly mixed. When the agar had solidified, an additional overlay of 4 ml. of sterile violet red bile agar was given to each plate.

All plates were incubated at 35°C for 24 hr.

C. Anaerobic Spore Counts

Anaerobic spore counts were made by heating the initial sample dilution to 80°C for 10 min., making additional dilutions if necessary, transferring 1 ml. of each dilution used to duplicate plates, pouring with anaerobic agar (BBL), and incubating the plates at 35°C in a Brewer's anaerobic jar.

Clostridium perfringens would not likely be isolated by this method because of its general failure to produce spores in a medium that is not sugar-free. Some additional counts specifically designed to determine Cl. perfringens are being planned. In these, 1-ml. portions of appropriate dilutions will be transferred to petri dishes poured with sulfite-polymyxin-sulfadiazine agar. Clostridium perfringens produces black colonies on this medium.

D. Salmonella

Of several techniques tried in pre-trial determinations, the following procedure appeared to give more consistent results, especially where numbers of Salmonella were low.2/

An initial 1:10 dilution was made by adding 50 gm. of food material to 540 ml. of lactose broth. This mixture was then blended in a Waring blender. The initial suspension of food sample and lactose broth was then divided into five 100-ml. samples and transferred to sterile bottles. Five 10-ml. samples, five 1-ml. samples and five 0.1-ml. samples were also transferred to tubes containing 10 ml. of sterile lactose broth. All tubes and bottles were incubated 24 hr. at 35°C.

At the end of 24 hr., a loop of material from each tube or bottle was transferred to tubes containing 1.0 ml. of selenite-cystine broth. These tubes were then incubated for 7 hr. at 35°C. At the end of the incubation period, each of the tubes was streaked onto brilliant green agar. The brilliant green plates were also incubated at 35°C for 24 - 48 hr. This is essentially the technique used by the U. S. Public Health Service. The most probable numbers (MPN) per gram of sample can be obtained by reference to the procedure in Recommended Methods for the Microbiological Examination of Foods.1/

When sample size was limited, the following alternative was followed: weigh one 10-gm. sample into 100-ml. lactose broth in a bottle or flask and also weigh one each of 1.0 gm. and 0.1 gm. into 10 ml. of lactose broth in screw-capped tubes. Incubate these samples for 24 hr. at 35°C. After 24 hr., transfer 1.0 ml. of each lactose broth culture to 10-ml. portions of selenite-cystine broth in screw cap tubes and incubate 24 hr. at 35°C.

Following growth in the enrichment tubes, a loop of each was streaked onto previously hardened and dried brilliant green agar plates. The plates were incubated 24 - 48 hr.

Colonies, which appeared to be Salmonella on the brilliant green plates, were checked biochemically by streaking the slant and stabbing the butt of triple sugar iron agar.

E. Studies of Microbial Survival

Studies were initiated to evaluate the lethality of the freeze-dry process for several species of bacteria on several kinds of food.

In these first studies, commercially canned products were used (No. 10 cans). The cans were opened aseptically and the juices drained. The food was then soaked in sterile distilled water and this also drained. A second wash was then given: also with sterile distilled water. Fruits were given a third washing.

After the product had been washed, sterile water was added again and after a few minutes of soaking, the appropriate bacterial suspension was added. After addition of the inoculum, the food was turned often to insure uniform contamination. After 30 min., the food was drained and placed in sterile trays for freezing. Before the foods were frozen, samples were taken to determine the initial numbers.

The trays of food were then placed on the plates in a RePP freeze-dehydrator and frozen at a temperature setting which would give the desired rate of cooling (0 to -50°); i.e., rapid or slow.

Samples were taken after each product was thoroughly frozen and again after the product was dried.

For study of storage influences on survival, the dried products were packed into No. 202 cans and either sealed in air or in vacuum (less than 2 per cent residual air). Parts of each canned product were then stored at each of three storage temperatures, 100, 70 and 40°F.

F. Survival Studies with Millipore Membranes

If the effects of a substrate on survivability of bacteria are to be accurately assessed, adequate controls are necessary. In these studies, bacteria on millipore membrane filters served as control.

Several experiments were performed, both with and without a nutrient medium.

One series was designed to determine the effect of the total freeze-dry process on cells frozen on the membranes

A millipore filter pad was wet with 1.5 ml. of sterile distilled water (in a petri dish). A sterile millipore filter was then placed on the surface of the pad. When the filter was saturated with water, 0.05 ml. of a dilution of cells (usually there were at least four dilutions) was pipetted to the center of the membrane. The drop was then spread over the surface of the membrane by use of a bent glass rod (hockey stick). A sufficient number of membranes were prepared so that each step to be evaluated was represented by at least three plates for each dilution used.

When all membrane surfaces had been inoculated, the plates were divided into groups and placed in the freeze-dehydrator. Thermocouples were placed at random in uninoculated pads for temperature control. The following groups were evaluated:

1. Controls - inoculated but not frozen or dried.

2. Frozen - frozen but not dried.

3. Dried - frozen and dried.

Counts of the bacteria on the membranes were made by placing the membrane on the surface of a previously hardened medium, in a petri dish. In this manner, the errors of dilution and replating were reduced.

In addition to distilled water, the effects of freezing bacteria in the presence of chicken broth and brain-heart infusion were studied. These tests were handled the same as those using cells in distilled water.

A second series of experiments were done to assess the damage of freezing to cells which were already dry. In these studies, dilution and inoculation of membrane surfaces were the same as for the previous studies. However, after the membranes were inoculated, they were placed in a desiccator jar, over a desiccant, and allowed to dry for 24 hr. At the end of this time, they were taken from the desiccator and subjected to freeze dehydration. Estimates of survivors were made at three stages:

1. Immediately after inoculation.

2. Immediately after desiccation.

3. Immediately after freezing (two rates of freezing evaluated).

G. Bacterial Cultures

All inoculations of bacterial suspensions, whether into foods or to nonnutrient surfaces, were made with 24-hr. cultures. These cultures were transferred daily and were carried on Trypticase soya extract agar.

Six species of bacteria were used in the studies reported here:

1. Salmonella oranienburg

2. Staphylococcus aureus

3. Alkaligenes faecalis

4. Aerobacter aerogenes

5. Escherichia coli-B

6. Paracolobactrum intermedium

III. RESULTS AND DISCUSSION

Thirty-five Quartermaster rations or ration items were surveyed to determine the degree of bacteriological contamination. Counts were made for total aerobic populations, for members of the coliform group, for total anaerobic spores and for Salmonella sp. The results of this survey are presented in Table I.

The highest aerobic counts were made on cabbage slaw; shredded cabbage alone carried a population only 1/17 that of the slaw. In general, the leafy vegetables were high in numbers compared to other items - with the exception of the meat mixtures. Seed and seed-pod foods appeared to have reasonable numbers of bacteria especially when calculated to a rehydrated basis.

All fruits were low in total aerobes.

Meat combinations such as stews and hashes were variable in numbers. They all tended to have aerobic populations which were at a level around 20,000/gm. Beef hash was the exception to this having a population of less than 1,000/gm. The counts on the meat combinations, viewed against the population found on the vegetable items and the meats alone, would suggest that the vegetable items in the stews are contributing the bulk of the microbes found in the meat-vegetables mixtures.

Sliced beef with gravy had a reasonable aerobic count from a standpoint of bacteriological quality; meat balls and gravy, however, had higher counts.

The degree of contamination due to bacteria initially carried by the foods and that picked up in handling and from the packaging materials could not be differentiated.

Only two items were found to carry appreciable numbers of coliform: cabbage slaw and vacuum canned carrots. Cabbage slaw had 250 coliform/gm and carrots 5,000/gm. The ratio of coliform to total aerobic count in carrots was 1:8.8 and in cabbage slaw was 1:1800. The counts on all other items were less than 10 cells/gm of food.

TABLE I

BACTERIOLOGICAL SURVEY OF QUARTERMASTER ITEMS

Numbers of Bacteria Per Gram (Dry Weight) of Food

Item	Total Aerobic Counts	Coliform	Total Anaerobic Spore Counts	Salmonella
Cocoa beverage	950	< 10	< 10	Neg.
Orange juice	132	< 10	< 10	Neg.
Fruit cocktail	333	< 10	< 10	Neg.
Green pea soup	467	< 10	< 10	Neg.
Apple sauce	37	< 10	< 10	Neg.
Rice	1,850	< 10	< 10	Neg.
Spinach	3,450	< 10	< 10	Neg.
Milk, dry	867	< 10	< 10	Neg.
Peaches	109	< 10	< 10	Neg.
Cabbage slaw	450,000	250	50	Neg.
Cabbage	26,400	< 10	< 10	Neg.
Sweet Potato	38	< 10	< 10	Neg.
Butterscotch pudding	273	< 10	< 10	Neg.
Grape juice	2,200	< 10	< 10	Neg.
Orange-grapefruit juice	53	< 10	< 10	Neg.
6 Mo. peas	533	< 10	< 10	Neg.
Green beans	13,400	< 10	< 10	Neg.
Peas	7,800	< 10	< 10	Neg.
Apricots	860	< 10	< 10	Neg.
Stewed prunes	600	< 10	< 10	Neg.
Sliced beef and gravy	14,200	< 10	< 10	Neg.
Macaroni and cheese	1,250	< 10	< 10	Neg.
Meat balls and gravy	91,000	< 10	< 800	Neg.
Corn and lima beans	23,000	< 10	120	Neg.
Chicken stew	22,000	< 10	700	Neg.
Beef stew	25,800	< 10	530	Neg.
Chicken and rice	2,800	< 10	30	Neg.
Beef hash	990	< 10	170	Neg.
Mashed potatoes	193	< 10	< 10	Neg.
Peas; vacuum canned	800	< 10	< 10	Neg.
Cooked beef; vacuum canned	80	< 10	< 10	Neg.
Onions; sliced, canned	6,000	< 10	< 10	Neg.
Carrots; vacuum canned	44,000	5,000	< 10	Neg.
Potatoes; diced	1,300	< 10	< 10	Neg.

A wide range of values was found for anaerobic spores in the survey. For the most part, the rations were found to contain less than 10 anaerobic spores/gm. However, this low figure may not be truly indicative of the total anaerobic population especially considering that Clostridium perfringens does not always sporulate well in laboratory medium unless it is free of sugar. Such may be the case in foods as well.

All the ration items shown in Table I were found to be free of Salmonella. In only one item was a suspect isolated. That isolation was from sweet potato. One, 1-gm. sample gave an isolation, although the 10-gm. and larger samples did not. Subsequent examination of other samples failed to produce any suspects. Since the single isolation was found to be serologically and biochemically related to the test strain in use for food inoculations, the conclusion reached was that the colony isolated from the sweet potato plate was indeed an accidental introduction. Because further examinations of the sweet potato have not revealed Salmonella, we have reported this item to be free of Salmonella. We are firmly convinced that it is.

The effect of initial numbers of bacteria on the per cent of survivors was determined using fruits and vegetables. From the figures presented in Table II, there is an indication that a six-fold increase in initial numbers did not affect the per cent of Salmonella cells which survived the freeze-dry process when corn was used as the substrate. However, when green beans were used as a substrate, an approximate two-fold increase in initial numbers resulted in a doubling of the per cent survivors. The point at which there is a diminishing return for increase in initial numbers has not yet been determined.

Staphylococcus aureus appeared to exhibit similar characteristics in relation to substrate, although to a slightly different degree, Table III.

The reduction in numbers of two test species by the various steps of the freeze-dry process is shown by the figures in Tables IV and V. Table IV is based on the reduction in numbers of Salmonella oranienburg in both fruits and vegetables.

TABLE II

EFFECT OF INITIAL NUMBERS OF SALMONELLA ORANIENBURG ON THE PER CENT WHICH SURVIVE FREEZE-DEHYDRATION IN VEGETABLES

(In 1,000's)

	Initial Count	Final Count	% Survival
Corn	650T	88T	13.5
	107T	10T	9.4
Green Beans	549T	252T	46.0
	208T	47.5T	23.0

TABLE III

EFFECT OF INITIAL NUMBERS OF STAPHYLOCOCCUS AUREUS ON THE PER CENT WHICH SURVIVE FREEZE-DEHYDRATION IN VEGETABLES

(In 1,000's)

	Initial Count	Final Count	% Survival
Corn	970T	50T	5.2
	100T	18T	18.0
Green Beans	121.5T	33T	11.2
	500T	160T	32.0

TABLE IV

DESTRUCTION OF SALMONELLA GRANIENBURG DURING THE FREEZE-DRYING PROCESS OF FRUITS AND VEGETABLES

Substrate	Average Total Reduction (%)	Range in Total Reduction	Average Reduction at End of Freeze Cycle (%)	Range Reduction at End of Freeze Cycle	Average Reduction at End of Drying Cycle (%)	Range Reduction at End of Drying Cycle
Corn	89	86-91	52	47-59	37	32-40
Green Beans	68	55-77	24	20-36	44	20-70
Peas	81	78-85	32	24-40	49	45-58
Pears	98	95-99	79	60-82	19	15-23
Peaches	99	94-99.9	96	94-99	3	1.8-5

TABLE V

DESTRUCTION OF STAPHYLOCOCCUS AUREUS DURING THE FREEZE-DRYING PROCESS OF FRUITS AND VEGETABLES

Substrate	Average Total Reduction (%)	Range in Total Reduction	Average Reduction at End of Freeze Cycle (%)	Range Reduction at End of Freeze Cycle	Average Reduction at End of Drying Cycle (%)	Range Reduction at End of Drying Cycle
Corn	95	93-99	47	35-52	48	40-51
Green Beans	85	79-89	49	38-59	36	30-41
Peas	51	42-61	39	28-51	12	10-14
Pears	69	51-79	24	19-32	45	36-57

The total reduction of numbers, for the cumulative process of freeze-drying, appeared to be greater when the cells were on green beans. The effect on corn and peas is about equal. Greatest total reduction occurred, however, when fruits were used as the test food.

When corn was used as substrate, nearly 50 per cent of the reduction occurred during the freezing cycle. With green beans, about 35 per cent of the reduction occurred during the freezing cycle. Freezing accounted for 80 per cent of the total reduction when pears were the substrate and Sal. oranienburg the test species. The reduction was even higher on peaches.

Although the total reduction of S. aureus appeared to be greater on corn than did Sal. oranienburg, the fraction that could be attributed to the freezing cycle was less: 49 per cent for S. aureus compared to 59 per cent for Sal. oranienburg. Total reduction of S. aureus on green beans was also less than for Sal. oranienburg; however, the portion of the kill caused by freezing was greater (58 per cent).

The destruction of S. aureus on peas was decidedly less than Sal. oranienburg on the same substrate (81 per cent for Sal. oranienburg and 51 per cent for S. aureus).

Pears were also found to offer a greater margin for survival for S. aureus than they did for Sal. oranienburg; only 34 per cent of the total reduction was accounted for by freezing.

Additional figures for survival (or reduction) of other bacterial species are presented in Table VI. The organisms presented here are gram-negative rods and their ability to survive the total freeze-dry process closely approximates that of Sal. oranienburg.

To date, only one series of nitrogen-packed foods has been examined; but the indications are that rapid decline in populations occur under nitrogen, and especially so at 70°F and 100°F.

The data in Table VII indicate that survival on nonnutrient surfaces may not be greatly different than survival in most vegetables. There is also an indication that the greatest influence of the food in controlling survival of bacteria comes into play during the freezing phase.

TABLE VI

DESTRUCTION OF THREE SPECIES OF BACTERIA DURING THE FREEZE-DRYING OF FOODS

Substrate	Reduction of Aerobacter aerogenes	Reduction of Escherichia coli	Reduction of Alkaligenes faecalis
Corn	83%	-	69%
Green Beans	73%	81%	86%
Peas	94%	83%	97%
Pears	98%	99.8%	99%

TABLE VII

SURVIVAL OF SALMONELLA ORANIENBURG AND STAPHYLOCOCCUS AUREUS WHEN FROZEN AND DRIED ON NONNUTRIENT SURFACES

Sample	Bacterium	
	Sal. Oranienburg	S. Aureus
Frozen to -10°F	83%	85%
Frozen to -50°F	67%	48%
Dried (from [illegible]zen)	11%	20%

The "freeze-death" is much more pronounced with the fruits than with vegetables. This, however, would be expected in that acidity of substrate is known to accelerate death during freezing and cold storage.

As a guide to use in future work, for determining the amount of inoculum when a certain range of survivors is desired, the average survivors for all test strains on all vegetables were prepared. This is

presented in Fig. 1, and is intended as a general guide only; i.e., when drying conditions are similar to those used in these studies:

1. Food frozen to -50°F.

2. A chamber pressure of 10μ or less.

3. A shelf temperature not to exceed 120°F.

IV. FUTURE WORK

Four foods, peas, pears, corn and green beans, have been inoculated, dried and stored at 40, 70 and 100°F under nitrogen and air pack. Evaluation of loss in viability of the bacteria on these foods will be made.

The foods supplied by the Quartermaster will be further surveyed for the presence of Fecal streptococci and Clostridium perfringens.

Fruits, vegetables and meats are to be inoculated with four additional bacterial species including Clostridium botulinum. Survival of these types, prior to freezing, during freezing and drying and after drying will be evaluated.

The influence of cooling rate is to be established.

V. EXPENDITURES

Approximately 35 per cent of the funds allocated for this project have been committed. With nearly one-third of the work completed, the remaining funds are sufficient to complete the work called for in the contract.

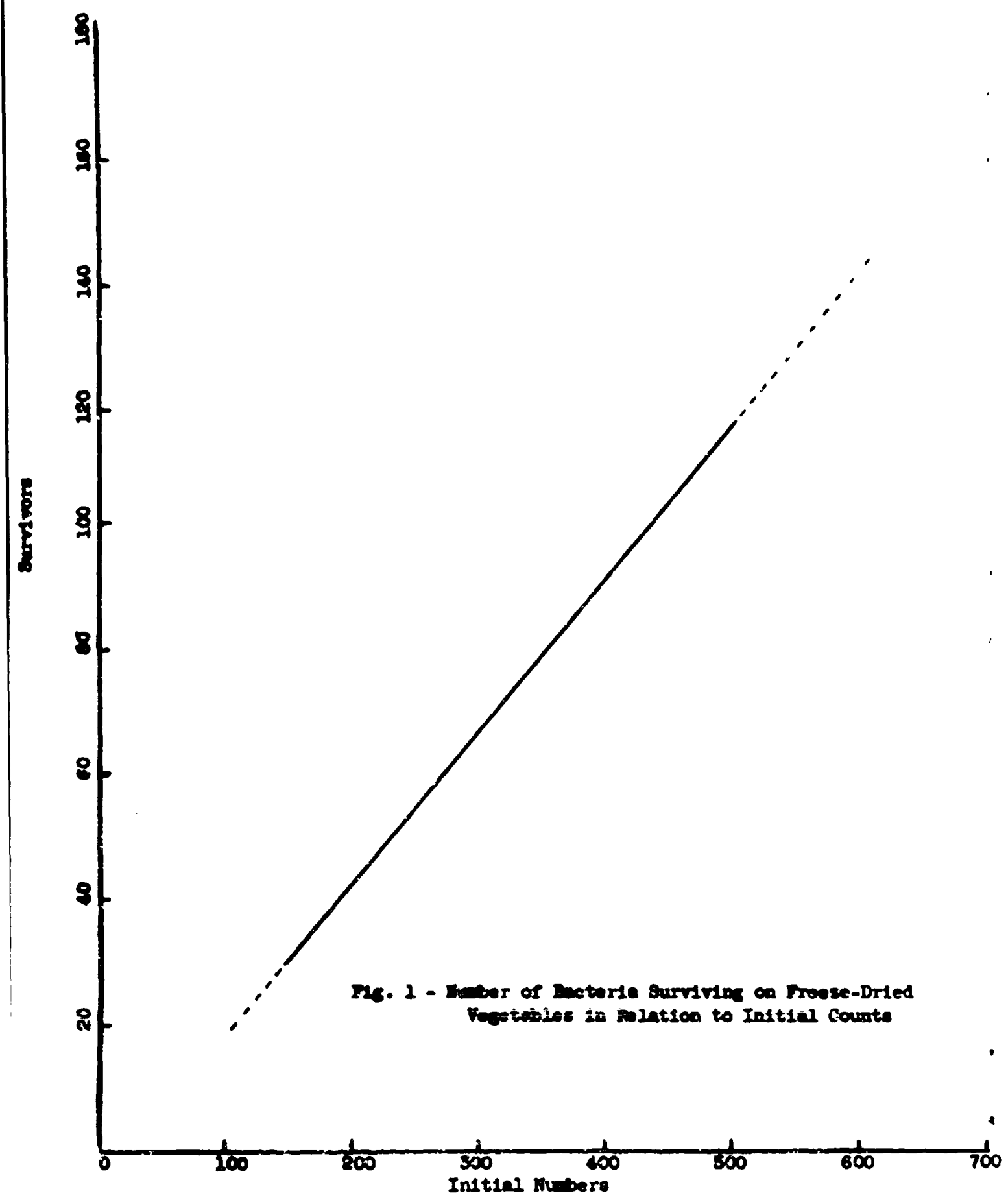

Fig. 1 - Number of Bacteria Surviving on Freeze-Dried Vegetables in Relation to Initial Counts

U. S. ARMY NATICK LABORATORIES
NATICK, MASSACHUSETTS

IN REPLY REFER TO

AMXRE-FPC 14 May 1964

Scientific and Technical Information Facility
ATTN: NASA Representative (SAK/DL)
P. O. Box 5700
Bethesda, Maryland 20014

Gentlemen:

Inclosed herewith are two (2) copies of Progress Report No. 1, Contract No. DA 19-129-AMC-206(N), Midwest Research Institute, Kansas City, Missouri, title "A Study of the Microbiology of Selected Dehydrated Food Products".

Karl R. Johnson

KARL R. JOHNSON
Project Officer

1 Incl.
Prog. Rept. #1 (in dupe)

REFERENCES

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2. Angelotti, R. (1963) Detection of Microbial Pathogens in Foods. In: Microbiological Quality of Foods. Academic Press, New York.

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