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INTRODUCTION

This report summarizes the second, and final, year's work on the project entitled "Stability of Viruses in Foods for Space Flight." As was stated in the first year's report, viruses sometimes contaminate food, but they apparently cannot multiply in it. This study has attempted to determine how likely a contaminating virus is to remain infectious until the food is consumed.

The first year's work employed poliovirus as a representative of the viruses of intestinal origin and influenza virus as a model respiratory agent. Several different foods served as model vehicles. The results indicated that poliovirus may persist in space foods for significant periods of time, but that influenza virus is relatively unstable. All of the experiments were carried out as if contamination had occurred during final packaging, or at least after the last processing step. Inactivation of poliovirus was generally much more rapid during storage at room temperature than in the refrigerator, and the rate also varied significantly among foods.

The present year's work was intended to test a broader range of viruses, to learn more about the effects of temperature, and to determine the reasons for the differences in poliovirus stability among foods. We had hoped to learn a good deal about the effect of freeze-drying viruses in foods, but it has only been possible to perform one such experiment. We had also hoped that our data would provide some basis for generalization, especially with respect to the effects of food composition upon virus stability. The problem in this case is that the experimental results were obtained under a limited variety of conditions, so we must avoid the temptation to over-generalize. This caution will be reiterated in a later section.

MATERIALS AND METHODS

Viruses are shed by their hosts in products of the host's body. We had supposed that this "body product" might influence the stability of the virus, so we tried to make the contaminants as natural as possible. The poliovirus used initially was obtained as feces of infants who had recently received the trivalent oral polio vaccine. As a rule, the fecal specimens selected were those which contained at least 10^6 plaque-forming units (PFU)/g.

ECHO virus type 6 (EC-6) and reovirus type 1 (Re-1), with which we wished to perform later experiments, were not available in infectious feces. These viruses had to be produced in primary monkey kidney (PMK) cell cultures and might then either be mixed with feces or with soft agar to contaminate food. Experiments reported below compared poliovirus shed in feces to poliovirus produced in PMK cultures and suspended in feces or 1% agar. The viruses proved approximately equal in stability in the foods used, under the conditions of these tests. Therefore, EC-6 and Re-1 (and frequently poliovirus types 1, 2, and 3 (Po-1, Po-2, Po-3) as well) were suspended in agar for subsequent experiments.

Influenza virus type A (strain PR-8) was propagated in the allantoic cavities of embryonated chicken eggs. As a food contaminant, it was mixed with boiled bovine respiratory mucus, the pH of which had been adjusted to near 7. Parainfluenza virus type 3 (strain SF-4) was propagated in PMK cultures and suspended in soft agar or simply in phosphate-buffered saline (PBS) at the

time that food was being contaminated.

Each of these five viruses was assayed by the plaque technique in PMK cultures. Three different agar overlays were required. Each was based upon Earle's balanced salt solution with neutral red and Noble agar. With added agamma calf serum, $MgCl_2$, cysteine, and protamine sulfate, this medium was used to assay the polioviruses, EC-6, and influenza virus. Parainfluenza virus 3 was inhibited by some of these ingredients but would form plaques in the presence of the agamma calf serum. Additives for Re-1 assay were pancreatin and skim milk.

The foods used as model vehicles were most frequently stock items from the space food menu. The data with which we were provided concerning the compositions of these foods were assumed accurate until proven otherwise. Deliberate modifications of foods included freeze-drying of bacon squares; rehydration of spaghetti with meat sauce; and addition of acid (anhydrous propionic), salt (dry PBS), and protein (lactalbumin) to banana pudding. It was usual to add 0.5 g of contaminant per unit of food. A unit might comprise one or two bites of a bite size food or as much as 30 g of a food intended to be rehydrated in the package. Each unit of food, with its contaminant, was packaged separately so that the entire contents of the package could be taken as the sample. The laminate package was evacuated and flushed with nitrogen twice, then evacuated again and heat-sealed. The time consumed in this operation was such that the virus in a "zero-time" sample had usually been in contact with the food for 1 to 2 hr before it was resuspended in diluent.

The sample was generally homogenized with from 100 ml of PBS for bite size foods to 200 ml for 20 to 30 g samples and tested at further dilutions of 10^{-1} and 10^{-2}. This has been called the dilution testing technique. Samples containing low levels of poliovirus or EC-6 could also be tested by a concentration technique which has been published elsewhere (Appl. Microbiol. 16:1564, 1968). No comparable methods are yet available for agents outside the enterovirus group.

RESULTS

Viruses. We found last year that poliovirus was quite persistent in a variety of foods and that influenza virus was relatively labile. It will be shown here that none of our test agents from outside the enterovirus group is stable under our experimental conditions. It had appeared that influenza virus might persist at 5C in contact with apricot cubes, gingerbread, and cream-style corn. The first two of these have relatively impervious coatings, so we looked mainly at the composition of the cream-style corn to see what conditions influenza virus would withstand. The corn's most unusual feature, when compared to the other foods tested, was its low acidity (nominal pH= 6.8). This seemed significant, in view of the known acid lability of influenza virus.

Banana pudding (said to be at pH 7.3) was chosen for further experiments. It was delivered in bulk cans in powder form. This food seemed more likely than most to be contaminated uniformly by virus applied to only one point in the sample, but this was not the case. If 25 g of the powder were placed in a laminate pouch and inoculated at one end, the far half of the contents remained virus-free even through the sealing cycle. This meant that, as with the other

foods, the entire contents of the package must be included in the sample if the inhomogeneity of contamination was not to cause problems. It also meant that the sample homogenate would contain approximately 10% food solids. We have no concentration method for influenza virus as yet, so it would have been convenient to be able to test the homogenate undiluted. Unfortunately, the undiluted sample homogenate killed the tissue culture cells. All subsequent homogenates of banana pudding were tested at further dilutions of at least 10^{-1}.

A more extensive set of banana pudding samples was inoculated with influenza virus and stored at 5C and room temperature. Compared to the 0-time samples, inactivation was approximately 90% and 95% complete in 1 and 3 days at 5C, respectively. No virus was detected at 7 days or thereafter (> 99.95% inactivation) at 5C or at room temperature. We conclude that influenza virus is not likely to persist long enough in dried foods to present a hazard to the consumer.

Parainfluenza virus type 3 was tested next. Banana pudding (20g/sample) was inoculated and stored at 5C. Two zero-time samples contained 1.1×10^4 and 1.2×10^4 PFU of virus respectively. No virus (i.e., $< 1.2 \times 10^3$ PFU)was detected after 3 and 7 days' storage. The experiment was repeated with a higher starting level of virus: 3.2×10^4 and 2.9×10^4 PFU in two zero-time samples. Again, no virus was detectable after 3 days at 5C. Finally, the level of inoculation was increased still further. The zero-time samples (10 g in this experiment) contained 1.2×10^5 and 1.4×10^5 PFU. Two samples taken after 1 day at 5C contained 1.2×10^3 PFU (the lower limit of detection by the dilution technique), and no virus could be detected at 4 days. There was a significant loss of virus in the zero-time samples and another 10^{-2} reduction in 1 day at 5C. This suggests a very low stability in food for parainfluenza virus type 3.

The name "reovirus" was coined as an acronym of Respiratory and Enteric Orphan virus; however, it seems to be shed most frequently by the enteric route and was used here as an alternate model intestinal virus. Though larger and more complex than the enteroviruses, the reoviruses have shown quite similar responses to environmental factors such as salt and are relatively stable at elevated temperatures. A half-life of 3.7 days at 4 C in tissue culture maintenance medium and good acid stability have also been reported for reoviruses. Re-1 in agar was inoculated into beef bites and stored at 5 C. The zero-time samples were found to contain 2.6×10^6 and 1.7×10^6 PFU. After 1 and 2 weeks at room temperature and at 5 C, no virus (i.e., $< 1.2 \times 10^3$ PFU/sample) was detected. The zero-time samples in a second experiment yielded 3.1×10^5 and 3.2×10^5 PFU. After 1 day at 5 C, a sample contained 2.7×10^4 PFU. Samples after 1 and 3 days at room temperature and after 3 days at 5 C were negative. These findings indicate to us that Re-1 is not a stable virus in beef bites nor, perhaps, in other foods either. Thus, of three supplemental virus models tested, none has proven nearly as stable in foods as poliovirus.

Mode of contamination. All of the experiments described below were performed with poliovirus or another enterovirus, EC-6. It was stated above that we had to determine whether viruses from tissue culture and from human intestines were similar in stability before results with these two agents could be compared. Poliovirus was the only agent available to us in both forms. Beef bites were contaminated with fecal virus or with Po-1 of tissue culture origin mixed with feces or with 1% agar. These were sampled repeatedly during 16 weeks' storage

at 5 C. The initial levels of contamination apparently were not identical, but the rates of inactivation were rather similar (cf. Fig. 1). The curves shown were derived by the linear least squares method. Their slopes are -0.0042 day $^{-1}$ for fecal virus, -0.0048 day $^{-1}$ for tissue culture virus in feces, and -0.0035 day $^{-1}$ for tissue culture virus in agar. We conclude that these modes of contamination are interchangeable at this temperature in this food. A similar experiment was done with bacon squares stored at room temperature, except that the set contaminated with tissue culture virus in feces was omitted. The tissue culture virus was Po-3. The difference in rate of inactivation was again minimal (cf. Fig. 2), suggesting that mode of contamination was not a significant variable under these conditions, either.

Temperature effects. Temperature has proven a very significant factor in experiments with poliovirus. Virus inactivation has been much more rapid at room temperature than at 5 C in most foods, and inactivation at the latter temperature has sometimes been too slow to measure. This was shown in an experiment begun during the first project year and continued into the second. Bacon squares were contaminated with fecal poliovirus and at 5 C and at room temperature. Among the samples at 5 C, an apparent increase in titer was observed toward the end of the first year's studies. This evidently was due only to sampling error (cf. Fig. 3). The least squares slope for samples during 8 months at 5 C was + 0.0014 day $^{-1}$, which does not differ significantly from zero. Inactivation at room temperature was significant, rapid, and perhaps not linear. At least the first two samples at room temperature which failed to yield virus probably resulted from inexperience on the part of the operator. This study was begun fairly early in the first year's work, and such findings became unusual later.

Somewhat similar results were obtained with EC-6 in agar as the contaminant, though the total period of storage was not as long. No virus was detected by the dilution method after 4 weeks or by the concentration method after 6 weeks at room temperature (cf. Fig. 4). This represents an inactivation rate of $\sim$ -0.17 day^{-1}, while the slope of the curve for 5C storage was +0.0014 day $^{-1}$. EC-6 was somewhat more persistent at room temperature in beef bites (cf. Fig. 5). The slope of the linear least squares curve for the room temperature samples was -0.061 day $^{-1}$, while that for the 5C samples was + 0.0016 day $^{-1}$. EC-6 data tend to be somewhat more variable than those for poliovirus where assay is carried out in more than one lot of PMK cultures; however, the data show that EC-6 is as stable an agent as poliovirus. Whether the room temperature curves in Figs. 4 and 5 differ significantly is questionable; the two experiments were not designed to permit this kind of comparison.

Enterovirus inactivation at room temperature was too rapid, and at 5C often too slow, to permit some of the comparisons which we wished to make in studying effects of food components. We therefore sought an intermediate temperature at which the inactivation rate might also be intermediate. Cheese sandwiches and Po-1 in agar comprised the model system. As is shown in Fig. 6, inactivation at 12 C was intermediate to those at 5 C and at room temperature. This conclusion can be drawn from the points themselves, as well as the least squares curves, which have been plotted with misgivings. One can see that the entire inactivation curve at 5C or at 12 C is not straight, though a straight segment appears to begin at 1 week at each temperature. If each of these linear segments is extrapolated back to the Y-axis, it appears that only 1% of the initial virus takes part in the process then being observed. More will be said about this later.

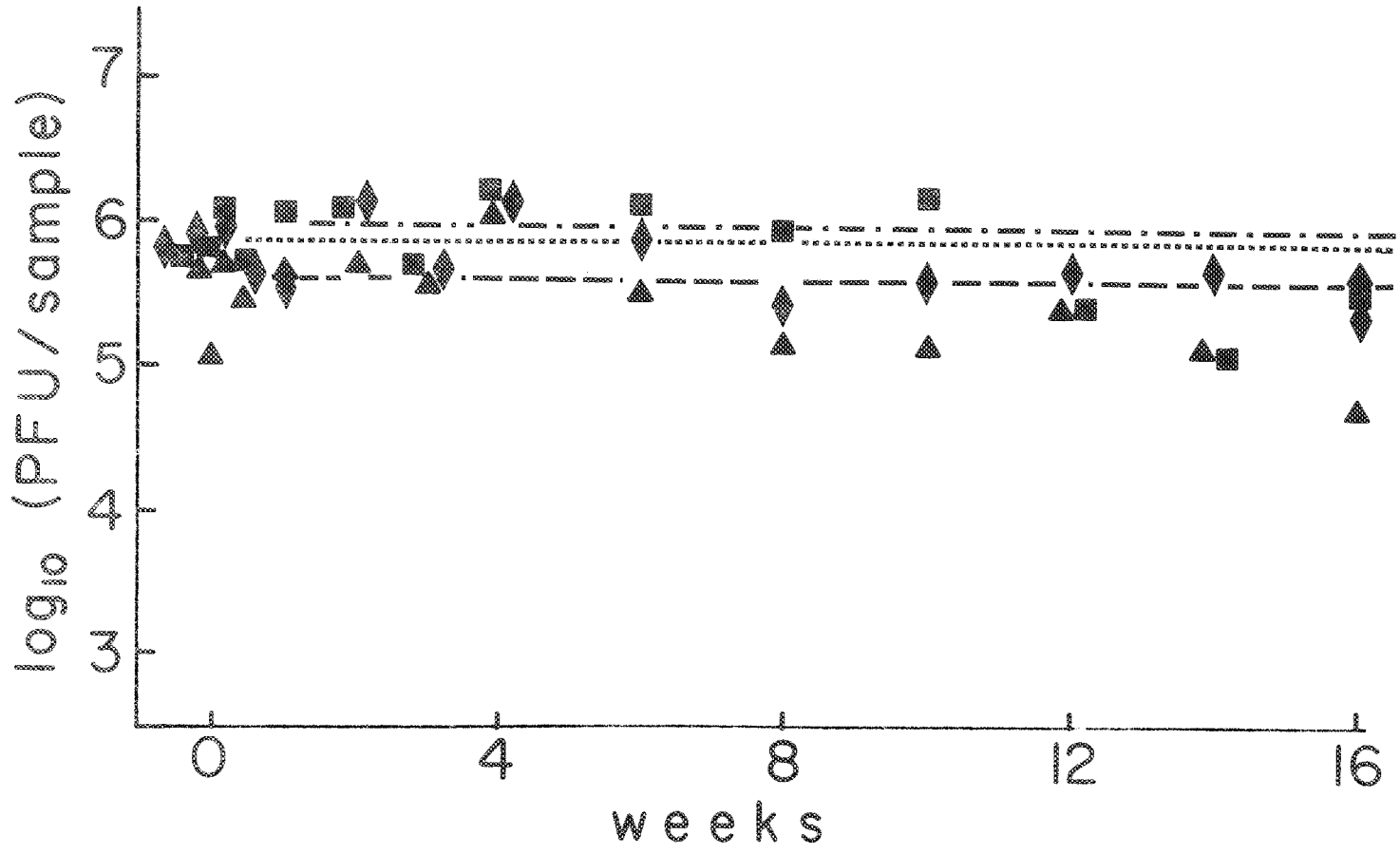

Fig. 1. Inactivation of poliomyelitis viruses in beef bites at 5 C ▲ fecal poliovirus; ■ Po-1 from tissue culture, in feces, ◆ Po-1 from tissue culture, in 1% agar)

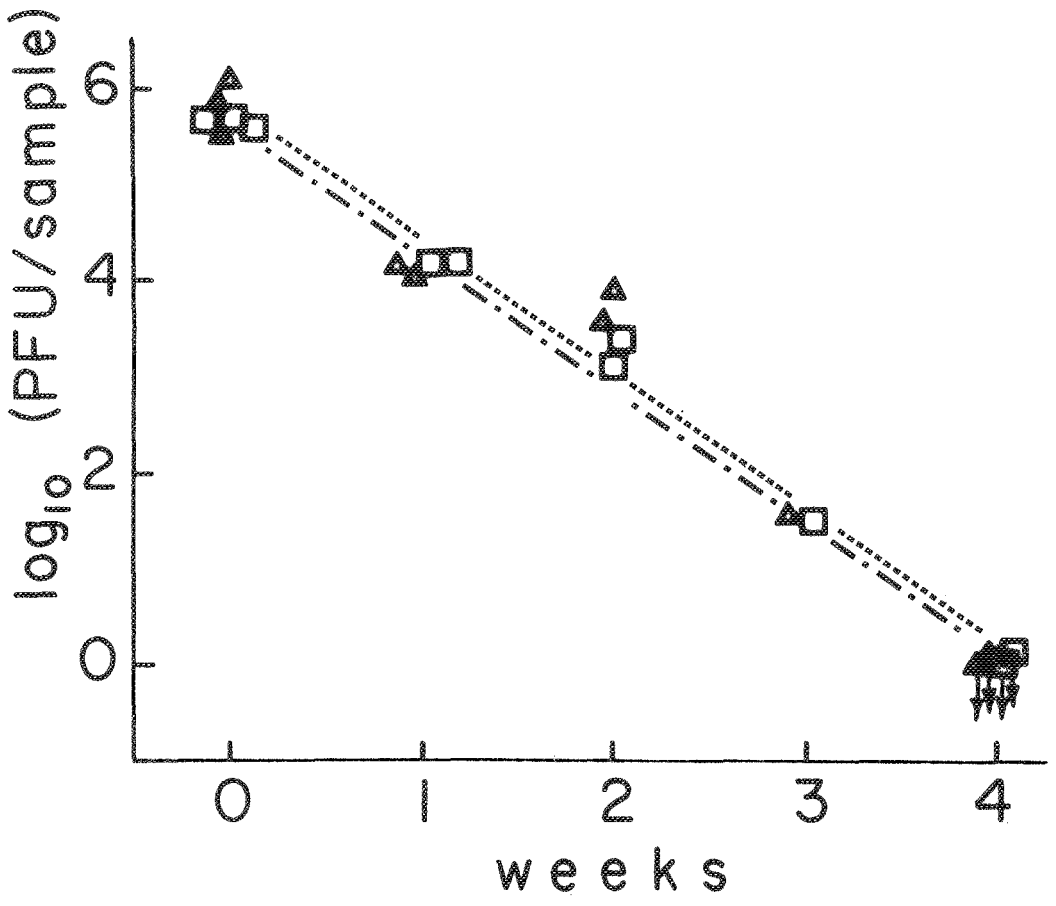

Fig. 2. Inactivation of poliomyelitis viruses in bacon squares at room temperature (▲ fecal poliovirus; □ Po-3 from tissue culture, in 1% agar; ↓ < indicated value)

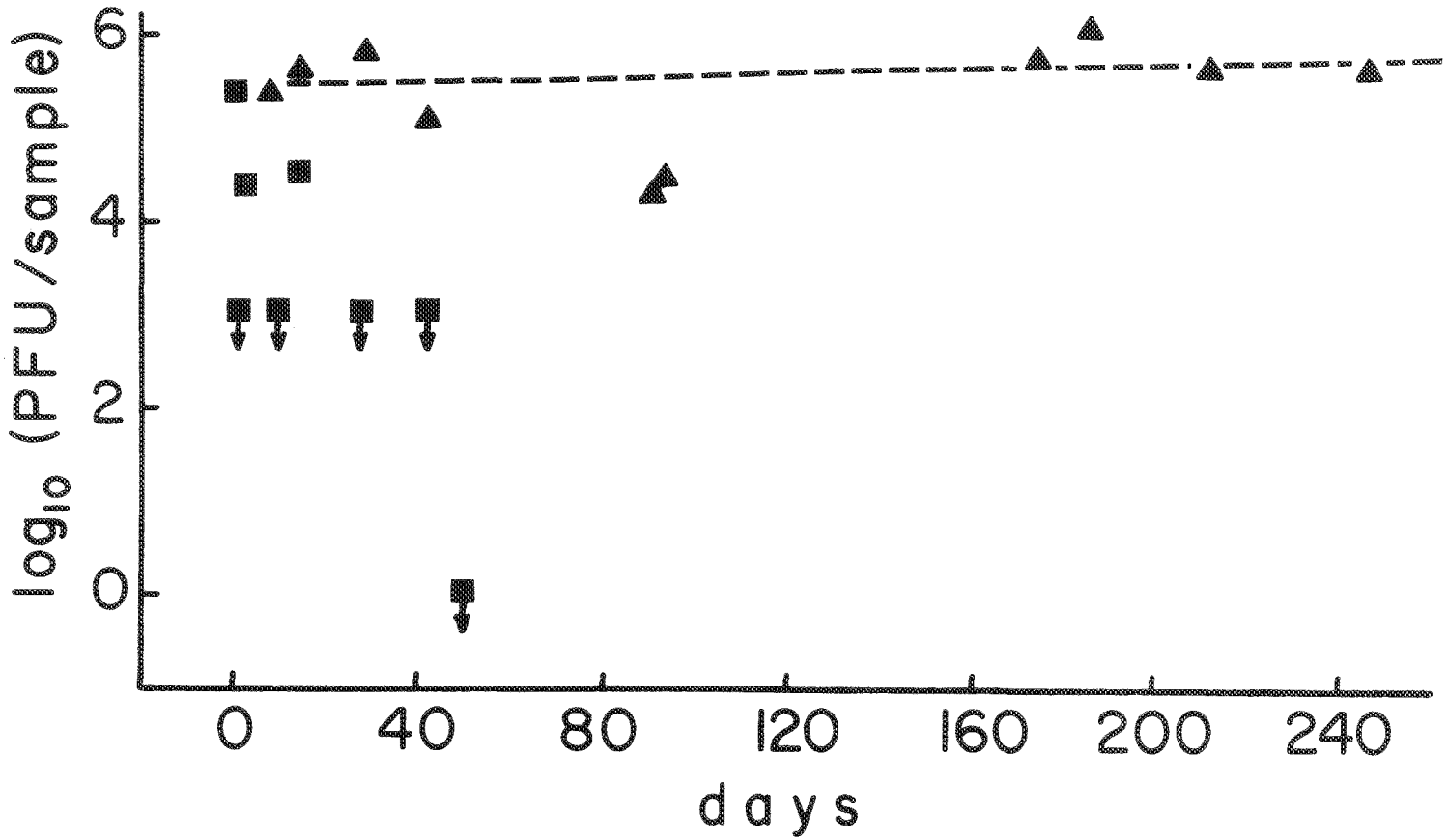

Fig. 3. Inactivation of fecal poliovirus in bacon squares as a function of storage temperature (■ room temperature, ▬▬▬▲ 5 C. ↓ < indicated value)

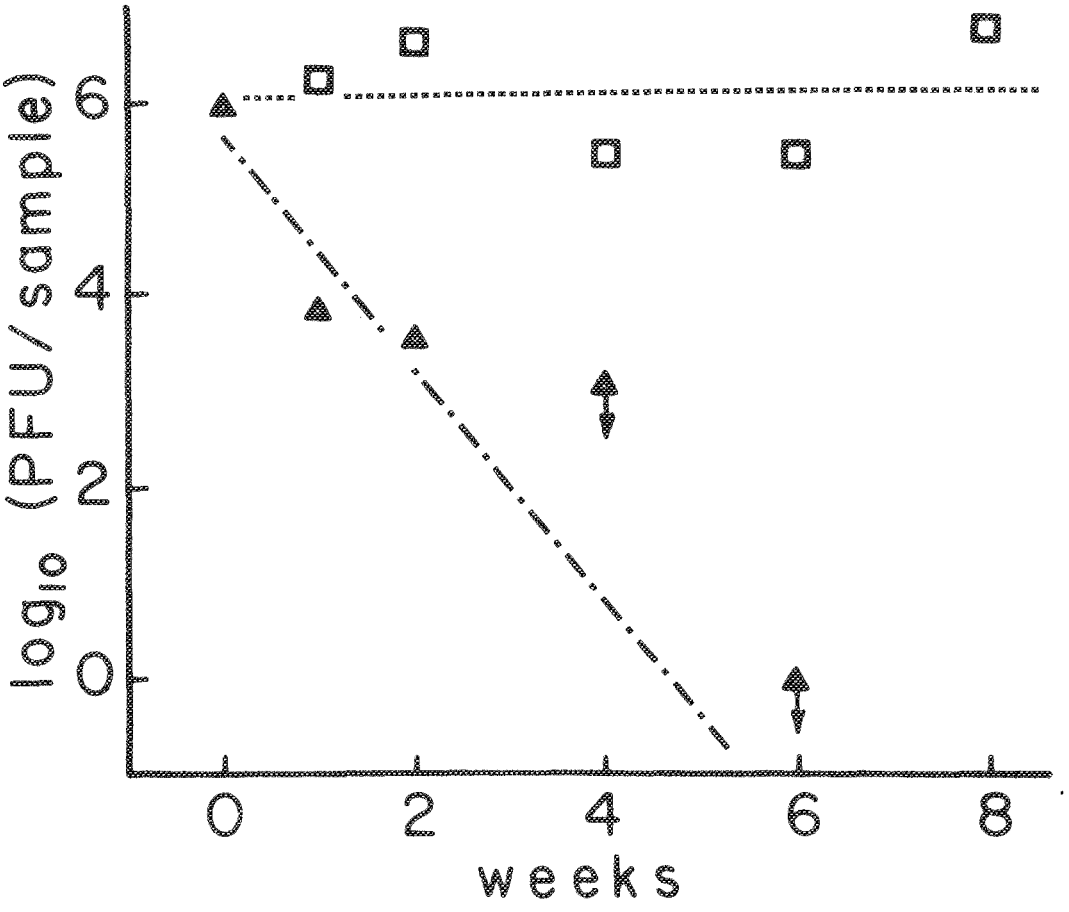

Fig. 4. Inactivation of EC-6 in dry banana pudding as a function of storage temperature (·—·—·—·▲ room temperature, ▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪ □ 5 C, ↓ < indicated value)

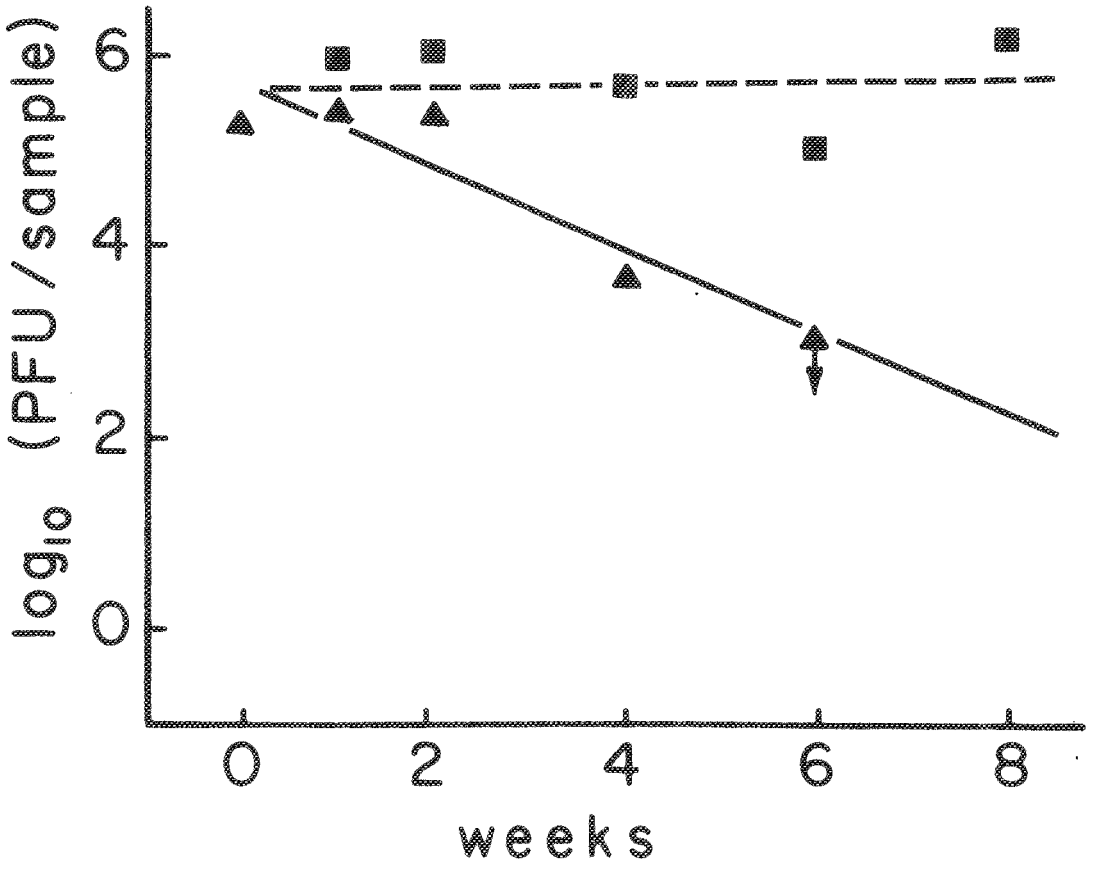

Fig. 5. Inactivation of EC-6 in beef bites as a function of storage temperature (▲ room temperature, ■ 5 C, ↓ < indicated value)

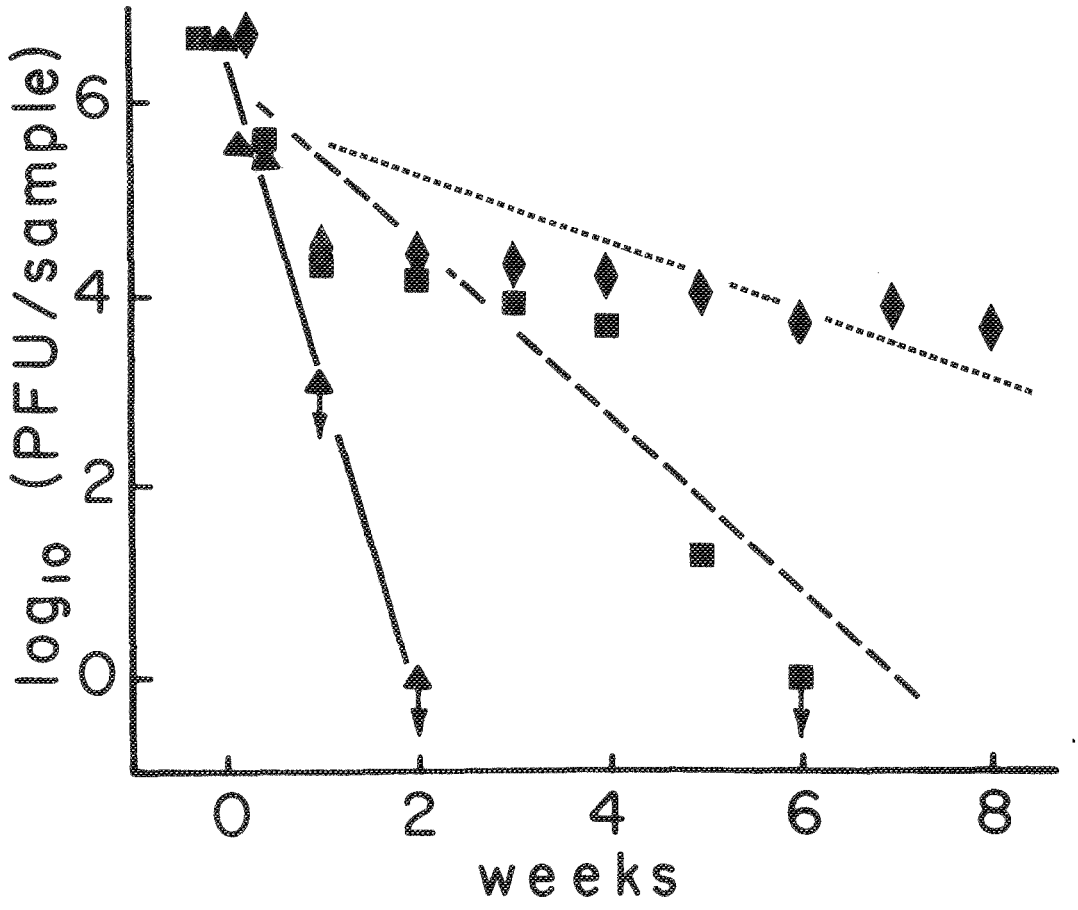

Fig. 6. Inactivation of Po-1 in cheese sandwiches as a function of storage temperature (——▲—— room temperature, — — — —■ 12 C, ▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪ ◆ 5 C, ↓ < indicated value)

We were also interested in the effects of fluctuating temperatures, for it seems that foods for space flight are refrigerated in storage, but not always when in transit. Poliovirus in bacon squares served as the model system. On the basis of the data in Fig. 3, we expected essentially no inactivation during periods of storage at 5 C. The results of the first such experiment were approximately as expected (cf. Fig. 7). Inactivation in sample set 1, held continuously at room temperature, gave a least squares slope of $-0.21\ day^{-1}$, and periods spent at room temperature by sample sets 2 and 3 resulted in inactivation at roughly comparable rates. Infectivity levels were approximately stable during storage at 5 C, except for the fourth week of set 2. Each of the 4 week samples was tested by the concentration technique, which is not as quantitative as the dilution technique. The experiment was repeated, with the results shown in Fig. 8. The findings during the first 2 weeks were quite in agreement with the hypothesis; precision again suffered thereafter because the concentration technique had to be used to test the samples. At storage temperatures alternating between 5C and room temperature, the amount of inactivation probably represents the total time spent at room temperature, but it may be greater.

Food composition. Poliovirus had proven to vary greatly in stability in the foods tested during the first year's studies. We hoped to determine which components of foods had a significant influence upon the rate of inactivation of contaminating enterovirus. Stability had been greatest in bacon squares, especially at 5 C, so we examined the tables of composition to determine which of its components might be significant. It was said to be relatively high in protein (54.9% by weight) and ash (10%) and intermediate in acidity (pH 5.2). Its only unique feature was its moisture content, which at 13.4% was the highest on the space food menu.

We applied for some bacon squares with a lower level of moisture. These were prepared by Dr. Robert Pavey of Swift and Co. A portion of a batch of bacon squares was freeze-dried (~ 4.5% moisture) and these and some standard bacon squares of the same batch (~ 12.9% moisture) were delivered to us. Both were inoculated with fecal poliovirus. Virus in samples stored at 5 C showed significant inactivation during the period of the study (cf. Fig. 9), which contrasts with the results presented in Fig. 3. Computed linear least squares slopes were $-0.0022\ day^{-1}$ for the standard and $-0.0029\ day^{-1}$ for the freeze-dried bacon squares. The close agreement in the values of the samples after 302 days' storage shows that the rate of inactivation did not differ much between these two levels of moisture. The residual virus in each of these samples was approximately 7% of that which was present initially.

Inactivation was more rapid at room temperature. There was a suggestion that virus was more labile in the freeze-dried bacon squares (cf. Fig. 10), though the interval until no virus could be detected by the concentration method was 4 weeks for both sets. The room temperature portion of the preceding experiment was repeated with three variations: the contaminant was Po-3 in agar, the sampling intervals were changed, and at least two samples were taken from each set each time (one of the 3-week samples was lost in titration). The results indicated that moisture differences, over this range, had little influence upon poliovirus stability in bacon squares at room temperature (cf. Fig. 11).

The range of values for food components in these studies has generally been restricted to those found at the time in the space food menu. However, we

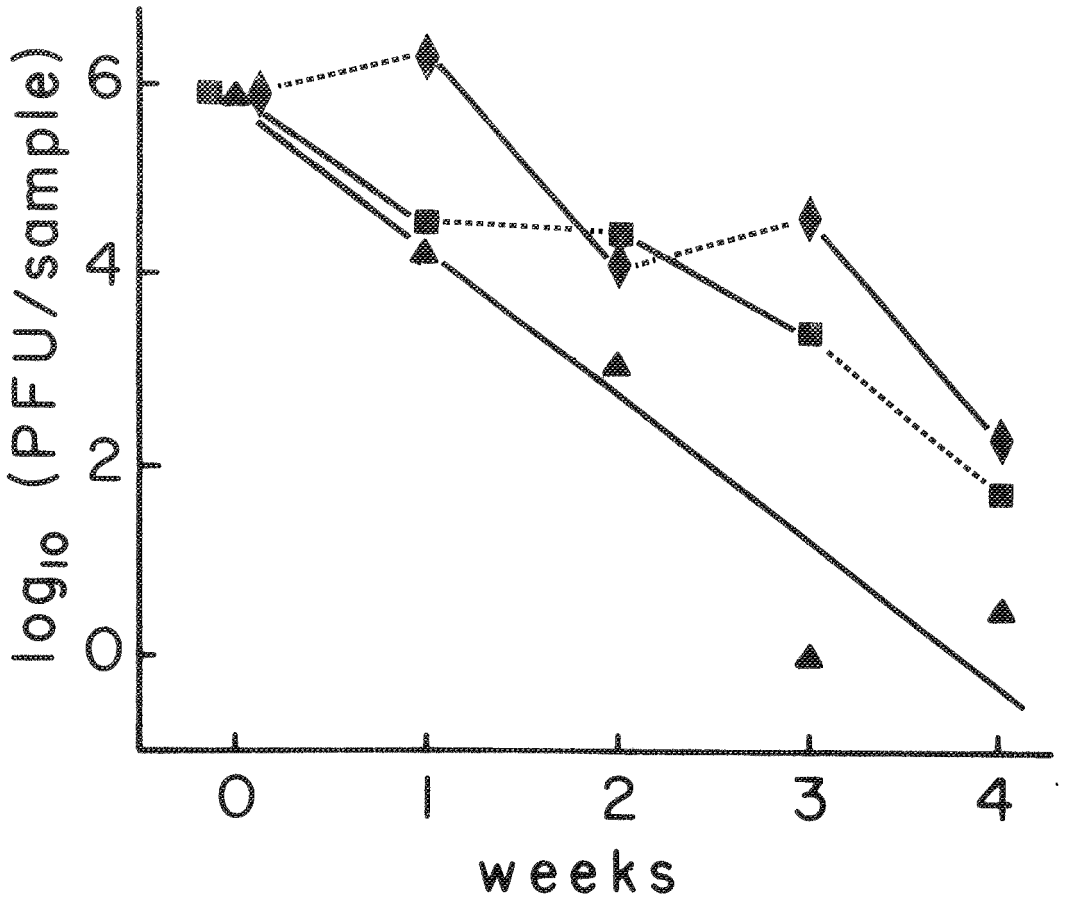

Fig. 7. Inactivation of fecal poliovirus in bacon squares as a function of storage temperature (▲ sample set 1, ■ 2, and ◆ 3; storage at ——— room temperature and ▪▪▪▪▪▪▪▪▪▪▪▪▪▪ 5 C)

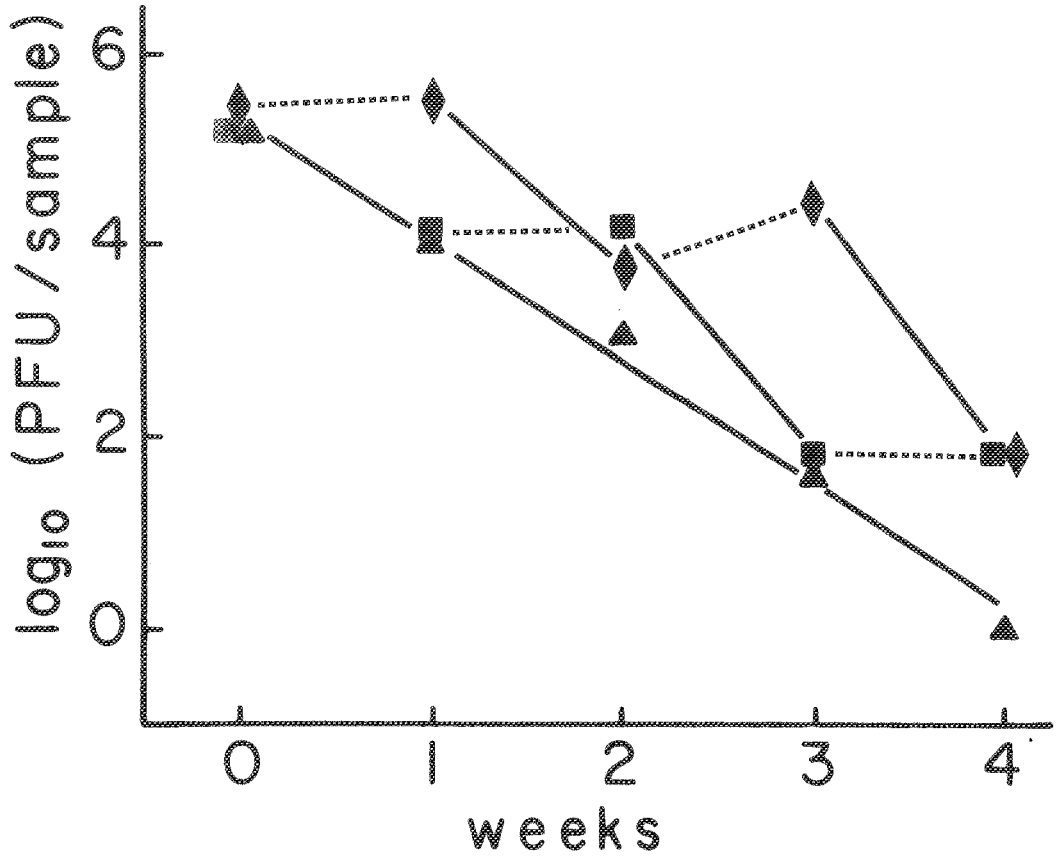

Fig. 8. Second experiment: inactivation of fecal poliovirus in bacon squares as a function of storage temperature (▲ sample set 1, ■ 2, and ◆ 3; storage at ——— room temperature and ---------- 5C)

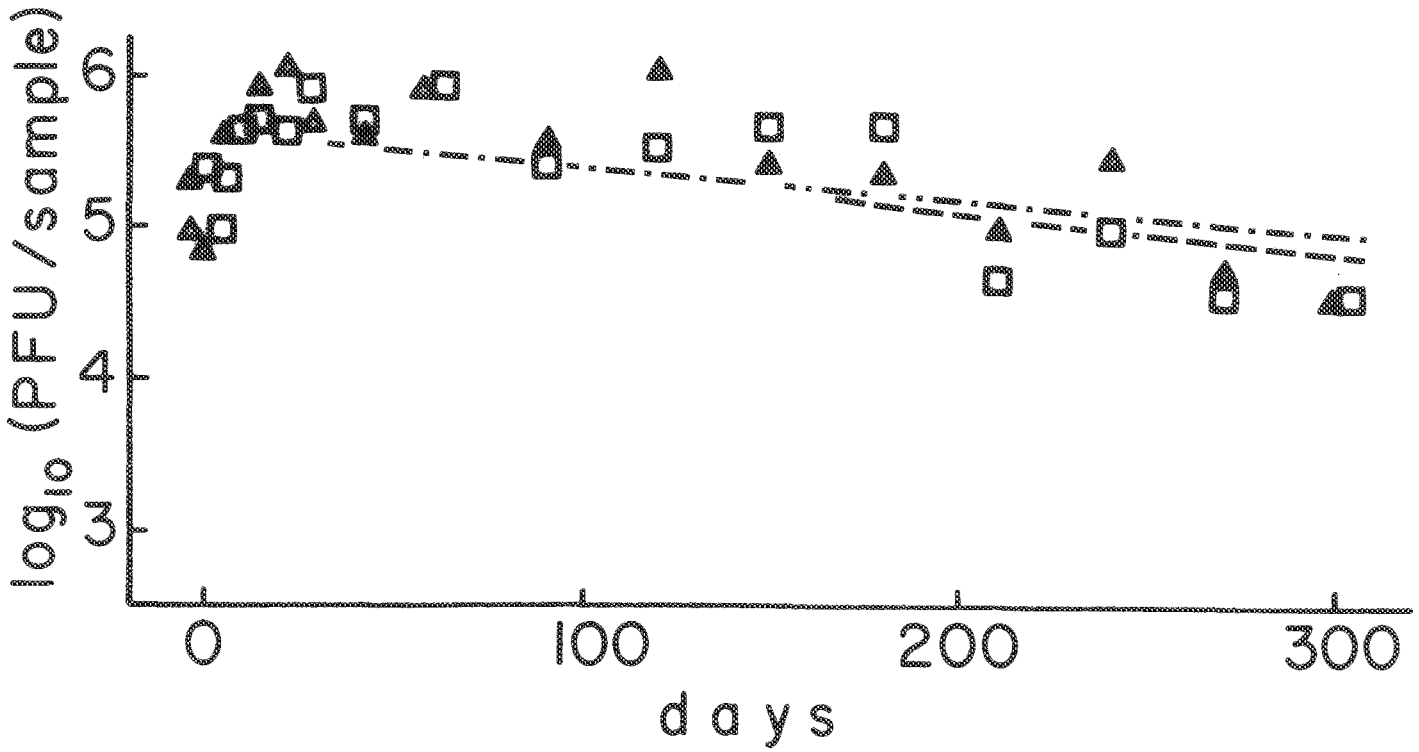

Fig. 9. Inactivation of fecal poliovirus during storage at 5 C as a function of moisture level in bacon squares (▲ standard, ~ 12.9% moisture; □ freeze-dried, ~ 4.5% moisture)

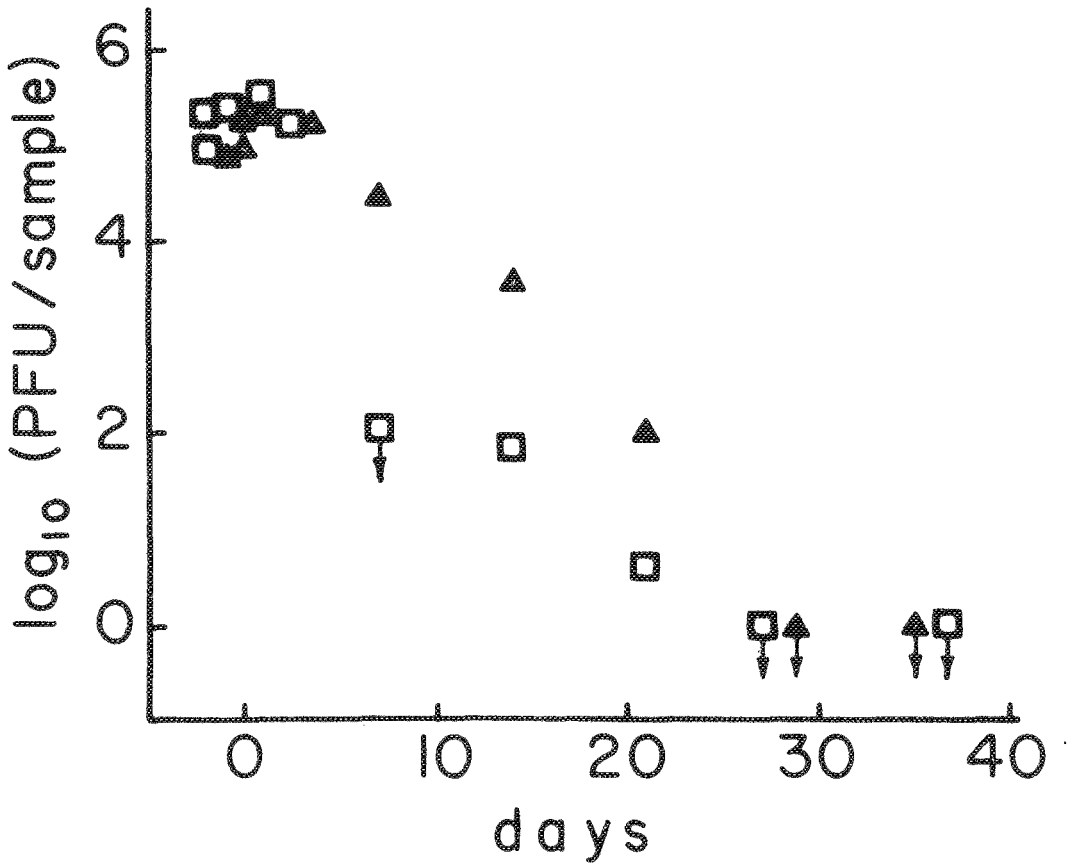

Fig. 10. Inactivation of fecal poliovirus during storage at room temperature as a function of moisture level in bacon squares (▲ standard, ~ 12.9% moisture; □ freeze-dried, ~ 4.5% moisture; ↓ < indicated value)

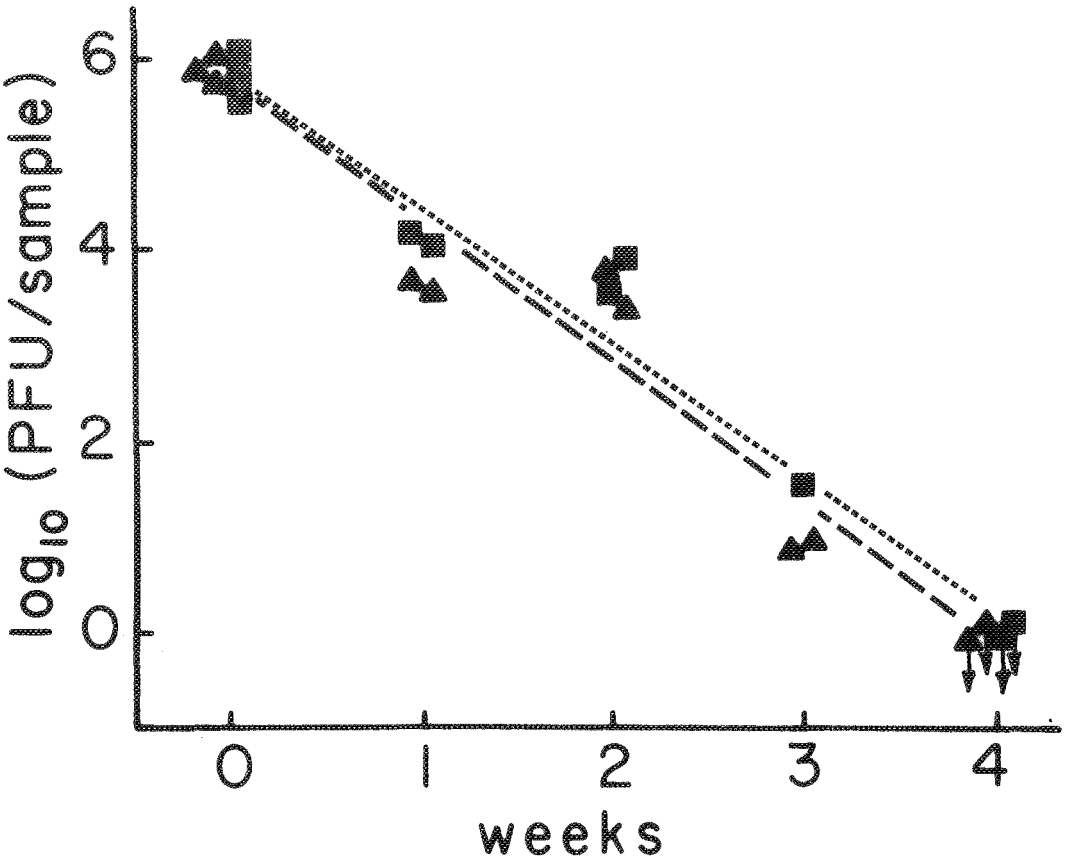

Fig. 11. Inactivation of Po-3 during storage at room temperature as a function of moisture level in bacon squares (▲ standard, ~ 12.9% moisture; ■ freeze-dried, ~ 4.5% moisture; ↓ < indicated value)

knew that this work would someday have to be expanded to include foods of normal moisture content and that at that point microbiological stability, particularly at room temperature, would be lost. We decided to do one preliminary experiment, using a rehydrated space food. We chose spaghetti with meat sauce because there was enough of it on hand. Rehydrated according to directions, the calculated moisture content was 81%. The contaminant was fecal poliovirus; this meant that both the microflora of the food and of the feces were present. Half of the samples received 2% tetracycline. The presence of the tetracycline had a great influence upon the decomposition of the food, but little upon the inactivation of the virus (cf. Fig. 12). The packages without tetracycline were gassy and foul-smelling within a week, and liquefaction of the food solids was far advanced by the third week. Only mild gas production and a slight odor of putrefaction were observed within 3 weeks in the presence of the antibiotic. The rates of virus inactivation were -0.12 and -0.10 day^{-1} with and without tetracycline, respectively, or somewhat slower than most of those observed with low moisture foods at these temperatures. This still did not explain the relatively high stability of poliovirus in bacon squares, which had been unaffected by reducing moisture.

We decided to select a bland food and add components to it to determine which influenced enterovirus stability. Banana pudding, with tabulated values of 1.1% moisture, pH 7.3, 2% protein, and 3.5% ash, was chosen. The pH of the batch we received was approximately 7.6 when water was added. We expected that any other desired pH could then be reached by adding acid. Anhydrous propionic acid was selected: it is a liquid over the entire range of temperatures being studied so it can be measured and mixed without adding water; it is neither hygroscopic nor extremely volatile; and it is strong enough that a small volume will produce the desired pH, but not so strong that the food is charred or decomposed at the first point of contact. This does not mean that propionic acid is typical of all the substances which make foods acid, but it was not bad for a start. In 10 g of banana pudding, 0.42 ml of propionic acid would reduce the pH (on rehydration) to 5.5, while 1.0 ml yielded pH 4.0. Slightly more acid was sometimes needed to achieve the same pH when other additives were present, but never more than an extra 10% (e.g., 1.1 ml total for pH 4.0). In a preliminary comparison, inactivation at 5 C of Po-3 in agar in banana pudding was increased slightly at pH 5.5 and a good deal more at pH 4.0 (cf. Fig. 13). The slopes of the least squares curves shown were -0.047, -0.079, and -0.16 day^{-1}, in decreasing order of pH.

Protein was the next food component examined. The nominal 2% protein concentration in the banana pudding was ignored, and lactalbumin was added to levels of 0, 15, 30, and 60%. One set of samples was at pH 7.6, and another was adjusted to pH 5.5 with propionic acid. We contaminated the samples with Po-2 in agar and stored them at 12 C (in order to speed inactivation somewhat). The inactivation rates at pH 7.6 were somewhat slower (cf. Fig. 14) than that in the previous experiment, despite the increase in storage temperature. The least squares curves for the 0 and 60% protein samples were so nearly identical that they could not be plotted separately. Slopes ranged from -0.0091 (for 30% protein) to 0.017 day^{-1} (for 60% protein) and did not appear to differ significantly. This trend was evident when tests of the 4 week samples were completed, so half of those remaining were stored at room temperature beginning at 5 weeks, and all were tested at 7 weeks. Based upon least squares estimates of the titers at 5 weeks, inactivation rates at room temperature ranged from -0.05 (for 60% protein) to -0.15 day^{-1} (for 0% protein). Inactivation at pH 5.5 was even more rapid, varying

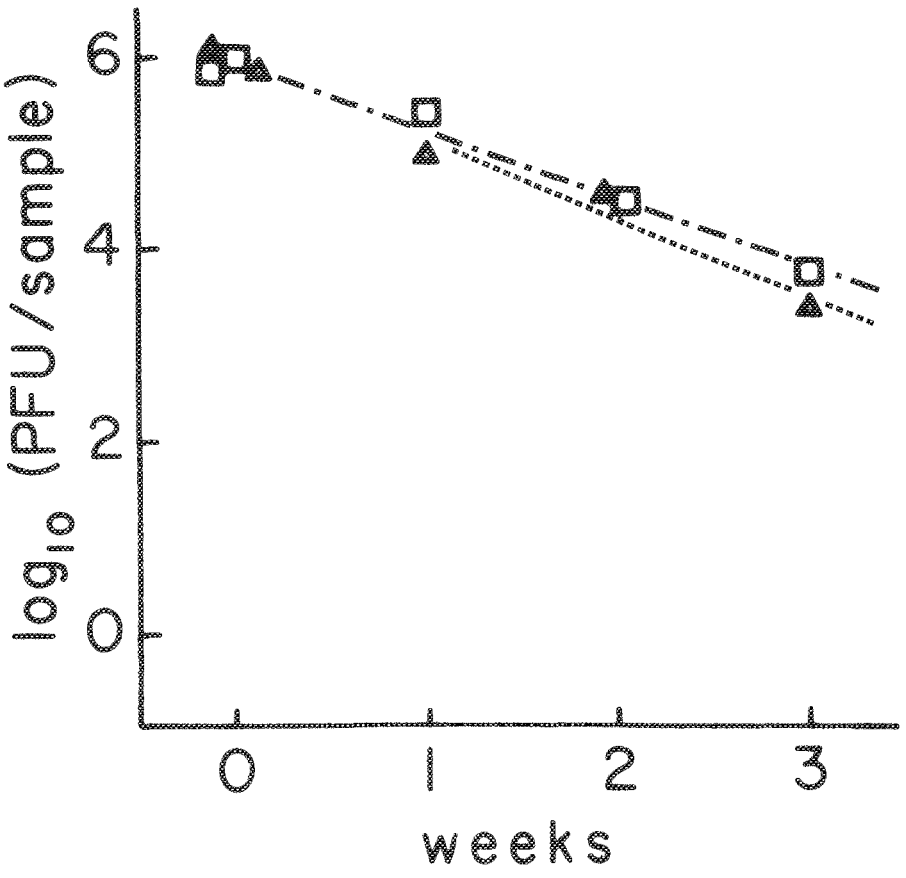

Fig. 12. Inactivation of fecal poliovirus at room temperature in rehydrated spaghetti with meat sauce (0% and 2% tetracycline added)

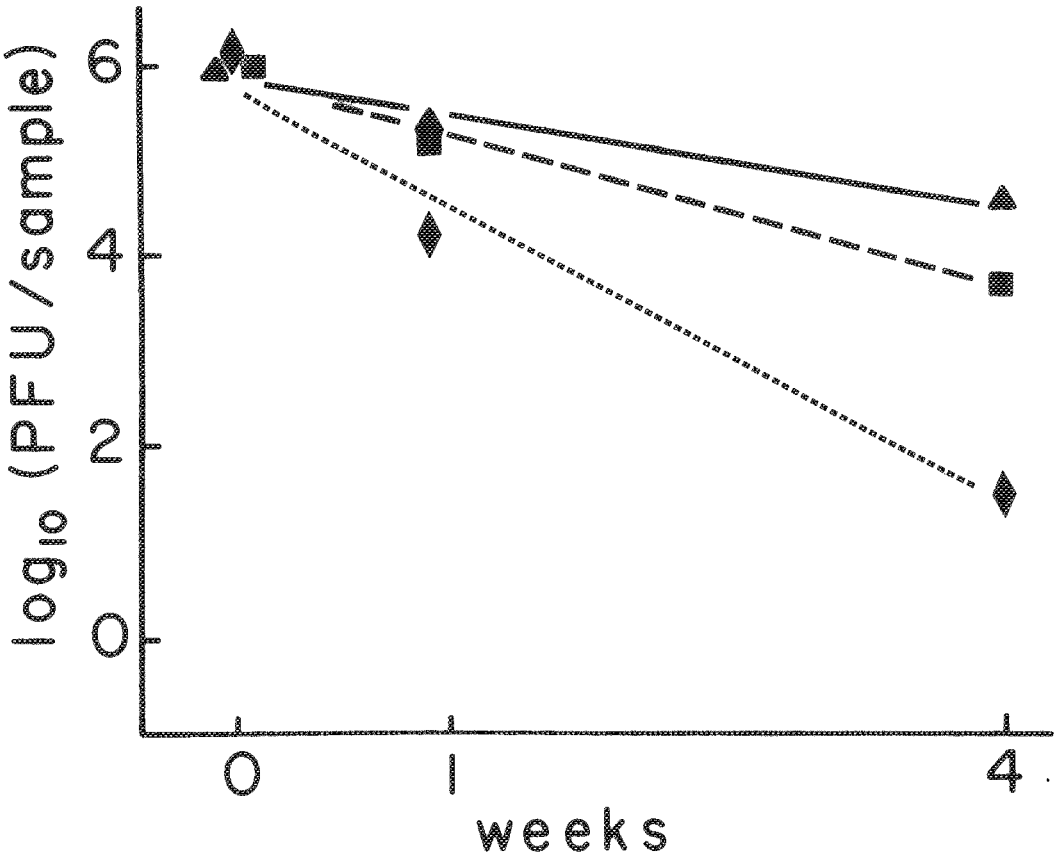

Fig. 13. Inactivation of Po-3 at 5 C in dry banana pudding as a function of acidity (▲ pH 7.6, ■ pH 5.5, ♦ pH 4.0)

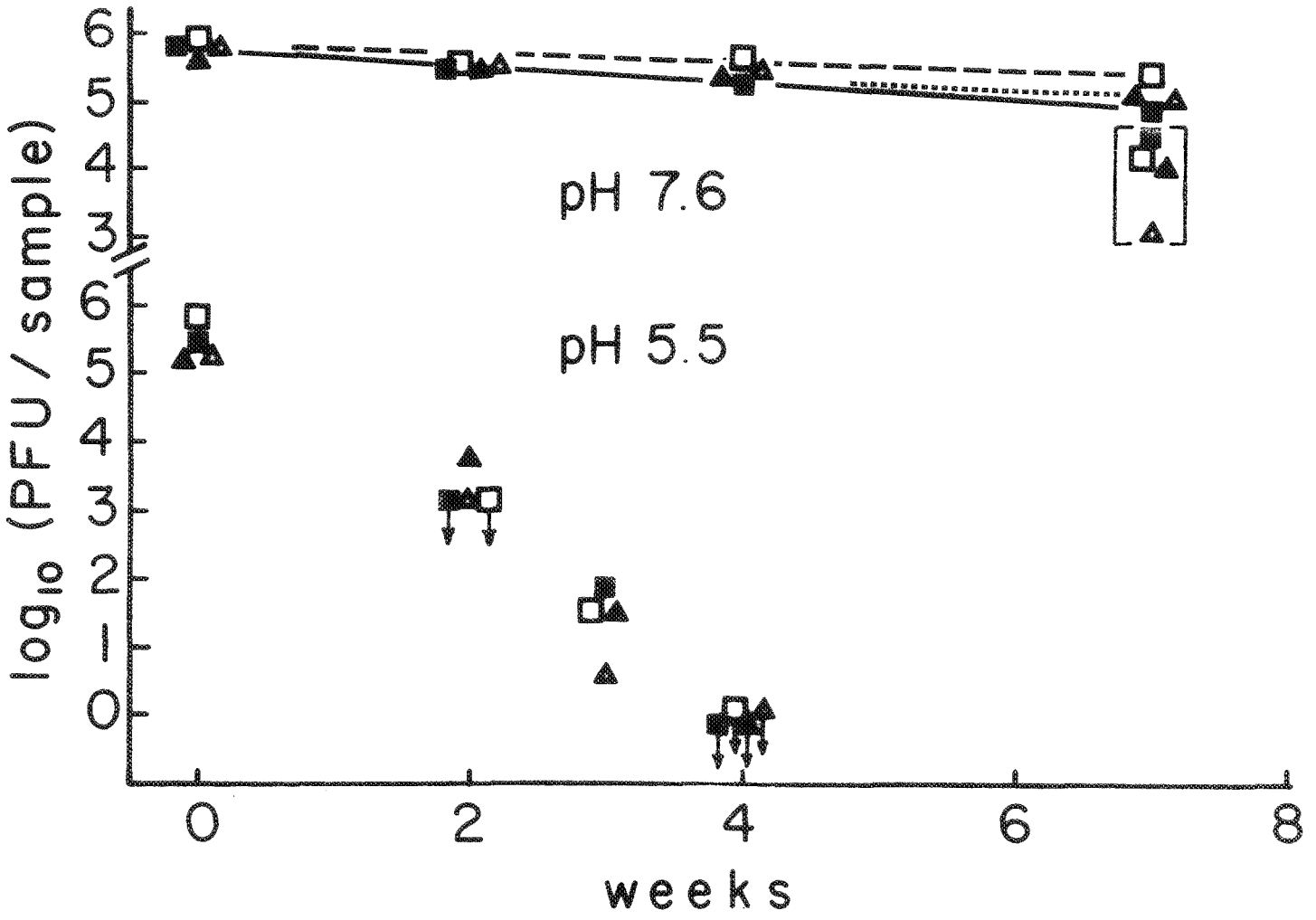

Fig. 14. Inactivation of Po-2 at 12 C in dry banana pudding as a function of added protein (——▲—— 0%, – – – ▲ – – – 15%, ····□···· 30%, and ——▩—— 60% lactalbumin; ↓ < indicated value; bracketed points, samples stored last 2 weeks at room temperature)

about a value of -0.2 day^{-1}. The data were not consistent enough or numerous enough to warrant estimating curves for the samples at pH 5.5. We surmise that protein level was not a very important factor in food-borne virus stability at pH 7.6 and temperatures of 12 C or below, under the conditions of this experiment. Virus inactivation was probably more rapid in the absence of added protein at pH 5.5, or at room temperature at pH 7.6.

The effect of salt also appeared to be pH dependent. One cannot account for all of the salts present in a food on the basis of the analytical values presented in the tables. The ash content of banana pudding is said to be 3.5%, of which about 70% can be identified in the tables, whereas bacon squares, which are excellent virus stabilizers, have 10% ash. We added 6.5% dry phosphate-buffered saline (PBS) mix to a portion of banana pudding and dispensed this and some of the original product into individual samples. The samples were contaminated with Po-2 in agar and stored at 5 C. As is shown in Fig. 15 (upper points), there was essentially no difference in inactivation rate with and without added salt. Only the former is shown as a curve; its slope was calculated to be -0.0061 day^{-1}. Another set of samples was prepared in the same manner, except that the pH of the food was adjusted to 5.5. The lower curve is for pH 5.5 without added salt; its slope is -0.0058 day^{-1}, or essentially the same as that shown above. Inactivation with high salt at low pH obviously was much more rapid (though non-linear) than under any of the other test conditions. One never knows whether to be surprised when an inactivation curve is linear or to apologize when it is not. Clearly, both conditions obtain in nature, though the linear curves are far the easier to compare to each other.

We also examined the interaction of salt and protein content. These components had had little influence upon virus stability at reduced temperatures when the pH was 7.6. Since this experiment was to be run at 12 C, the pH selected was 5.5. The food was banana pudding, with and without 50% lactalbumin and 6.5% dry PBS mix; and the contaminant was Po-1 in agar. Not unexpectedly, inactivation was fairly rapid in the high protein-low salt and low protein-high salt combinations (cf. Fig. 16). The third sample from each of these two sets had to be tested by the concentration method and somewhat earlier than we had originally planned. The slopes of these inactivation curves were so nearly the same (-0.25 and -0.24 day^{-1}, respectively) that space required that they be represented by a single line in the figure. The high protein-high salt combination is near in gross composition to the freeze-dried bacon squares; but inactivation is perhaps more rapid in the former (-0.025 day^{-1}) than would be predicted from the latter (-0.0029 day^{-1}), even in view of the increase in storage temperature from 5 C to 12 C. The control samples (low protein-low salt, represented by open triangles in Fig. 16) have counterparts in the experiments represented in each of the preceding three figures, allowing only for differences in storage temperature. At 5 C, the observed inactivation rates have been -0.079 (Fig. 13, middle curve) and -0.0058 day^{-1} (Fig. 15, lower curve). The open triangles in the lower part of Fig. 14 suggested an inactivation rate at 12 C of roughly -0.2 day^{-1}, which certainly does not agree with that seen here (-0.033 day^{-1}).

Five factors have been included in the studies reported here under "food composition." These were moisture, temperature, pH, protein content, and salt content. The last four of these have been studied by adding "factors" to banana pudding, though not all of the possible combinations could be tested. The experimental data indicated that Po-1 (and presumably other enteroviruses) is stable at reduced temperatures in low moisture foods which are low in acid, but protein may be necessary for stability at room temperature. At pH 5.5, which is about the

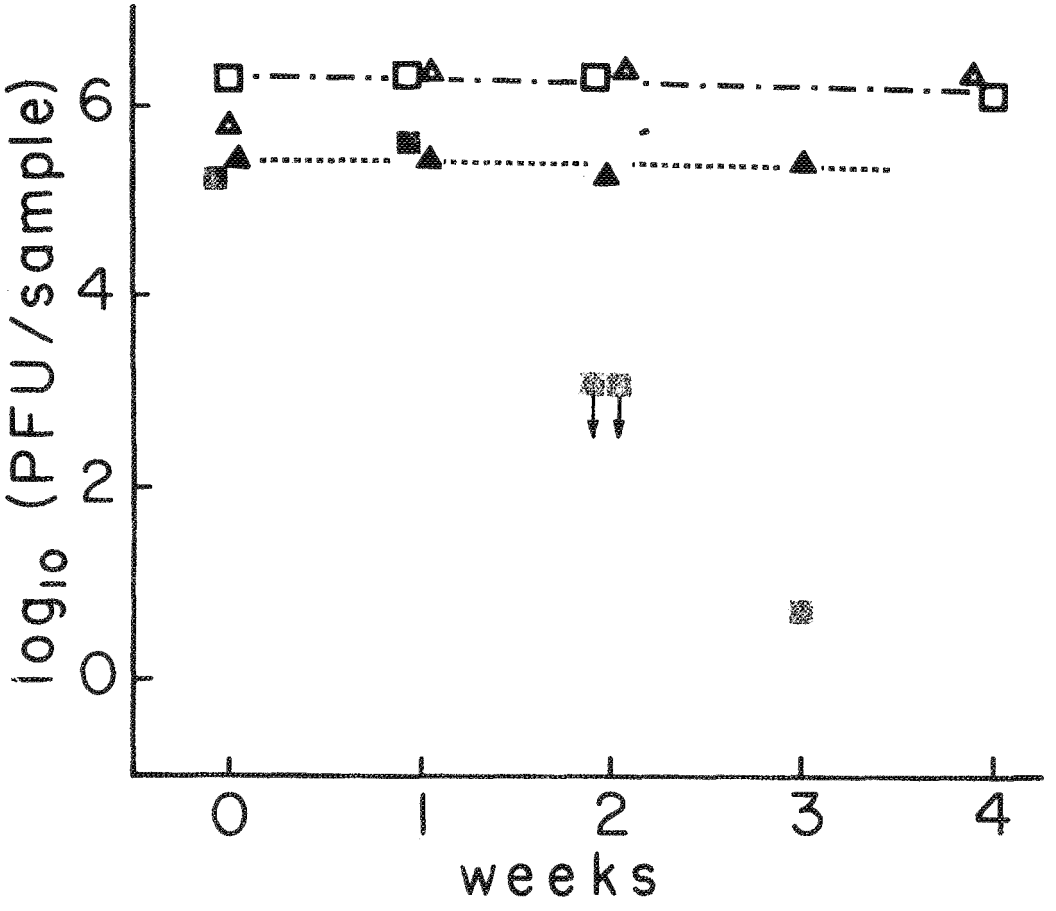

Fig. 15. Inactivation of Po-2 at 5 C in dry banana pudding as a function of added salt (pH 7.6, ▲ 0% and —·—·—·□ 6.5% added dry PBS; pH 5.5, ▪▪▪▪▪▪▪▪▪▪▪▪▪▪ ▲ 0% and ▩ 6.5% added dry PBS; ↓ < indicated value)

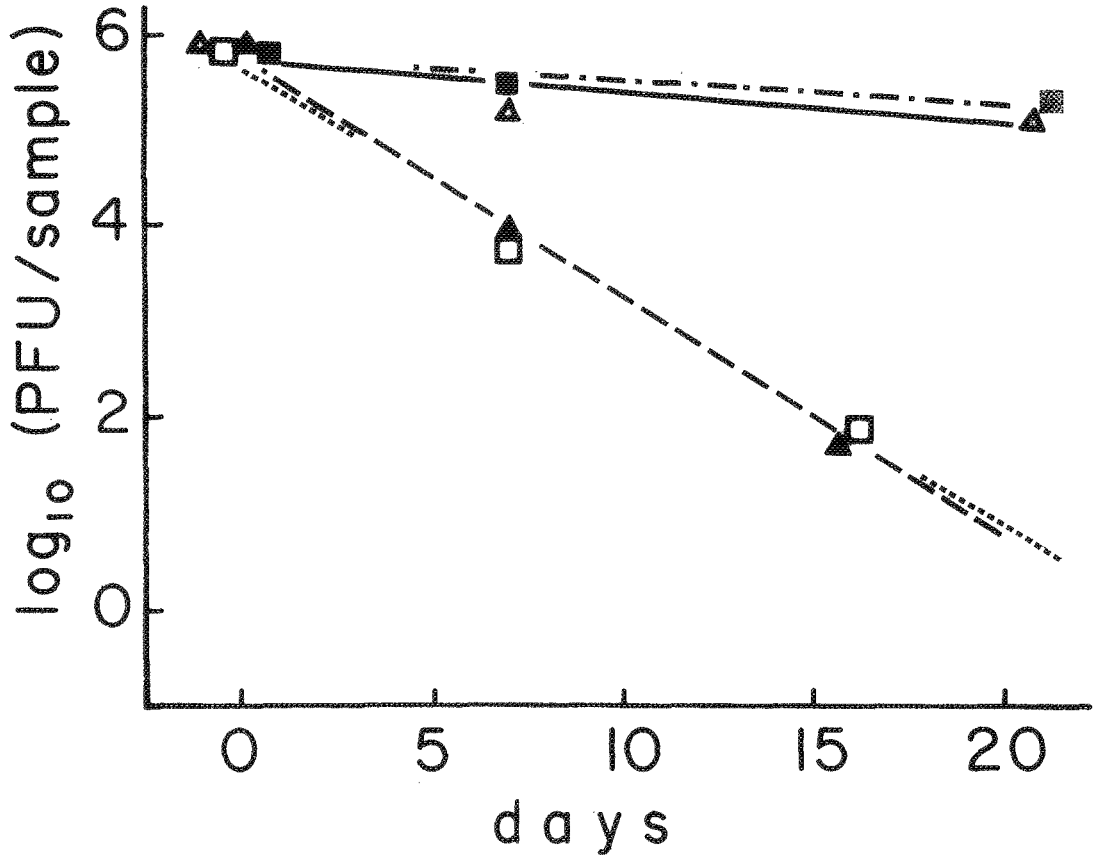

Fig. 16. Inactivation of Po-1 at 12 C in dry banana pudding adjusted to pH 5.5, as a function of added salt and protein (added dry PBS and lactalbumin: ——▲ 0% and 0%; – – – –▲ 0% and 50%; ·····□ 6.5% and 0%; and –·–·–▩ 6.5% and 50%, respectively)

mid-range value for acidity in space foods, high salt or (perhaps) high protein appears to speed virus inactivation, but the two together stabilize the virus.

Freeze-drying virus-contaminated food. Most of the experiments described were carried out in low moisture foods, and most of the low moisture foods had been freeze-dried. Once we determined that poliovirus (and probably other enteroviruses) could persist for long periods in some freeze-dried foods, we wanted to know whether the virus could withstand freeze-drying in food and what effect this had upon the stability of the virus in subsequent storage. Cream style corn was chosen as the model food vehicle because the "pre-mix" form of this food is a grocer's shelf item (canned cream style corn). Feces containing poliovirus were weighed onto laminate squares (0.5 g per square) and shipped frozen to Dr. M. C. Smith at NASA Houston. These were added to cream style corn in molds, freeze-dried, vacuum packaged, and shipped to Madison for assay. A sample was taken after each processing step, rehydrated, and frozen. The greatest loss of virus evidently occurred during the freeze-drying step (cf. Fig. 17). A further apparent loss of infectivity in transit from Houston to Madison seems not to have been real; the titer of the sample after freeze-drying is identical to the least squares estimate of the zero-time titer of the stored samples. Contaminated food samples stored at 5 C since arrival in Madison have shown slow loss of infectivity; the slope of the least squares curve shown in Fig. 17 is $-0.0032\ day^{-1}$. One sample was divided into small volumes and frozen after rehydration. Portions of this, tested after periods of up to 12 weeks at -20 C, have shown an apparent inactivation rate of $-0.0008\ day^{-1}$. This probably does not differ significantly from zero; more than 3 years would be required for 90% inactivation at this rate. The stability of the virus at 5 C in this food should be due principally to the lack of acid; the pH shown for this food in the tables is 6.8. The loss in freeze-drying was of the order of 10^{-2}, which is within the range of our previous experience with virus inoculated into already dried foods of several kinds (cf. Fig. 6). The stability of the virus in storage ranks among the very highest that we have seen.

DISCUSSION

We have identified several factors which seem to influence the stability of viruses in foods for space flight. Preeminent among these is the kind of virus; reovirus type 1 and two myxoviruses (influenza A and parainfluenza virus type 3) were rapidly inactivated in foods at 5 C. Enteroviruses, including all three types of polioviruses and EC-6, were quite stable in some foods and were invariably more persistent than the agents listed above. There was no apparent difference in stability among enteroviruses, nor did source (tissue cultures or the human intestines) nor carrier (feces or 1% agar) influence the rate at which the enteroviruses were inactivated. The emphasis here has been upon freeze-dried foods, but there has been no indication that enterovirus stability is directly dependent upon moisture content. Most of the inactivation rates which we have observed have been within the range of those reported by others who were working with foods of normal moisture content. Stability of enteroviruses in bacon squares and beef bites at 5 C in our experiments has been greater than in any food studied by others, but this seems not to be due to their low moisture content. It should be mentioned that the beef bites used in this year's work differ from those tested previously in that they do not have the waxy coating which seemed to limit interaction of the virus with the food.

Temperature proved a very significant factor in virus inactivation. Virus was inactivated more rapidly at room temperature than at 12 C or at 5 C,

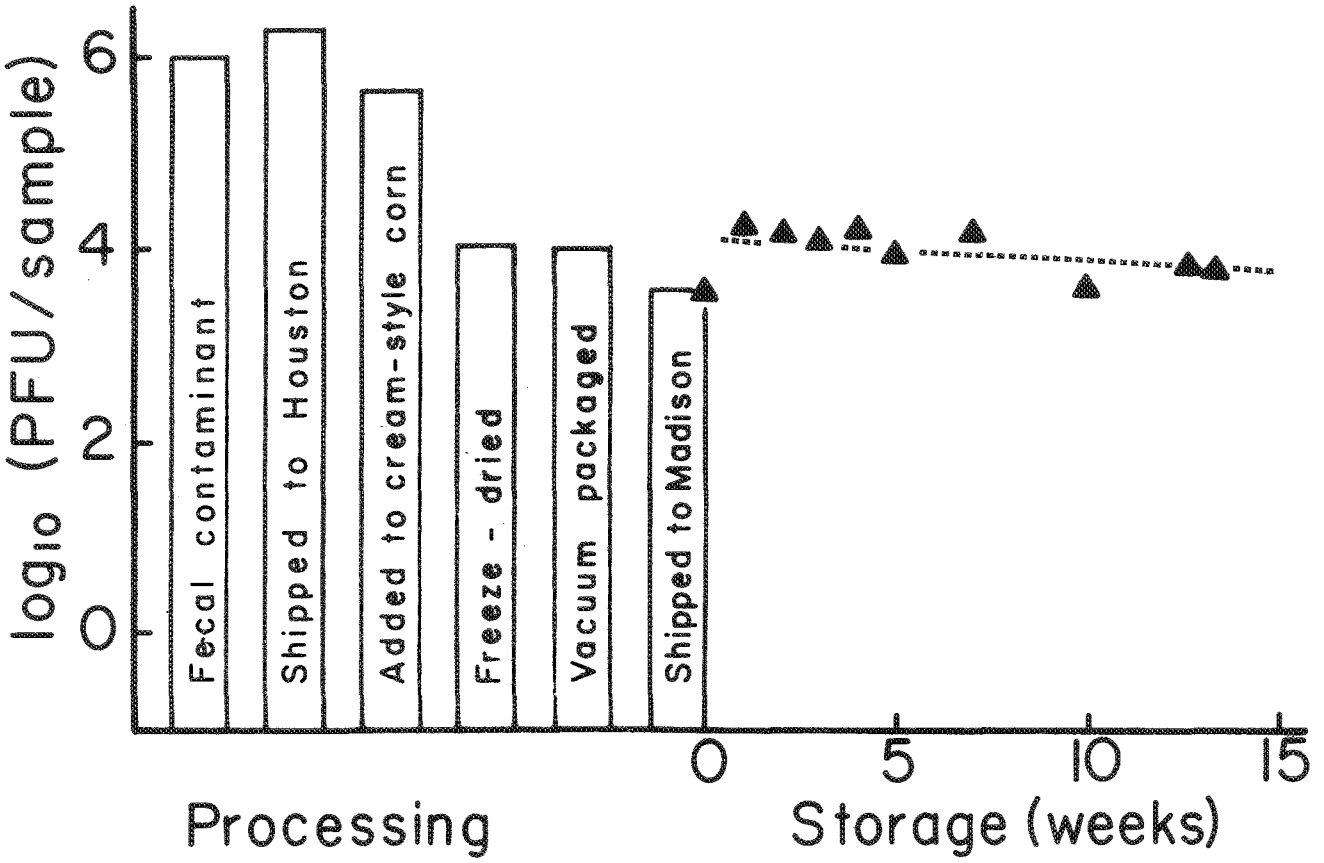

Fig. 17. Inactivation of fecal poliovirus in processing and during subsequent storage at 5 C

and may not have been inactivated at all at -20 C. Only virus in bacon squares was tested by cycling it between room temperature and 5 C. The amount of inactivation was apparently equal to, or perhaps slightly greater than, could be attributed to the time spent at room temperature. Though inactivation in bacon squares at 5 C has been negligible in short-time experiments, poliovirus has been less stable in bacon squares than in some other foods at room temperature. Therefore, we cannot assume that what we have learned of the influence of food composition on virus stability at reduced temperatures is valid at room temperature or above.

Acidity appears to be the next factor, in order of precedence, influencing the stability of enteroviruses in foods at reduced temperatures. Inactivation in foods at pH 7 or slightly above has generally been slower than in acid foods. Acidity also appears to have influenced the way in which other food components affect virus stability. Neither high protein nor salt was significant in neutral food, but the two together stabilized virus at pH 5.5. These statements should be hedged by pointing out that they are based upon data from one food (dry banana pudding) adjusted with one acid (propionic) to one pH (5.5) and supplemented with one protein (lactalbumin) and one form of salt (dry PBS). The data obtained from this experimental system have not been at odds with those from whole foods of varying composition, but they obviously have not covered all of the possibilities.

The data on freeze-drying poliovirus in cream style corn lend themselves to a rather circular interpretation. It appears that the virus is stable in storage in the dry form of this food at 5 C. Perhaps as a consequence, it was inactivated only to a limited extent (10^{-2}) during freeze-drying. Having been freeze-dried in the food apparently did not diminish the stability of the virus, for it was (as stated above) inactivated very slowly in storage at 5 C. This is not a very satisfying interpretation, but anything more definite awaits further experience with freeze-drying other foods.

One is tempted to expound further upon what we don't know. We have found that there are at least four potentially significant factors (temperature, pH, protein, and salt content) in the stability of enteroviruses in foods for space flight. If one envisions the quantity of each of these being represented in a four-dimensional plot, we have been working principally along the axes rather than into the space which they define. A real knowledge of the influence of these factors upon virus inactivation requires that a much broader set of combinations of these factors be tested. We have already detected one anomaly: the effect of salt upon virus stability in acid food evidently varies with protein level. If additional work is done, there are likely to be several more surprises.

SUMMARY

Reovirus type 1, influenza virus type A, and parainfluenza virus type 3 persisted for $\leq$ 3 days in the space foods tested. The enteroviruses tested persisted > 2 weeks at room temperature and > 2 months in the refrigerator. Where storage temperature is not constant, time-temperature effects are roughly cumulative. Inactivation of enteroviruses proceeds at an intermediate rate in foods stored at an intermediate temperature of 12 C.

Polioviruses from feces and from tissue cultures are inactivated at

comparable rates in foods. The inactivation rate of another enterovirus (ECHO-6) was similar. Neither the presence of feces nor of the fecal microflora seemed to influence the persistence of food-borne poliovirus.

The moisture level in a food did not affect poliovirus inactivation under the conditions of these tests. At reduced temperatures, virus stability was great in foods at pH $\geq$ 7. At pH 5.5, there is a complex interaction of protein and salt content upon virus stability. If the contaminant is applied to a bite size food item with a truly impervious coating, the internal composition of the food is immaterial.

Poliovirus was inactivated 10^{-2} during freeze-drying of cream style corn. The remaining virus was quite stable during storage of the product at 5 C.

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