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"Stability of Viruses in Foods for Space Flights"

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Dean O. Cliver, Ph. D.
Associate Professor
Food Research Institute and Department of Bacteriology
University of Wisconsin
Madison, Wisconsin 53706

INTRODUCTION

The following is a brief account of studies performed during the six-month period ending 31 May 1969. As was indicated in the Final Technical Report of the first year's work, the present phase of the program is intended to generalize the results of the exploratory studies. We found a great difference in stability between our model enteric virus (poliovirus) and our model respiratory virus (influenza A virus); we want to know whether these differences are also seen with other enteric and respiratory viruses. We found a great difference in the stability of poliovirus in different foods; we want to know why. We found that poliovirus was usually inactivated at very different rates in the same food stored at different temperatures; we want to know more about this.

MATERIALS AND METHODS

The viruses used in this work have been of only two kinds to date. The enteric viruses have been represented by polioviruses, usually as shed in infant feces following oral polio vaccination. The respiratory viruses have been represented by influenza A (PR 8) virus produced in embryonated eggs (allantoic fluid). Influenza virus to be inoculated into food was first suspended in bovine respiratory mucus which had been centrifuged to remove gross particles and adjusted to pH 7 or slightly above.

Foods were contaminated with 0.5 g of infectious feces or with 0.5 ml of inoculated respiratory mucus per sample. A sample consisted of one or two bites of a bite-size food or 20 or 25 g of a food intended to be rehydrated in the pack. These were placed in laminate pouches, flushed twice with nitrogen, and sealed under vacuum.

Samples were stored at room temperature (~23 C) or in the refrigerator (5C). At the indicated times, the entire contents of a package were taken and sampled by one of two methods. The "dilution method" involved homogenization of a sample of bite-size food in 100 ml (200 ml for foods designed to be rehydrated in the pack) of phosphate-buffered saline (PBS) and testing at further dilutions of 10^{-1} and 10^{-2} in PBS plus 2% agamma chicken serum. The "concentration method" was essentially that of J. Herrmann and Cliver (Appl. Microbiol. 16:1564, 1968), except that concentration of each food extract was done directly in the ultracentrifuge. This required that one tissue culture be used to test the concentrate from each 30-35 ml of food extract. Our methods of preparing tissue cultures and using them to enumerate viruses have recently been described in detail (Cliver and R. Herrmann, Health Lab. Sci. 6:5, 1969).

RESULTS

Last year's studies had indicated that influenza virus was much less stable than poliovirus. Influenza virus in prepared bovine mucus was inactivated to the extent of 10^{-3} to 10^{-5} with 30 days at 5C. Of foods surveyed previously, extensive inactivation of the virus was noted within 24-72 hr at 5C in all but apricot cubes, gingerbread, and cream-style corn.

The first two of these have a relatively impervious coating, so it seems unlikely that the virus was greatly exposed to the food itself. Cream style corn ("corn bar") is listed at pH 6.8 in a table provided us, so these results were thought to agree with 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.

The CMC series of fecal specimens containing poliovirus was nearly depleted when the present year's work began. Another set (KLS) was provided by Dr. Smith at MSC. Thirty-four specimens were tested for virus titer (but not virus type) by the plaque technique. These were placed in five classes (where V = the number of plaque-forming units (PFU) of virus per gram of feces): $10^5 \leq V < 10^6$ (2 specimens), $10^6 \leq V < 10^7$ (19 specimens), $10^7 \leq V < 10^8$ (3 specimens), $V < 2 \times 10^4$ (6 specimens, all taken on or after 9/24/68), and 4 specimens (dated 9/4, 5, 9, and 10) which were toxic in tissue at dilutions of 10^{-6}.

The handling of the feces during contamination of a set of samples often included one or more freezings and thawings. Further, the 0-time samples taken in any series usually had been in contact with the food for about 1-1/2 hr (the time consumed in bagging and heat-sealing the samples) before the homogenates were prepared. These were two possible sources of virus loss which we felt should be investigated. A specimen was thawed and weighed as usual, but the virus titer was determined immediately. Another portion was weighed and then refrozen. It was thawed the next day and yielded a higher titer than the original sample. Additional samples which had been weighed and refrozen the day previous were placed in laminate pouches and tested after 1-1/2 hr at room temperature, having been flushed, evacuated, and sealed at the beginning or end of the 1-1/2 hr period or not at all. There was considerable "scatter" to these data, but there was no indication that virus was lost either in refreezing or sealing.

It was stated above that poliovirus was selected as a model for the enteric viruses. In fact, none of the other enteric viruses was readily available in feces, as shed in the course of an infection. We wish to use ECHO virus type 6 and reovirus type 1 in some comparative experiments, but virus of tissue culture origin was all we had of either. An experiment has been undertaken to determine whether tissue culture virus differs from fecal virus in persistence in foods and whether the feces have any effect upon the persistence of the virus. Beef bites were selected as the model food because they had shown a significant rate of early virus inactivation at 5C in an experiment reported last year. Three sets of sample were contaminated: one with virus as shed in feces, one with tissue culture virus mixed with sterile feces, and one with tissue culture virus mixed with soft agar. No inactivation of the poliovirus was observed in any of these during 6 wk of sampling. This was a surprise. We attribute the lack of inactivation to a difference in the food: last year's beef bites were coated, while these apparently are not. It seems likely that the substance of the beef bites has always been an efficient virus stabilizer (perhaps as much as the bacon squares) but that the coating was not. Enough samples remain to permit a total of 16 weeks' study. Meanwhile, there is no reason to believe that tissue culture virus in agar differs significantly from virus as shed in feces in this system.

Last year's work revealed that poliovirus is unusually persistent in bacon squares. No inactivation was detected during 6 mo at 5C, and there was some indication that the virus titer might have risen in the 5 and 6 mo samples. The last two samples of this series have since been tested (at 7 and 8 mo.). Their titers fell within the range of the previous determinations: higher than that at 0-time, but lower than that at 6 mo. An equation for the curve was derived by the linear least squares method. The slope of the curve was positive but did not differ significantly from 0. It was concluded that the virus titer of these samples had not changed during 8 mo at 5C.

The bacon squares were distinguished from other foods in the program by their exceptionally high content of water and salt. Either of these components, or these in combination with others, might have been responsible for the unusual stability of the poliovirus. A conference with Dr. Smith and with Dr. Robert Pavey of Swift and Co. indicated that the moisture could most easily be changed, so this was done first. A portion of a batch of bacon squares was freeze-dried (~ 4.5% moisture) and these and some regular (~ 12.9% moisture) bacon squares of the same batch were delivered to us. These were contaminated with infectious feces, and a portion of each was stored at room temperature and at 5C. A preliminary experiment had indicated that significantly more virus might be inactivated in the first 24 hr at room temperature in the freeze dried food. Results in the present experiment were somewhat different: demonstrable inactivation was seen only after the sample taken at 3 da at room temperature from each set. Room temperature samples at 7, 14, and 21 da were lower in titer (perhaps significantly) for the freeze dried set, but neither set contained virus detectable by the concentration method after 28 and 36 da at room temperature. The latest of the refrigerated samples were taken at 3 mo, and no inactivation had occurred in either the regular or the freeze-dried set. Sampling will be continued.

It did not appear that the stabilization of poliovirus by bacon squares in the refrigerator was due to their high moisture content. The salt content of the bacon squares would have been very difficult to adjust independent of the moisture level, so we have just begun another experiment in which salt has been added to banana pudding. The results of this experiment will be included in the year's-end report.

Each of the previous experiments had been conducted at storage temperatures which were as constant as possible. This makes the data easier to analyze, but it is not typical of the way foods for space flights are likely to be handled. We wished to experiment with storage alternating between room temperature and 5C. Bacon squares were selected as the model food in the hope that no virus would be inactivated during the periods at 5C. One set of samples was incubated continuously at room temperature. The other two sets were alternated between the two temperatures of storage at weekly intervals. One of these began at room temperature and the other at 5C. Over the 4 wk experimental period, the virus titer of the samples tored continuously at room temperature decreased about 1.5 "logs" per wk (linear least squares derivation). The mean decrease during four 1-wk periods at room temperature for the other two sets was 1.73, while that for the periods at 5C was 0.19. It is possible that experimental variation may have obscured some real differences, but the data obtained give no reason to suppose that the time-temperature effects are not simply additive. That is, if one knew the rate at which virus was inactivated at various temperatures in a given food, he could arrive at a fairly good approximation of total inactivation by adding the products of time at each temperature and inactivation rate at each temperature.

DISCUSSION

We are convinced that influenza A virus which contaminates food at any time up to final packaging is not likely to persist long enough to infect a consumer. We have scheduled an experiment with parainfluenza 3 virus in banana pudding to determine if the same is true for another respiratory virus.

The preliminary indication that tissue culture virus in soft agar is equivalent to virus shed in feces permits us to experiment with other enteric viruses. We have scheduled studies of reovirus type 1 in beef bites and of ECHO virus type 6 in beef bites and banana pudding for the next few months.

Several food components have been suspected of contributing to the observed differences in poliovirus stability. Among these are moisture, salt, acid, and protein. The complete stability of poliovirus at 5C in bacon squares appears not to be due to their high moisture content. An experiment to evaluate the effect of salt (in banana pudding) is already in progress. Because banana pudding is low in each of the potentially significant components listed above, it will also be used as the vehicle in experiments on acid and protein effects. Low moisture acid and protein will be added to banana pudding to determine if these modify the stability of the poliovirus. We hope eventually to be able to study the interaction of two or more factors as well.

Temperature effects have now been studied from two standpoints: the difference between constant high (~ 23C) and low (~ 5C) storage temperatures and the effect of changing the temperature of storage back and forth between these two levels. It appears that inactivation due to storage is additive, though these determinations need to be repeated for greater precision. We have also scheduled an experiment in which poliovirus in cheese sandwiches will be stored at constant temperatures of 23, 12, and 5C. Information on the relative effect of the intermediate (12C) storage temperature will be useful in interpreting the results of varying the storage temperature.

Several sources of experimental variation are known to be at work in these studies. It is impossible to contaminate all of the samples in one experiment with exactly the same quantity of virus. We determined during last year's work that the distribution of virus in a single fecal specimen was unbiased, but it does vary considerably. It seems possible that this variation can be reduced by using tissue culture virus in soft agar as the model contaminant in the storage studies. It has not been determined whether the same substitution could properly be made in other kinds of experiments. The distribution of virus within a food sample has been shown to be biased, but this is not a problem since the entire food sample is homogenized with diluent at the time of testing. One source of variation which is probably quite significant is the difference in sensitivity of various batches of primary monkey kidney tissue cultures. The durations of these experiments are such that the same batch of cultures cannot be used to test successive samples at the time they are taken. To determine the significance of this factor, we are freezing the homogenates of one series of samples so that all can be thawed and tested on the same day in the same batch of cultures. The results of these determinations will be compared to those obtained when each sample was assayed on the day it was taken.

Another experiment indicated that one could weigh out fecal contaminant and refreeze it before an experiment with no loss of virus. A set of preweighed contaminants has been prepared and sent to MSC to be freeze-dried in a food. When we receive these samples, we will attempt to determine the effects of the freeze-drying upon the infectivity of the virus and upon its stability during subsequent storage.

SUMMARY

It has been shown that influenza A virus is not likely to be transmitted in foods for space flight. Poliovirus persists for weeks at room temperature and (in some foods) for months at 5C. It seems not to be inactivated at all in bacon squares at 5C.

The influence of food components on virus stability has begun to be explored, and the study of temperature effects has been broadened. A limited selection of secondary model virus agents has been made. These will be used in forthcoming studies to determine if the results of experiments with influenza A and poliovirus are generally applicable to other virus agents.

Dean O. Cliver

D. O. Cliver, Ph. D.
Project Director

cc: Dr. Malcolm C. Smith, MSC (2)
Mr. Lon Van Ess, U. W.