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PROJECT DIRECTOR: WILLIAM B. BEARDMORE, Ph.D.

FEBRUARY 2, 1976 TO FEBRUARY 2, 1977

ANNUAL REPORT

R & D FOR AN IMPROVED HEAT RESISTANT LIVE ATTENUATED MEASLES VACCINE

Contract AID/afr-C-1192

Annual Report

February 2, 1976 to February 2, 1977

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NATURE OF INVESTIGATION

The workscope of this contract consists of a research and development program to develop a method to produce an improved live attenuated measles vaccine which shall drop no more than 1 log in titer when held without refrigeration for at least five days before reconstitution and for an additional 12 hours after reconstitution at ambient temperature of 40°C. Ideally, this improved vaccine will retain immunizing potency levels for several months at ambient temperature of 40°C.

The thermal lability of measles virus grown in chick embryo fibroblast cultures is well characterized. This viral instability has been attributed in part to the structure of the measles virion in which peripheral structural lipoproteins are altered in freezing, resulting in degredation of the particle with concomitant loss of infectivity. The physical principles of drying virus by sublimation in vacuo also influence the stability of the final preparation. The molecular arrangement of water and the state of the virus matrix markedly influence energy required for drying with consequent inactivation of the virus. The specific reasons for the continuing loss of infectivity of the dried virus upon storage at ambient temperatures have not been established. Loss of virus infectivity may also occur when dried virus is rehydrated. Differential expansion of the several components of the virus may disrupt the spatial relationships of basic structures by osmotic shock.

We have studied the influence of the addition of many well known cryoprotective substances (plus a number of novel substances thought to have potential protective effects) on the Schwartz strain of attenuated measles virus, the virus licensed for vaccine use in the U.S.A., before freeze-drying and to the fluid to be used for reconstitution of the virus to determine if any

of these protect the virus to the required degree from the effects of freeze-drying per se, from further loss of infectivity following extended storage at 40°C and to minimize loss of infectivity following reconstitution. Our selection of potential cryoprotective substances was limited to those which would be acceptable for parenteral administration, thus eliminating such substances as bovine serum, milk, dimethylsulphoxide, peptones, Bentonite, etc., which have been reported to have significant virus stabilizing properties.

All virus titrations were carried out by a modification of the method described by DeMaeyer (1960 Plaque Formation by Measles Virus. Virology 11, 634. New cells, a cell line originally obtained from the kidneys of African green monkeys (Cercopithecus aethiops), were substituted for the HEp-2 cells in this procedure.

EVALUATION OF PROGRESS AND PREDICTION OF POSSIBLE LEVEL OF ACHIEVEMENT FOR END OF PROJECT PERIOD

The objective of this contract is to develop a stable vaccine which can be used in an area with poor refrigeration and freezer facilities under field conditions. The existing live attenuated measles virus strain licensed for vaccine use has been demonstrated to be safe and efficacious by extensive clinical evaluation. It was decided, therefore, that this approved virus strain should not be modified per se, to improve the virus stability by cell passages at different temperatures and/or recombination with other viruses, as this would entail prohibitively expensive and time consuming clinical trials. Our approach, therefore, has been to discover a means to improve the stability of the licensed virus strain (Schwartz strain) by the addition of substances which prevent or slow down thermal inactivation.

We have screened many substances for some unique agent with cryoprotective and thermoprotective properties greater than have heretofore been reported. We have also tested many combinations of substances which we have demonstrated to possess some stabilizing effects to determine if there is an additive or synergistic action. It has been necessary to approach this work by largely emperical means because the mechanism of virus inactivation in general and the extremely labile measles virus in particular, is still but poorly understood.

Three distinct methanisms of virus inactivation during freezing and thawing are osmotic shock, low temperature salt denaturation and eutetic crystallization. Numerous compounds have been reported to stabilize viruses against the deleterious effects of freezing or freezing and storage at low temperature. These protective compounds function in part or in whole by: a) competition for the gas-liquid interphase at which protein denaturation takes place, b) producing an initial

dehydration prior to freezing, c) the formation and preservation of open channels in the solid matrix which act as escape passages for particulates and d) changes in the rates of formation, dissolution and redistribution of ice. Steele (1975) has recently demonstrated that the mechanism of traditional cryoprotective additives is not due to the colligative mechanism of lowering the concentration of other salutes in equilibrium with ice at any temperature, as was formerly believed.

We have found various combinations of substances which effectively stabilize measles virus during freezing, drying in-vacuo, and thawing or rehydration with an insignificant loss of infectivity.

We had anticipated that the stabilization of the dried virus for a comparatively long time at elevated temperatures would be the most difficult phase of this contract. However, toward that goal, we have made excellent progress and have improved the stability of the dried virus significantly. A total of 101 different conventional stabilizing mixtures combined with virus and held at 40°C for five days have shown less than 1.0 log 10 PFU/ml loss. The best of these lost only 0.27 log 10 PFU/ml and many other solutions lost less than 0.5 log 10 PFU/ml. We have not yet found any single substance with stabilizing properties appreciably greater than have been reported for other viruses, although we found that the particular combinations and concentrations are critical for maximum stabilizing effects. Virus combined with various concentrations of sucrose, sodium glutamate, phosphate buffer (mono and di-potassium) and hydrolized gelatin, snap-frozen, and freeze-dried has been the most stable upon storage of the dried virus at 40°C for five days.

The details of our findings are discussed under the following section of this report. In very brief summary, sucrose in the range of 20-40% has greater

stabilizing action than do concentrations above and below this range. The sucrose probably prevents virus damage by substituting for water in the virus structure, by retaining water by hydrogen bonding to water, or by providing a protective coat for the virus. We have found no other sugar to be as effective a stabilizer as is sucrose and there is no additive effect in combining other sugars with sucrose.

It is more difficult to assess the optimum concentration of sodium glutamate as this appears to be influenced by the nature and concentration of the other substances with which it is combined. In general, however, 1% or 2% appears to be as effective as concentrations up to 5% but with no toxicity or decreased protection at the higher glutamate concentrations. The sodium glutamte may function in stabilizing the virus by reacting preferentially with carbonyl groups in the medium and thus protect the virus protein which in the dried state is sensitive to denaturation by carbonyl groups.

The primary role of the K_2HPO_4 and KH_2PO_4 is probably to buffer the medium although the ions may also have some other action on the viral lipo-protein coat. These salts do not have nearly as much influence in stabilizing the virus as does the sucrose.

Gelatin appears to be the most effective single stabilizing substance we have found. A range of 4-7% appears to have the greatest stabilizing action with concentration above 7% having appreciably less action. This hydrolized gelatin probably acts as a cryoprotectant by providing a protective coat for the virus. It may also prevent inactivation of the virus particles which can occur when they reach the gas-liquid interface, by saturating the gas-liquid interface with another protein thereby preventing virus access to the surface. The use of hydrolized gelatin has a number of advantages over other substances which

might have a similar action, such as bovine or human albumin with especial reference to cost and to potential sensitization of the vaccinee.

While we have improved the stability of the dried virus significantly, we have not yet been able to maintain a satisfactory infectivity titer in in dried virus which has been rehydrated and held in the fluid state for 12 hours at 40°C. Many of these virus-stabilizer combinations still possess considerable infectivity after 5 days at 40°C dried plus 12 hours at 40°C after rehydration, but all of them have lost more than 1.0 log 10 PFU/ml in-toto. Our data suggest that concentrations of substances required to maintain maximum infectivity during freeze-drying and subsequent storage in the dried state are ineffective or even deleterious to the virus after it is rehydrated and held for the relatively short time of 12 hours at 40°C. This suggests that a biphosic stabilizing system is required in which those stabilizers optimum for freeze-drying and storage at elevated temperature in the dried state would be combined with the virus prior to freezing and drying. Those stabilizing substances optimum for virus stability in the fluid state would be incorporated into the fluid used to rehydrate or reconstitute the dried virus. It is possible that substances which are not effective in stabilizing measles virus infectivity in drying and in the dried state may have appreciable stabilizing action in the fluid state. We are in the process of re-evaluating certain of the substances originally screened and found to be relatively in-effective in the dried virus to determine if some of these substances would be effective stabilizers to virus in the fluid state.

Thermal inactivation of viruses in the fluid state occurs by two-component inactivation curves. Protein damage results at high temperatures (43°-61°) with nucleic acid damage occurring at physiological temperatures. It seems that most research and studies on the kinetics and mechanisms of virus thermal inactivation have been concerned primarily with the virus protein damage

rather than RNA stabilization - probably because it is simpler but also because maintenance of the intact normal virus lipoprotein capsid may protect the internal RNA from the actions of ribonucleases, heavy metal ion contaminants, etc. This is discussed at some length in the following section of this report. This discussion of the mechanism of thermal inactivation of viruses provides the background and rationale for additional work we now have in progress.

The stabilizing effects on a wide variety of viruses (including measles virus) of monovalent and divalent cations and anions are well established. The discussion portion of this report reviews a number of relevant published reports. Although the mechanism of the action of these salts (primarily $MgCl_2$, NaCl, $MgSO_4$, Na_2SO_4) has not been entirely characterized, it appears to result in from an action on the viral protein. It is possible that this action is separate from and independent of the action of the other substances which protect the virus protein, and may be additive.

Another approach we intend to pursue is the stabilization of measles virus by the addition of chelating substances to the virus preparation both prior to drying and in rehydration. EDTA (ethylenedianinetetraacetic acid) has been reported to stabilize Japanese encephalitis virus RNA - a virus closely related to measles virus. Rabies virus (another RNA ether-sensitive myxovirus) has also been reported to be stabilized by both EDTA and EGTA (ethyleneglycol-bis-(β-aminoethylether) tetraacetic acid). The mechanism by which EDTA and EGTA protects virus against heat inactivation has not been determined. The chelating effect may act by removing cations that: 1) act as necessary cofactors for degradative enzymes, 2) non-enzymecally catalyze decomposition of virus components, or 3) poison endogenous virus enzymes necessary for replication. Michalski, et al., (1976) have reported that serum added to rabies virus suspended in a buffer solution containing EDTA protects the virus at physiological temperatures under

conditions where serum added to virus in cell culture medium is totally without effect. The explanation for these observations is not clear but as Michalski, et al., conclude, cell culture medium is a poor milieu for the manipulation of rabies virus in the laboratory. It may well prove that cell culture fluid is a poor medium for the preparation of measles virus vaccine. Current commercially available measles vaccine is basically measles virus and additives in cell culture medium. In addition to evaluation of EDTA and EGTA as stabilizing agents under a variety of conditions, we intend to study the influence of whole serum as well. Serum is not permissible in a vaccine but if significant stabilizing action can be demonstrated in the presence of fetal calf serum and/or adult bovine serum, other leads may be suggested, such as the presence of enzyme inhibitors or some type of serum protein other than albumin.

Another approach to the stabilization of measles virus infectivity is to solve the problem of the loss of infectivity in a fluid state by not rehydrating the dried virus. This may be accomplished by suspending the dried virus in a water-free or almost water-free liquid such as peanut oil or glycerin. We have carried out some preliminary studies with extremely encouraging results. In one instance we reconstituted a dried virus-mixture of 14% sucrose, 0.3% K_2HPO_4, 0.1% KH_2PO_4, 1% sodium glutamate and 1% human serum albumin with peanut oil and held duplicate vials for 12 hours at 40°C and at 4°C. The titer of the dried virus rehydrated with water immediately prior to assay was $10^{-3.03}$, the titer of dried virus reconstituted with peanut oil and held 12 hours at refrigerator temperature was the same while the titer of the virus reconstituted with peanut oil and held 12 hours at 40°C was $10^{-2.30}$ for a loss of only 0.73 log 10. In another trial, we dried virus in a mixture of 1 M $MgSO_4$, 0.01 M EDTA, 1% sodium glutamate, PO_4 buffer, 30% sucrose, 4% gelatin and peanut oil. The water was removed from the mixture through the peanut oil in the drying

process and left an oily residue. Duplicate vials of this material were titrated for infectivity without exposure to elevated temperature and after holding at 40°C for 5 days and 12 hours. The titer of the unheated virus was $10^{-2.81}$ while the titer of the heated virus was $10^{-3.00}$. This rather unconventional technique may prove to be the most productive in developing a highly heat stable vaccine. A stable vaccine not requiring rehydration would have many advantages over the current vaccine available.

In conclusion, we feel that our approach to the stabilization of measles virus has been very productive. We have developed a stable dried virus preparation and believe that this material can be made even more stable by alteration of the basic drying cycle we have employed and substituting argon for the nitrogen under which all vials were sealed in the drying chamber. Adoption of the biphasic stabilizing concept should result in improvement of the infectivity stability in a fluid state. Evaluation of basic amino acids such as arginine, histidine and lysine, which should have a greater affinity for carbonyl groups than does glutamate, should also improve the virus stability. If the effectiveness of various ions and chelating substances in protecting viral protein and RNA apply to measles virus, additional gains in stabilization should result. The potential value of a water-free, inert medium in which dried measles virus is suspended is obvious. Peanut and sesame oil, isopropyl myristate and glycerin, all approved for parenteral administration, will be evaluated in detail.

Much work remains to be done. Even if a major breakthrough occurs any day, we still must refine the composition of the product to make it as simple, inexpensive, non-reactive, efficacious and easy to administer a vaccine as practical. We hope but cannot at this time guarantee that we will be able to meet all of these objectives adequtely by the end of the contact period in August, 1977.

RESULTS AND DISCUSSION

The Schwartz strain of measles virus, which is approved for vaccine use in the United States was used for this work. Approximately 18 liters of crude virus fluid was prepared in primary chicken embryo fibroblast cultures from this virus seed. The bulk virus was distributed into small volumes and held frozen at -70°C for our stabilization studies.

One and half liters of this virus was concentrated in a L4 zonal centrifuge in a sucrose gradient to obtain a highly purified virus concentrate free of tissue culture, which might contain exogenous enzymes or some other factors resulting from replication of the virus in the chick embryo fibroblast which could affect the virus stability. (Michalski, et al., 1976, has reported that serum added to buffer protects rabies virus at physiological temperatures under conditions where serum added to cell culture medium is totally without effect. They concluded that cell culture medium is a poor milieu for the manipulation of rabies virus (which is related to measles virus) in the laboratory). The cuts collected from the centrifuge rotor bowl were monitored by ultraviolet absorption. No virus concentration was detected by this technique but the fractions with the appropriate sucrose density which theoretically should have contained most of the virus were combined and a portion of this pool containing the sucrose gradient was filled into vials and freeze-dried. The remaining fraction pool was filtered through a Sephadex G-25 column to remove the sucrose and was freeze dried. Very little infective virus could be detected in either the zonal centrifuge "concentrate" or in the sucrose-free preparations. It appears that this concentration-purification method is not practical for commercial vaccine production.

Additive Tested	Drop in PFU/ml (log_{10})
sucrose-gelatin*	0.55
sorbital (10%)	1.60
sorbital (5%)	1.59
sorbital (1%)	>2.88
gelatin (1%)	1.54
gelatin (0.5%)	2.26
gelatin (0.1%)	>3.02
lactose (10%)	1.76
maltose (25%)	0.63
raffinose (8%)	1.18

Most of the other additives tested had little or no effect with little detectable virus infectivity.

* Composed of the following:

Basic Solution

distilled water	800 ml
KOH	0.548 g
l-glutamic acid	1.440 g
K_2HPO_4	2.508 g
KH_2PO_4	1.030 g
sucrose	150 g (equivalent to 15% sucrose in final preparation)

Gelatin Solution

A 20% solution of gelatin (USP Low Bloom 112 Knox type 2321) in distilled water autoclaved at 15 lb. for two hours. (This hydrolyzes the gelatin to render it safe for injection without potential for hypersensitive reactions.)

One part of the gelatin solution was added to four parts of the Basic Solution. Equal volumes of the resulting mixture and measles virus were combined and dried.

Since it appeared that the sucrose-gelatin additive was the most effective, a controlled study comparing the additive effect of the more promising of the above listed substances (plus arabinose in place of raffinose) was carried out. The dried virus-stabilizer mixtures were held at 40°C (104°F) for 48 hours and titrated immediately after rehydration. Unheated preparations were also titrated simultaneously. While the objective of this contract is to develop a procedure to maintain virus infectivity of the dried preparation within 1 log after five days storage at 40°C plus 12 hours at 40°C after reconstitution, we limited the storage at 40°C to two days for these screening studies. Those substances which appear to be the most promising by these tests will then be studied more extensively over the entire five day period alone and combined with other substances for possible additive effect. A summary of the drop in infectivity titers in this study is given in the following table:

de Telus page 11.

A large variety of additives have been used for many years alone or in mixtures to prevent inactivation of frozen and freeze-dried virus suspensions. We have evaluated various concentrations of the following representatives of these classes of substances known to possess stabilizing action under specific conditions:

human serum albumin	dulcitol
hydrolized gelatin	mannitol
sucrose	inositol
maltose	sorbitol
lactose	glycine
raffinose	soluble starch
arabinose	polyvinylpyrrolidone
inulin	methocel
glycogen	polyethylene glycol, 4,000 cps
	polyethylene glycol, 6,000 cps

Polyvinylpyrrolidone, soluble starch, gelatin, sorbitol, sucrose, maltose, raffinose, methocel and polyethylene glycol had the greatest protective action for measles virus infectivity upon freeze-drying and rehydration without exposure to elevated temperatures. All material which retained adequate infectivity following the drying operation were held at 40°C (104°F) for 48 hours and retested immediately after rehydration. Of all of the substances tested, only the following showed any significant stabilizing effect:

Stabilizer	Drop in PFU/ml (log_{10})
*Basic buffer, gelatin, 15% sucrose	0.63
Basic buffer, gelatin, 30% sucrose	0.32
Basic buffer, gelatin, 25% maltose	0.99
Basic buffer, gelatin, 15% sucrose + 25% maltose	0.66
Basic buffer, gelatin, 5% sorbitol	>2.84
Basic buffer, gelatin, 15% sucrose + 5% sorbitol	1.02
Basic buffer, gelatin, 25% maltose + 5% sorbitol	1.27
Basic buffer, gelatin, 10% arabinose	2.66
Basic buffer, gelatin, 10% arabinose + 15% sucrose	2.72
Basic buffer, gelatin, 10% arabinose + 5% sorbitol	2.75
Water + 5% sorbitol	complete loss
Water, 5% sorbitol + 15% sucrose	2.01
Water, 5% sorbitol + 25% maltose	1.36
Water + 25% maltose	1.07
Water alone	complete loss

*Basic buffer composition

distilled water	800 ml
KOH	0.548 g
l-glutamic acid	1.440 g
K_2HPO_4	2.508 g
KH_2PO_4	1.030 g

These results demonstrate that substances which most effectively maintain measles virus infectivity through the freeze-drying process do not necessarily protect the measles from loss of infectivity upon subsequent storage at elevated temperature. Most of the substances we have screened individually and in various combinations are relatively ineffective in protecting virus infectivity through both freeze-drying and exposure to 40°C. Those substances which do show activity in this regard are very promising. On the basis of these results, sucrose, hydrolized gelatin and a phosphate buffered solution containing KOH and l-glutamic acid are highly effective in stabilizing measles virus infectivity. We therefore initiated a study of various concentrations of glutamate alone and with the basic buffered salt solution, sucrose, gelatin and human serum albumin.

Virus was mixed with equal volumes of each of the 11 different solutions described in Table 1 (see addendum attached). These virus mixtures were filled into 2 ml vials (0.5 ml/vial), snap-frozen in dry ice and alcohol, dried by the standard drying cycle used throughout this project to date and the vials were sealed under a negative pressure of five inches Hg. The dryer chamber was reduced to this pressure with dry nitrogen. These vials were held at 40°C for two days or for five days after which they were rehydrated with water and immediately titrated along with vials which were frozen but not dried and with vials which were dried but not held at 40°C. The two time intervals were used because we did not want to miss the endpoint if the virus were to be inactivated completely after five days and also to get some idea of the relative rates of inactivation which could not be obtained with only one time interval. The results of this study are summarized in Table 2 and Table 2A. All 10 solutions were

significantly superior to the control buffer solution containing only phosphates, KOH and l-glutamic acid (Solution No. 11). A relatively slight decrease in infectivity resulted from the freeze-drying operation with all 10 stabilizer solutions with the solution of 30% sucrose, 1% sodium glutamate and gelatin (Solution No. 8) decreasing in titer only 0.08 $\log_{10}$. All of the solutions had an appreciable drop in titer, however, after storage at 40°C for two days with Solution No. 8 and Solution No. 10 (same as Solution No. 8 but contains in addition 1% human serum albumin) dropping 0.56 and 0.66 $\log_{10}$ respectively. These two stabilizing solutions were also superior after five days at 40°C, with losses of 0.82 and 0.72 logs respectively. As summarized in Table 2A, most of these solutions had slightly more than 1 log loss after five days.

Six of these stabilizing solutions (as described in Table 1) were tested at intervals of 2, 3, 4 and 5 days storage of the dried virus mixtures at 40°C. In addition, the dried virus mixtures were rehydrated with water after five days storage at 40°C and the rehydrated virus was held at 40°C for 12 hours and titrated. The results of all of these tests are summarized in Tables 3 and 3A. These results demonstrate that the decrease in measles virus infectivity occurs gradually over the five day storage at 40°C and confirms the superiority of Solution No. 8 which showed a virus loss of only 0.33 logs. The complete loss of all virus infectivity in all solutions held dried for five days at 40°C plus 12 hours at 40°C after rehydration was not anticipated. The one study reported last quarter in which only 0.10 log infectivity was lost after 12 hours at 40°C had led us to believe that stabilization of the dried

virus for extended periods would present more of a problem than would the relatively short storage of the rehydrated virus.

A very large study of evaluating 74 different stabilizing solutions was then done. The composition of these solutions is given in Table 4. These consist, basically, of three different concentrations of sucrose (7, 14 and 30%) each containing permutations of 1, 2, 3, 4 and 5% sodium glutamate, 1, 2, 3, 4 and 5% human serum albumin, with and without hydrolized gelatin. The bulk of this report covers testing of these stabilizing solutions under various conditions. Because of personnel-time limitations, we cannot titrate more than 50 samples in a given day. The results of each day's titrations are summarized in separate tables. Tables 5 through 9A list the results of our assays of stabilizing Solutions 1 through 72. Equal volumes of measles virus were mixed with each of the stabilizing solutions, freeze-dried, titrated before and after storage of the dried virus for five days at 40°C and after an additional 12 hours at 40°C following rehydration with water. These results indicate that 34 of the stabilizing solutions prevent a loss of >1.0 $\log_{10}$ PFU after the dried virus-stabilizer mixture is held at 40°C for five days but in only 10 instances did any infective virus survive the additional 12 hours at 40°C after rehydration. It was noted that in general there did not appear to be any correlation between the titer of virus surviving the five day period and the 12 hour period post-rehydration. These results suggested that: a) storage of the dried virus at elevated temperature reduced the stability of the virus upon subsequent rehydration, b) the stabilizers with maximum effectiveness in stabilizing

dried measles virus are not necessarily the most effective in stabilizing fluid measles virus, or c) the particular combination of stabilizing substances, while protecting virus infectivity in freezing and drying and storage at high temperatures actually have a deleterious effect on the virus after rehydration. To determine which if any of these explanations are valid, and to determine appropriate corrective measures, a series of studies were carried out.

The results of one experiment are summarized in Tables 10 and 10A. In this study selected dried stabilizing solution-virus mixtures were titrated before and after storage at 40°C for five days. In addition, these preparations were assayed following rehydration with water and storage at 40°C for 12 hours without previous storage in the dried state at 40°C. It appears, therefore, that virus is relatively unstable after 12 hours at 40°C in the fluid state even though the dried virus had not been held at an elevated temperature prior to rehydration.

One possible explanation for instability of fluid virus is the pH of the virus mixture. A pH on the acid oralkaline side conceivably could affect the virus stability independently of other inherent physical-chemical stabilizing properties of the stabilizing solution. We had assumed that the concentration of phosphates in the basic solution used to prepare most of these stabilizing solutions (0.123% K_2HPO_4 and 0.051% KH_2PO_4) would satisfactorily buffer the solutions at around neutrality as this phosphate concentration has commonly been used in many reported studies of virus stability in the literature. We determined the pH of all of the stabilizing solutions described in Tables 1 and 4 of this

report. These are listed in Table 11. It is apparent that the pH varies from 6.0 to 7.0, with most of the solutions having a pH on the low side. In every solution in which the hydrolized gelatin was included, the pH was 6.0, a pH which would not seem to be desirable for maximum stability.

In order to resolve this apparent fluid virus instability, therefore, we attempted to determine the phosphate concentration which would buffer the stabilizing solutions studied at about pH 7.0 and compare the titers of virus reconstituted with water and with the buffer solution after holding the reconstituted virus preparations 12 hours at 40°C. We prepared and tested a series of varying concentrations of K_2HPO_4 and KH_2PO_4 in water in preparing stabilizing solutions containing up to 30% sucrose and the usual gelatin concentration. Phosphate concentration of 0.615% K_2HPO_4 and 0.255% KH_2PO_4 was required to maintain the pH at 6.8-7.0. This phosphate concentration is five times that which was used in the preparation of the stabilizing solutions described in Tables 1 and 4. Accordingly, we ran a small study of a series of three stabilizing solutions (each with a pH of 6.0 and which we would have expected to be more effective stabilizers than previous results indicated if the pH were at neutrality) as planned, reconstituting one set of dried vials previously held five days at 40°C with the high phosphate solution (henceforth referred to as Buffer) and the other set of dried vials reconstituted with water. These reconstituted vials were held for 12 hours at 40°C or 4°C and then were titrated immediately. The results of this study are summarized in Tables 12 and 12A. These results indicate that use of the Buffer does slightly

improve the stability of the virus subsequently held at 40°C but has little effect on the virus subsequently held at 4°C. The drop in virus titer at 4°C, however, was much less than at 40°C. The five days storage of dried virus at 40°C prior to reconstitution did not appear to influence the subsequent stability after reconstitution in confirmation of data presented earlier in this report.

A more extensive study of selected stabilizing solutions comparing the effectiveness of water and Buffer in reconstituting the dried virus was carried out and the results are summarized in Tables 13 through 15A. These results further confirm the greater stability of the virus reconstituted with both water and Buffer at 4°C over the corresponding virus preparations held at 40°C. There were significant differences in the stability of the virus in the different stabilizing solutions. The virus loss in the virus reconstituted with water and held at 4°C for 12 hours ranged from 0 to 0.51 logs; comparable preparations held at 40°C had virus losses ranging from 1.42 to 3.73 logs. The virus loss in the virus reconstituted with Buffer and held at 4°C for 12 hours ranged from 0 to 0.34 logs; comparable preparations held at 40°C had virus losses ranging from 1.26 to 2.37 logs. The mean $\log_{10}$ virus titer losses are summarized in the following table:

Rehydrating Fluid	Mean PFU/Ml (log 10) Loss	
	40°C	4°C
H_2O	2.20	0.17
Buffer	1.85	0.16

These average figures tend to confirm the results of the preliminary experiment summarized in Tables 12 and 12A in that the Buffer does improve the stability of the virus held at 40°C but has little effect on the virus held at 4°C. Average figures can be misleading, however, as improvement in stability at 40°C with a few of the stabilizing solutions was much greater than the averages of 0.35 logs. For example, stabilizing Solution 37 showed an improvement of 1.46 logs, Solution 29 had an improvement of 1.36 logs and Solution 57 had an improvement of 0.76 logs. It would appear that each combination of stabilizing substances must be evaluated individually and empirically.

One additional study of comparing the effects of Buffer and water reconstitution on stability at 40°C after rehydration was performed. A group of 14 Stabilizing Solutions, selected on the basis of their pH, were held dried at 40°C for five days after which they were reconstituted with either water or Buffer and held an additional 12 hours at 40°C and titrated. Those virus mixtures which had a pH of 6.0 were reconstituted with Buffer while those which had pH values of 6.4 to 7.0 were reconstituted with water. In addition, one stabilizing solution (#66) with a pH of 6.0 was reconstituted with both water and Buffer, held 12 hours at 40°C and 4°C and titrated. The results of this study are summarized in Tables 16 and 16A. These results further confirm that those virus solutions reconstituted with Buffer are more stable than those reconstituted with water. The resulting stability is not satisfactory, however, and factors other than pH appear to be interracting in the various stabilizing solution mixtures.

Gelatin appears to be the most effective single stabilizing substance we have tested. While this gelatin solution becomes acidic upon hydrolysis, we have subsequently noted that the high phosphate buffer required to maintain the pH at neutrality causes a precipitation of the gelatin solution and may alter its stabilization capacity.

While the study reported in Tables 16 and 16A was in progress (it takes two weeks to complete an assay and it is not productive to await the results of each study before starting another study), another study of Stabilizing Solutions with original pH values of 6.0-6.4 with reconstitution with Buffer only was carried out. These dried virus mixtures were held at 40°C for five days after which they were reconstituted with Buffer and held 12 hours at 4°C or 40°C and titrated. A similar test of a production lot of released measles vaccine (Attenuax® Lot 1608V - Expiration Date June 21, 1977) rehydrated with fluid provided by Merck Sharp & Dohme, was included in this study. The results are summarized in Tables 17 and 17A. These results further indicate that adjustment of the pH with the high phosphate buffer in reconstitution, under the conditions of this study, will not stabilize the virus adequately through the 12 hour storage at 40°C. The lot of commercial Measles Vaccine tested lost all detectable virus infectivity during the 12 hours at 40°C after rehydration with the fluid provided by the manufacture for reconstitution of the dried vaccine.

It was thought that perhaps physical-chemical changes of the stabilizing mixtures occurred during the freeze-drying process with consequent loss of stabilizing capacity after rehydration and storage at 40°C in the fluid state. A total of 16 virus-stabilizer mixtures were reconstituted with the original stabilizing solution combined with the virus prior to

freeze-drying. These vials were held in duplicate, at 4°C and at 40°C, for 12 hours and titrated. The results are summarized in Table 18. Since the virus mixtures held at 4°C would be presumed to have higher titers than those held at 40°C, they were diluted 1:10 for plaque assay while those held at 40°C were tested undiluted. As the results demonstrate, practically no virus was lost after 12 hours at 4°C. The virus mixtures were toxic to the tissue cultures in the assay system when tested undiluted, therefore, no valid comparative titers after storage at 40°C were obtained in this study.

As noted previously, gelatin appears to be the most effective single stabilizing substance we have tested. Prior to this time, only one concentration of gelatin was evaluated in combination with the various other potential stabilizing substances. This concentration of gelatin (one part of 20% hydrolized gelatin + 4 parts of stabilizer) had been found to be effective in Parke-Davis research programs with other viruses. In order to determine if other gelatin concentrations might be more effective in stabilizing measles virus infectivity, a series of different gelatin concentrations was used in rehydrating selected dried virus preparations in duplicate. These rehydrated virus preparations were then held at refrigerator temperature and at 40°C for 12 hours and were then titrated for infectivity. The results of this study are summarized in Tables 19, 20, 21 and 21A. These results indicate that with the most of the stabilizing solutions evaluated in this comparison, proportions of stabilizer to gelatin of 3+1 and 2+1 are superior to higher concentrations of gelatin, with these two proportions being equivalent.

Since sucrose was tested only at concentrations of 7, 14 and 30%, with 30% appearing to have a greater stabilizing action than the 7% and 14% under the conditions of evaluation, two large freeze-drying runs were made of virus

combined with a range of sucrose concentrations (50%, 40%, 30%, 20% and 10%), with each sucrose concentration containing 1%, 2%, 3%, 4% and 5% sodium glutamate and a range of gelatin concentrations (0, 4+1, 3+1, 2+1, 1+1, 1+2, 1+3 and 1+4). Two hundred separate and distinct mixtures were combined with equal volumes of measles virus and freeze-dried. Table 22 describes these samples. All of these samples were held for 5 days at 40°C in the dried state, were then rehydrated with water and then held for an additional 12 hours at 40°C in the fluid state. The results of titrations of these samples at the beginning and end of storage at 40°C are summarized in Tables 23 through 30. These samples were also tested at 0 time and after 12 hours at 40°C following rehydration (without storage of the dried virus at 40°C) and the results available to date are summarized in Tables 31 through 35. All of the results listed in Tables 23 through 35 are given in Table 36 re-calculated in terms of virus infectivity loss.

In order to make these large amounts of data easier to assimilate, Tables 37 through 42 present the data arranged in an organized comparative fashion. Analyses of the data in these tables in terms of the three methods of storage follow.

1. Stability of dried virus for 5 days at 40°C

   When the results of the 5 separate sodium glutamate dilutions are combined together for an overall comparison of the effects of the different sucrose and gelatin concentrations as shown in Table 37, the range of 20-40% sucrose has greater stabilizing properties than do concentrations of 50% and 10% at gelatin proportions of 4+1 and 3+1 but the 50% sucrose is more effective than the other sucrose concentrations at a gelatin proportion of 2+1. The results in Table 38 in which the individual sodium glutamate results are tabulated confirm that the sucrose range of 20-40%

is most effective. It is interesting to note that the combination of 20% sucrose and 2% glutamate is most effective at a gelatin proportion of 4+1, 30% sucrose and 2% glutamate at gelatin 3+1 and 40% sucrose and 1% glutamate at gelatin 2+1. This suggests that the greater the concentration of gelatin, the more sucrose is required for maximum stabilizing action. All other factors being equal of course, the less material in the vaccine the better from standpoint of solubility and reactivity as well as cost. Since the most stable preparation contained 30% sucrose and a gelatin proportion of 3+1 (a loss of 0.28), this would be the most desirable for a potential vaccine. There does not appear to be a significant difference in the stabilizing properties of the five different sodium glutamate concentrations, therefore it would be advantageous to use 1% or 2% at most.

2. Stability of virus held dried for 5 days at 40°C plus 12 hours at 40°C after rehydration

These results are incomplete but the overall comparison of the effects of the five concentrations of sucrose at eight different gelatin concentrations, as summarized in Table 39, indicate that the stabilizing effect of gelatin is considerable, with extreme lability of the virus in the complete absence of gelatin and with decreasing effectiveness of the stabilizing action as the gelatin concentration increases beyond a proportion of 1+2. The most effective stabilizing range of the gelatin is 4+1 to 1+1 with the greatest stability shown with 20% sucrose and a gelatin proportion of 3+1. The relationship of the concentration of sucrose and gelatin concentrations previously mentioned exists in this study also at gelatin proportion of 4+1, 3+1, 2+1 and 1+1, but it breaks down at the higher gelatin concentrations of 1+2, 1+3 and 1+4 where stabilizing effects are greatly reduced. The results in Table 40 in

which the individual sodium glutamate results are tabulated differ markedly from those presented in Table 38 which apply only to the stability of the dried, non-rehydrated virus samples. The most stable virus preparation (losing 1.61 logs) was that containing 50% sucrose and 2% sodium glutamate with 1+1 gelatin. The preparation containing 50% sucrose and 2% sodium glutamate with 2+1 gelatin was also relatively stable, losing only 1.96 logs. Overall the concentration of sodium glutamate did not seem to be critical. These results suggest that a stabilizer combination that is the most effective in protecting the infectivity of dried measles virus is not the most effective in maintaining the virus infectivity in a fluid state at 40°C.

3. Stability of virus not held at 40°C in the dried state but held at 40°C after rehydration

These results are incomplete also but the overall comparison of the effects of the five sucrose concentrations at eight different gelatin concentrations, as summarized in Table 41, show the same pattern with respect to sucrose-gelatin concentrations previously discussed. It would be premature to reach any conclusions on the data presented in Table 42 in which the individual sodium glutamate results are tabulated because of paucity of data at present. The most stable preparation for which we have results contains 40% sucrose, 1% glutamate and gelatin 2+1, This preparation lost 1.20 logs at 40°C for 12 hours. A good stability was also shown by a number of other samples: 30% sucrose and 4% glutamate plus gelatin 2+1 (-1.30 logs), 40% sucrose and 2% glutamate plus gelatin 2+1 (-1.36 logs), 30% sucrose and 3% glutamate plus gelatin 3+1 (-1.36 logs), with a number of other samples having only slightly greater losses of infectivity. Because these results are incomplete, they cannot yet be compared with those being obtained for the dried virus held 5 days at

40°C, and the dried virus held 5 days at 40°C plus 12 hours at 40°C after rehydration to compare the effectiveness of the various combinations of stabilizers under these different conditions.

As discussed previously, our data suggest that stabilizers which are effective in maintaining the infectivity of measles virus in the dried condition may not be the most effective in protecting virus infectivity inthe fluid state. Perhaps incorporation of substances in the rehydrating fluid will protect virus infectivity in the fluid condition even though they are relatively ineffective in the dried state. Various results have been reported to protect the infectivity of a number of heat-labile viruses by many investigators. The following review of some of the more relevant literature provides the background and rationale for our studies of the stabilizing properties of such salts.

Burnet and McKie (1930) reported that monovalent cations enhanced the inactivation of bacteriophage by heat but that the addition of low concentrations of divalent cations partially prevented this inactivation. Lark and Adams (1953) reported stabilizing effects on bacteriophage of Mg ions at 0.000001 M and of Na ions 0.1 M. They suggested that a dissociable complex was formed by the ions and vulnerable sites on the phage particles, the complex being more heat resistant than the cation-free site. Adams (1949) reported that bacteriophage could be stabilized at 37°C for 24-48 hours with 0.001 M concentrations of many cations (Ba, Ca, Sn, Mg, Mn, Co, Ni, Zm, Cd and Cu). Allisbaugh (1957) reported that hypertonic NaCl, KCl or NH_4Cl in high concentrations (5 to 40%) stabilized smallpox virus to the extent that the virus remains viable about twice as long at 5°C as virus suspended in glycerinated diluent. Wallis and Melnick (1961) reported that the use of high concentrations (one molar) of divalent cations in stabilizing poliovirus Mg^{++} protects poliovirus at 50° for 1-2 hours with

only gradual loss of infectivity over the next 24 hours. Molar Mg^{++} protects poliovirus at room temperature (25°C) for 25 days, and at 37° for over 3 days, without any detectable loss of infectivity. They found that 1 or 2 M NaCl protects poliovirus at 50°C for shorter periods (30-60 minutes) but at 37° it enhances viral inactivation. That inactivation of virus at 50°C proceeds differently from that at 37° has been suggested by Younger (1957).

Wallis and Melnick (1962) subsequently reported that M Mg^{++}, M Ca^{++} or 2M Na^{+} also stabilize ECHO and Coxsackie viruses to heat treatment of 50°C for 1 hour. They found that the myxoviruses (influenza, mumps and parainfluenza), Sindbis virus and polynoma viruses are more sensitive to heating at 50° in molar Mg^{++} than in distilled water. Divalent but not monovalent cations have also been reported to enhance the thermolability of vaccinia, herpes simplex, adeno and SV-40 viruses. Wallis and Melnick (1962) emphasize that while the divalent cations stabilize enteroviruses at all temperatures between 4° and 50°, the monovalent cations are selective in their temperature range. At 50°, M Mg^{++} or Ca^{++} stabilizes enteroviruses for 1 - 3 hours, whereas the stability offered by 2 M Na^{+} begins to fall sharply after 1 hour. At 37°, M Mg^{++} or Ca^{++} stabilizes these viruses for at least 3 days, conversely 2 M Na^{+} enhances their inactivation at this temperature. Wallis and Melnick conclude that only viruses with cubic symmetry, RNA core and free of essential lipids are stabilized by molar magnesium.

Nakamura and Ueno (1964) reported a study on the stabilization of Japanese B Encephalitis (JBE) virus and found that water, 1.0 M Na_2SO_4 and 1.5 M $MgSO_4$ protected JBE virus from inactivation at 50° whereas 0.1 M and 1.0 M NaCl, 0.1 M Na_2SO_4 as well as 0.15 M $MgSO_4$ inactivated the virus. However, the JBE-RNA was more stable in the presence of a high concentration of NaCl than in H_2O and other salts tested. Since there was no indication that divalent cations

had any greater effect than monovalent cations, they suggested that the differences in effect are related to the kind of anion present. This speculation is supported by the data of Wallis, Yang and Melnick (1962) which show that NaCl protects some but not all DNA viruses from thermal inactivation indicating that the anion is the determining factor. Nakamura and Ueno (1964) further suggest that the effect of anions on inactivation of virus is associated with viral surface protein, but not viral ribonucleic acid.

Rhinoviruses are more stable at 56° than are enteroviruses: Dimmock and Tyrrell (1964); Ketler,et al. (1962) and the stability of rhinoviruses is not increased by high concentrations of magnesium chloride as much as the stability of enteroviruses. Dimmock (1967) considered therefore that the thermal inactivation of a representative member of each group should be studied in detail at various temperatures. It was thought possible that the RNA of the virus might be inactivated either by direct thermal damage or by the action of cellular ribonuclease which gains access to the particle by penetrating the virus protein coat. Damage to the protein moiety might prevent adsorbing or uncoating and protein damage was detected by failure to combine with specific neutralizing antibody. Dimmock cited Woese (1960) who pointed out that the activation energy of the reaction was higher when the inactivation occurred at high rather than low temperatures and believed that viruses became noninfectious when their nucleic acid becomes non-infectious and that the nucleic acid is rendered noninfectious at high and at low temperatures. Dimmock feels that this cannot generally be true however, for when Murray Valley encephalitis virus or the picornavirus foot-and-mouth disease virus are inactivated at temperatures of around 55°, the infectivity titer of the RNA extracted from the virus particle may be only slightly

reduced although the infectivity of the heated virus is greatly depressed (Ada and Anderson 1959; Bachrach 1961). On the other hand, Pollard (1953) suggested that infectivity of viruses was inactivated at higher temperatures when some protein component was denatured. Dimmock (1967) found that inactivation of the infectivity of a rhinovirus and a poliovirus between 20° and 60° took place by two thermodynamically different reactions which for the rhinovirus intersected at 39° and for the poliovirus at 44°. With the addition of M $MgCl_2$ to the poliovirus, the intersection temperature was raised to 47°. At temperatures below the intersection, inactivation was associated with relatively small thermodynamic values ("low" temperature inactivation) and above the intersection by much larger values ("high" temperature inactivation). RNA infectivity was thermally inactivated at about the same rate as virus infectivity at "low" temperatures but was far more stable than virus infectivity at "high" temperatures. Poliovirus RNA, but not rhinovirus RNA became susceptible to ribonuclease after the virus was heated at high temperature; M $MgCl_2$ prevented inactivation of RNA infectivity within the virus but not of isolated RNA and therefore was thought to strengthen the structure of the virus capsid. At "low" temperature the antigen associated with virus infectivity was more stable than infectivity, but at "high" temperature it was destroyed at the same or at a slightly slower rate. He concluded that a) rhinovirus and poliovirus infectivity could be inactivated through damage to both the RNA and the protein moieties and that b) the stabilities of these moieties vary with temperatures independently of each other so that c) inactivation at a particular temperature takes place through whichever component is the least stable at that temperature. He thinks that the primary reason for inactivation at high temperature is damage to virus protein and at low temperatures it is damage

to RNA. Both reactions probably take place at all temperatures but one is more important quantitatively than the other at a particular temperature except at the temperature where the two reactions intersect. The effect of M $MgCl_2$ on heat inactivation of the picornavirus HGP (rheovirus) and poliovirus type 1 (LSc) can be incorporated into this hypothesis. There was no evidence of any effect of $MgCl_2$ ions on the stability of viral RNA. There was, however, both direct and indirect evidence that they stabilized viral protein. The only marked effect $MgCl_2$ ions had on unpurified virus was on the high temperature reaction in which protein destruction was believed to be the key event. The protective effect was more marked in the case of the LSc poliovirus in which the protein appeared to be more severly damaged. The decrease in entropy of high temperature inactivation of LSc on the addition of M $MgCl_2$ suggests that possibly the ions increased the bonding between adjacent protein components.

Wallis, Smith and Melnick (1964) reported that the infective titer of reovirus could be increased 4 - 8 times by heating at 37°-55° for 5 - 15 minutes in 2 M $MgCl_2$. Only Mg ions were found to enhance the titer of reovirus at 50°; other divalent cations and NaCl were without effect. The maximum titers at 37°C were attained in 256 minutes, at 45°C in 16 minutes; at 50° in 8 minutes; and at 55° in 2 minutes. After varying periods, depending upon the temperature, the activity of the virus declined. The infectivity of virus held at 55° dropped sharply and was 1 log below the original titer after 64 minutes. The virus held at 50° and 45°C was reduced 0.5 log after 9 hours while the virus held at 37° was still 0.25 log higher than the original titer after 9 hours. The non-$MgCl_2$ containing virus preparations lost infectivity rapidly at all temperatures tested. 1 log of virus was lost in 16 minutes at 55°, 128 minutes at 50°, 256 minutes at 45° and 0.5 log was lost after 9 hours at 37°.

The Mg ions altered the state of the virus in another unusual way so that the virus becomes highly sensitive to subzero temperatures, especially between -20° and -40°, where less than 0.1% of the virus may survive. This inactivation cannot be attributed to ice formation alone (if at all) for at -30° where a 2 M $MgCl_2$ solution does not freeze, only a small fraction of the virus population survives. In its inactivation by heat, reovirus is unlike the enteroviruses, which remain fully active when held at -20° in $MgCl_2$ solution.

The mechanism of this altered response to temperature change is not understood.

Rapp, et al. (1965) reported a study of the effect of temperature on the infectivity of virulent and attenuated strains of measles virus maintained in the absence or presence of $MgCl_2$, $MgSO_4$ and other salts. They found that the infectivity of measles virus in water is rapidly destroyed at 37° and higher temperatures. More than 50% of the infectivity is lost after 1 hour at 25°C and almost 90% loss of infectivity occurs within 24 hours at 4°. Magnesium chloride enhances the inactivation of the virus at all temperatures tested. Addition of either magnesium or sodium sulfate, however, protected the virus against thermal inactivation. The stabilizing effect is demonstrable at temperatures ranging from 4° to 56° but is especially pronounced through 45°. Prolonged storage (up to 6 weeks) of the virulent virus at 4° in 1 M $MgSO_4$ permits retention of substantial infectivity whereas storage at 4° in either water of 1 M $MgCl_2$ results in a loss of infectivity approximately 99% after 2 weeks. Magnesium chloride also enhances inactivation of the attenuated vaccine strain of measles virus. The attenuated virus, however, is strongly protected by magnesium sulfate against thermal inactivation, and retention of infectivity for long periods at 4° seem feasible when the virus is kept in 1 M $MgSO_4$.

Hopkins (1967) and Coria (1972) have reported that infectious bronchitis virus (which is another RNA, ether sensitive myxovirus) is stabilized by $MgSO_4$ at 50° for 80 minutes.

All of these reports suggest that monovalent or cationic ions or perhaps merely SO_4 ions can enhance the thermostability of measles virus. In view of Rapp's promising results with $MgSO_4$ and Na_2SO_4, we felt that first consideration should be given to a study of the stabilizing properties of these two salts. Accordingly, a number of previously dried virus-stabilizer preparations were rehydrated with 1 M $MgSO_4$ and 1 M Na_2SO_4 and held at 4°C and 40°C for 12 hours and titrated. The results of this study are summarized in Table 43. The Na_2SO_4 produced a precipitate in all samples including those without gelatin - the $MgSO_4$ did not produce a visible precipitate. Neither of these salts had any stabilizing affect - at 40°C, the virus lost more infectivity in the presence of these salts than it did in the complete absence of the salts.

Since Nakamura and Ueno (1964) reported that 1 M Na_2SO_4 and 1.5 M $MgSO_4$ protected JBE virus from inactivation at 50°C but 0.1 M Na_2SO_4 and 0.15 M $MgSO_4$ inactivated the virus, we felt that perhaps our poor results with these salts was because because the only concentration we had used (1 M) was not optimal for this measles virus. A series of doubling dilutions of both of these salts ranging from 1.0 M to 0.06 M were evaluated in rehydrating a number of vials of the same dried virus preparation. The results of this study, summarized in Table 44, demonstrate no stabilizing action at any dilution tested. The reason these salts do not show any measles virus stabilization, in contrast to the results published by Rapp, et al., is unknown. Perhaps the salts are ineffective at 40°C although Rapp found them to stabilize at 45°C. Rapp studied the thermal stability of measles virus in relatively pure preparations while the virus preparations we have

studied with these salts contain a complex mixture of additives. Some chemical reactions in this milieu may alter the stabilization which might otherwise operate.

A freeze-dry run of $MgSO_4$ and Na_2SO_4 combined with various other stabilizing substances combined with measles virus was carried out. The solutions used in this study are listed in Table 45. The results of infectivity titration of these samples held dried 5 days at 40°C, held dried 5 days at 40°C plus 12 hours at 40°C after rehydration; and after 12 hours at 40°C following rehydration, with no storage of the dried virus at 40°C are summarized in Tables 46-50. All of these results are expressed as virus lost in Table 51. These results confirm our earlier findings that neither $MgSO_4$ nor Na_2SO_4 have any measles virus stabilizing action under our experimental conditions.

A comparison of the virus infectivity loss of the non-dried samples (freeze-controls) and the dried virus samples rehydrated with water is listed in Table 52. It is interesting to note that with but three exceptions, all of the non-dried samples lost more titer on storage at 40°C for 12 hours than did their non-dried counterparts. This suggests that the drying process may render the virus more heat-labile. The three exceptions noted were with stabilizing solutions 26, 27 and 28. The only thing these three samples have in common is the lack of 1% sodium glutamate. Since those samples containing $MgSO_4$ and $NaSO_4$ with equivalent composition with respect to the 1% sodium glutamate are more stable non-dried than dried at 40°, and since without exception all non-dried samples were more stable at 4°C than were the dried samples, the absence of the sodium glutamate in the three exceptional samples is of questionable significance. This observation of the apparent decreased thermal stability of dried virus when held at elevated temperature after rehydration has never before been reported.

It is becoming increasingly apparent that stabilization of the attenuated measles vaccine virus to meet the specifications required - to lose not more than 1 log infectivity after holding the dried virus for five days at 40°C plus 12 hours at 40°C after reconstitution - is not as simple as it first seemed. We had expected the first phase - stabilization of the dried virus for a comparatively long time - to be the most difficult. We have made significant progress in this aspect of our investigations, however, and have improved the stability of the dried virus significantly. A total of 101 different stabilizing solutions mixed with virus and held at 40°C for five days have had less than 1.0 $\log_{10}$ PFU/ml loss. The best of these stabilizing solutions in this respect lost only 0.27 $\log_{10}$ PFU/ml and many other solutions lost less than 0.5 $\log_{10}$. We have not yet, however, been able to maintain a satisfactory infectivity titer in fluid virus preparations for 12 hours at 40°C. While many of these virus-stabilizer combinations still maintain some virus infectivity after 5 days dried plus 12 hours fluid at 40°C, all of them have lost more than 1.0 $\log_{10}$ PFU/ml in-toto. Our data actually suggest that substances required to maintain infectivity upon freeze drying and subsequent storage in the dried state are ineffective or even deleterious to the virus after it is rehydrated and held for the relatively short time of 12 hours at 40°C. Conversely, it is possible that substances which are not effective in stabilizing measles virus infectivity in drying and in the dried state may have appreciable stabilizing action in the fluid state. To explore this, a study of the influence of a number of different additives to the fluid used in reconstituting the dried virus is indicated. This was touched on in the review of literature relevant to ion stabilization presented earlier in this report. Some possible mechanisms for the stabilizing properties of these ions were discussed. Most authorities seem to agree that thermal damage of viruses occurs by two mechanisms: protein denaturation at high temperature

(43°-61°) and nucleic acid destruction by chemical or enzyme action at lower temperatures. The protective effects of the Mg and Na salts are attributed to an effect on the virus protein, a role presumably played by gelatin sucrose and sodium glutamate as well. If the mechanism of this effect is different, the stabilizing effects may be additive and therefy improve the stability of the virus.

Another approach to the stabilization of measles virus infectivity is the evaluation of chelating agents as measles virus stabilizers. Nakumeura and Ueno (1964) reported that sodium EDTA at concentrations of 0.002% to 0.2% stabilized Japanese encephalitis virus (JBE) RNA either when EDTA was used as a homogenizing solution of infected mouse brain or when it was added to the RNA extracted from virus to which no sodium EDTA had been added. They speculated that the lability of JBE-RNA is not due to contamination of protein which contained RNAase but due to heavy metal contamination of the preparations.

Michalski, et al., 1976 studied the thermal inactivation of rabies virus (an RNA, ether sensitive myxovirus). They found that bovine serum protein components in complex cell culture media stabilized virus at 56°C but at temperatures of $\leq$ 37°, sodium tris (hydroxymethyl)-aminomethane (NT) buffer containing ethylenediamenetetraacetic acid (EDTA) (NTE) was a much more efficient stabilizer of virus infectivity. Chelating agents EDTA and EGTA ethyleneglycol-bis(β-aminoethyl ether) tetraacetic acid were equally efficient in protection of rabies virus infectivity; the effect of each was lost when excess Ca++ was added. Biphasic inactivation curves were observed when the virus was treated at 56° in diluents other than PBS or NT buffer. It was determined that diluents containing serum protein constantly afforded some protection to viruses heated at 56° whereas protein-free diluents did not.

At temperatures of 37° and below, NTE provided a remarkable degree of protection whereas the protein containing diluents based upon complex cell culture medium were totally inefficacious. These results suggested that different mechanisms of inactivation are operative at 56° and at lower physiological temperatures. A similar pattern detectable during heat inactivation of picornaviruses was previously suggested by: 1) observation of two-component inactivation curves at high temperatures as reported by Dimmock (1967) and by Woese (1960); 2) stabilization of infectivity by treatment with 1 M Mg^{++}, preferentially at high temperatures as reported by Dimmock (1967); 3) inactivation of infectivity of virus and nucleic acid at different rates at high temperature but at similar rates at lower temperature (Dimmock 1967); and 4) isolation of virus variants whose stability was increased at high temperature but unchanged at physiological temperatures (Youngner 1957). Dimmock 1967 has suggested that these data are compatable with virus inactivation primarily by protein damage at the higher temperatures and by disruption of nucleic acid at physiological temperatures. The mechanism by which EDTA protects rabies virus against heat inactivation has not been determined. The chelating effect may act by removing cations: 1) that act as necessary cofactors for degradative enzymes, 2) that non-enzymically catalyze decomposition of virus components, or 3) that poison endogenous virus enzymes necessary for replication. Michalski, et al., do not know which ion is critically important. The efficient protective effect of EGTA, which allegedly complexes only Ca^{++} and Ba^{++} suggests that Ca^{++} may be the critical cation. Two contrary observations are: 1) the fact that excess Mg^{++} greatly reduces the protective effect of EDTA despite the fact that Mg^{++} is not capable of displacing Ca^{++} from EDTA, and 2) a slight protective effect of EDTA and EGTA persists in the presence of 5 to 10 fold excess concentrations of Ca^{++}, despite the fact that these chelating agents can complex cations only on an equimolar ratio. The latter observations suggest that the added Ca^{++} and Mg^{++} may eliminate the protective effect of the chelating

agents by displacing some other cation, possibly present only in trace amounts.

Michalski reported that serum added to NT buffer protects rabies virus at physiological temperatures under conditions where serum added to cell culture medium is totally without effect. Serum and EDTA protective effects in NT buffer are not additive. The explanation of these observations is uncertain. It is possible that serum itself exerts a minor chelating effect or that serum and EDTA may act in a similar non-specific virus-complexing manner in simple diluents only. It is apparent that addition of EDTA or EGTA to the virus-suspending medium in minimal concentrations may be expected to stabilize effectively the infectivity of rhabdoviruses undergoing storage for research purposes or for use as live virus vaccines. Addition of serum to NTE is indicated for preparations to be frozen. Cell culture medium is a poor milieu for the manipulation of rabies virus in the laboratory.

Another major approach to develop a more stable measles vaccine may be more productive. This would consist of the development of a system in which the freeze-dried virus is suspended in a water-free metabolizable vehicle for administration without rehydration. Parke-Davis has the basic patent for this (U.S. Patent 3,100,178). We have developed a new form of smallpox vaccine based on this principle (Beardmore, 1973). We have obtained some very encouraging preliminary data, as discussed on pages 8 and 9 of this report and are in the process of evaluating peanut oil under various conditions. Peanut oil does create some problems in the drying process because of the layering of the oil on the surface of the virus-stabilizer mixture with consequent interference with the water removal, and distribution of the virus in a homogenous suspension for uniform dosage. The use of Span and Tween type compounds, along with cholesterol, will be evaluated to improve the dispersion of the dried virus in this oily vehicle. The application of

various concentrations of glycerin will also be studied in this regard. Since glycerine is water-miscible, it would have advantages over peanut oil, if it is effective as a stabilizer under these conditions. Other non-aqueous liquids which have potential in this regard are isopropyl myristate, sesame seed oil and propylene glycol.

REFERENCES

Ada, G. L. and S. G. Anderson 1959. Yield of infective ribonucleic acid from impure Murray Valley encephalitis virus after different treatments. Nature 183, 799-800.

Adams, M. H. 1949. Stability of bacterial viruses in solutions of salts. J. Gen. Physiol. 32, 579.

Allisbough, H. C. 1957. Patent No. 2,787,577. Stable smallpox vaccine. U.S. Patent Office, April 1957.

Bachrach, H. L. 1964. Foot-and-mouth disease virus: structure and mechanism of degradation as deduced from absorbance-temperature relationships. Proc. Soc. Exp. Biol. Med. 95, 147-152.

Barlow, D. F. 1972. The effects of various protecting agents on the inactivation of Foot and Mouth Disease virus in aerosols and during freeze-drying. J. Gen. Virol. 17, 281-288.

Beardmore, W. B. 1973. A new form of smallpox vaccine. J. Infect. Dis. 127, 718-719.

Burnet, F. M. and M. McKie 1930. Balanced salt action as manifested in bacteriophage phenomena. Austral. J. Exp. Biol. Med. Sci. 7, 183.

Coria, M. F. 1972. Stabilizing effect of magnesium sulfate on avian infectious bronchitis virus propagated in chicken embryo kidney cells. Applied Microbiol. 23, 281-284.

Dimmock, N. J. 1967. Differences between the thermal inactivation of Picornaviruses at "High" and "Low" Temperatures. Virology 31, 338-353.

Dimmock, N. J. and D. A. J. Tyrrell 1964. Some physiochemical properties of rhinoviruses. Brit. J. Exp. Pathol. 45, 271-280.

Hopkins, R. S. 1967. Thermal stability of infectious bronchitis virus in the presence of salt solutions. Avian Dis. 11, 261-267.

Ketler, A. V. V. Hamparian and M. R. Hilleman 1962. Characterization and classification of ECHO 28-rhinovirus-coryzavirus agents. Proc. Soc. Exp. Biol. Med. 110, 821-831.

Lark, K. G. and M. H. Adams 1953. The stability of phages as a function of the ionic environment. Symposia on Quantitative Biology 18, 171.

Michalski, F., N. F. Parks, F. Sokol and H. F. Clark 1976. Thermal inactivation of rabies and other Rhabdoviruses: Stabilization by the chelating agent ethylenediaminetetraacetic acid at physiological temperatures. Infection and Immunity 14, 135-143.

Nakamura, M. and Y. Ueno 1964. Infectious ribonucleic acid (RNA) of Japanese B Encephalitis (JBE) Viruses: High yields of RNA and its stabilization. Proc. Soc. Exp. Biol. Med. 117, 700-704.

REFERENCES
(cont'd)

Pollard, E. C. 1953. The Physics of Viruses. Academic Press, New York.

Rapp, F., J. S. Butel and C. Wallis 1965. Protection of measles virus by sulfate ions against thermal inactivation. J. of Bacteriology. 90, 132-135.

Scott, E. M. and W. Woodside 1976. Stability of Pseudorabies virus during freeze-drying and storage: Effect of suspending media. J. Clin. Microbiol. 4, 1-5.

Steele, P. R. M. 1976. Prevention of low temperature denaturation injury in T4Bo phage by low concentrations of traditional cryoprotective additives. J. Hyg. Comb. 76, 453-458.

Turner, G. S. and C. Kaplan 1967. Some properties of fixed rabies virus. J. Gen. Virol. 1, 537-551.

Wallis, C. and J. L. Melnick 1961. Stabilization of poliovirus by cations. Texas Rept. Biol. Med. 19, 683-700.

Wallis, C. and J. L. Melnick 1962. Cationic stabilization - a new property of enteroviruses. Virology 16, 504-525.

Wallis, C., C. Yang and J. L. Melnick 1962. Effect of cations on thermal inactivation of vaccinia, herpes simplex and adenoviruses. J. Immunol. 89, 41-46.

Wallis, C., K. O. Smith and J. L. Melnick 1964. Reovirus activation by heating and inactivation by cooling in $MgCl_2$ solutions. Virology 22, 608-619.

Woese, C. 1960. Thermal inactivation of animal viruses. Ann. N.Y. Acad. Sci. 83, 741-745.

Youngner, J. S. 1957. Thermal inactivation studies with different strains of poliovirus. J. Immunol. 78, 282.

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ADDENDUM

TABLES 1 - 52

TABLE 1

Stabilizing Solution Number	Composition	
1	sucrose	0.218 M (7.4%)
	K_2HPO_4	0.0071 M (0.123 g/100 ml)
	KH_2PO_4	0.00376 M (0.05 g/100 ml)
	sodium glutamate	1%
	human serum albumin	1%
2	One part of hydrolyzed gelatin (20% plus 4 parts of Solution 1	
3	distilled water	800 ml
	KOH	0.548 g
	l-glutamic acid	1.440 g
	K_2HPO_4	2.508 g (0.3135 g/100 ml)
	KH_2PO_4	1.030 g (0.12875 g/100 ml)
	sucrose	30%
4	One part of hydrolized gelatin (20%) plus 4 parts of Solution 3	
5	distilled water	800 ml
	KOH	0.548 g
	l-glutamic acid	1.440 g
	K_2HPO_4	2.508 g
	KH_2PO_4	1.030 g
	human serum albumin	1%
	sucrose	30%
6	One part of hydrolized gelatin (20%) plus 4 parts of Solution 5	
7	distilled water	800 ml
	K_2HPO_4	2.508 g
	KH_2PO_4	1.030 g
	sodium glutamate	1%
	sucrose	30%
8	One part of hydrolized gelatin (20%) plus 4 parts of Solution 7	
9	distilled water	800 ml
	K_2HPO_4	2.508 g
	KH_2PO_4	1.030 g
	sodium glutamate	1%
	human serum albumin	1%
	sucrose	30%
10	One part of hydrolized gelatin (20%) plus 4 parts of Solution 9	
11	distilled water	800 ml
	KOH	0.548 g
	l-glutamic acid	1.440 g
	K_2HPO_4	2.508 g
	KH_2PO_4	1.030 g

TABLF 2

Stabilizing Solution Number	PrU/ml (log 10)			
	Frozen (Not Dried)	Days Dried Virus Held at 40°C		
		0	2	5
1	4.0	3.61	2.69	2.48
2	3.95	3.55	2.84	2.49
3	3.98	3.41	2.17	2.25
4	3.96	3.75	3.28	2.83
5	3.87	3.48	2.79	2.61
6	3.95	3.87	3.06	2.92
7	3.82	3.47	2.57	2.13
8	3.85	3.77	3.21	2.95
9	3.74	3.60	2.91	2.63
10	3.89	3.72	3.06	3.00
11	2.83	1.30	0	0

TABLE 2A

Stabilizing Solution Number (See Table 1)	PFU Ml (log 10) Loss		
	Drying Step	Dried Virus	
		2 Days at 40°C	5 Days at 40°C
1	0.39	0.92	1.13
2	0.40	0.71	1.46
3	0.57	1.24	1.73
4	0.21	0.47	1.13
5	0.39	0.69	1.26
6	0.08	0.81	1.03
7	0.35	0.90	1.34
8	0.08	0.56	0.82
9	0.14	0.69	0.97
10	0.17	0.66	0.72
11	1.53	1.30*	1.30*

* No infective virus detectable

TABLE 3

Stabilizing Solution Number	PFU/Ml (log 10)						
	Frozen (Not Dried	Days Dried Virus Held at 40°C					12 Hours 40°C after Rehydration*
		0	2	3	4	5	
1	4.04	3.10	2.95	2.90	2.72	2.44	0
2	4.06	3.23	3.05	3.22	2.78	2.11	0
6	4.04	3.30	3.35	3.07	3.08	2.94	0
7	4.00	2.57	2.41	2.62	2.50	1.99	0
8	3.89	3.22	2.87	2.54	2.93	2.89	0
10	3.79	3.47	-	-	-	2.69	0

* Preparation reconstituted after storage at 40°C for five days

TABLE 3A

Stabilizing Solution (See Table 1)	PFU/ml (log 10) Loss					
	Drying Step	Days Dried Virus Held @ 40°C				12 Hours 40°C after Rehydration
		2	3	4	5	
1	0.94	0.15	0.20	0.38	0.66	3.10*
2	0.83	0.18	0.01	0.45	1.12	3.23*
6	0.74	0	0.23	0.22	0.36	3.30*
7	1.43	0.16	0.00	0.07	0.58	2.57*
8	0.67	0.35	0.68	0.29	0.33	3.22*
10	0.32	-	-	-	0.78	3.47*

* No infective virus detectable

TABLE 4

STABILIZING SOLUTION

Solution No.	Composition	
1	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
	human serum albumin	1%
2	One part of 20% hydrolized gelatin plus 4 parts of Solution #1	
3	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
	human serum albumin	1%
4	One part 20% hydrolized gelatin plus 4 parts of Solution #3	
5	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
	human serum albumin	1%
6	One part 20% hydrolized gelatin plus four parts Solution #5	
7	sucrose	14%
	K_2HPO_4	0.3135%
	KH_2PO_4	0.12875%
	sodium glutamate	1%
	human serum albumin	1%
8	One part 20% hydrolized gelatin plus four parts Solution #7	
9	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
10	One part 20% hydrolized gelatin plus four parts Solution #9	
11	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	2%
	human serum albumin	1%

12	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	3%
	human serum albumin	1%
13	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	4%
	human serum albumin	1%
14	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	5%
	human serum albumin	1%
15	sucrose	14
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
	human serum albumin	2%
16	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
	human serum albumin	3%
17	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
	human serum albumin	4%
18	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
	human serum albumin	5%
19	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	2%
20	One part 20% hydrolized gelatin plus four parts Solution #19	
21	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	3%

22	One part 20% hydrolized gelatin plus four parts Solution #21	
23	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	4%
24	One part 20% hydrolized gelatin plus four parts Solution #23	
25	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	5%
26	One part 20% hydrolized gelatin plus four parts Solution #25	
27	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	2%
	human serum albumin	2%
28	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	3%
	human serum albumin	3%
29	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	4%
	human serum albumin	4%
30	sucrose	14%
	K_2HPO_4	0.123%
	KH_2PO_4	0.151%
	sodium glutamate	5%
	human serum albumin	5%
31	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
32	One part 20% hydrolized gelatin plus four parts Solution #31	
33	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	2%
	human serum albumin	1%

34	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	3%
	human serum albumin	1%
35	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	4%
	human serum albumin	1%
36	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	5%
	human serum albumin	1%
37	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
	human serum albumin	2%
38	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
	human serum albumin	3%
39	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
	human serum albumin	4%
40	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
	human serum albumin	5%
41	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	2%
42	One part 20% hydrolized gelatin plus four parts Solution #41	
43	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	3%
44	One part 20% hydrolized gelatin plus four parts Solution #43	

45	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	4%
46	One part 20% hydrolized gelatin plus four parts Solution #45	
47	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	5%
48	One part 20% hydrolized gelatin plus four parts Solution #47	
49	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	2%
	human serum albumin	2%
50	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	3%
	human serum albumin	3%
51	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	4%
	human serum albumin	4%
52	sucrose	7%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	5%
	human serum albumin	5%
53	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
54	One part hydrolized gelatin (20%) plus four parts Solution #53	
55	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	2%
	human serum albumin	1%

56	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	3%
	human serum albumin	1%
57	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	4%
	human serum albumin	1%
58	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	5%
	human serum albumin	1%
59	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
	human serum albumin	2%
60	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
	human serum albumin	3%
61	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
	human serum albumin	4%
62	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	1%
	human serum albumin	5%
63	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	2%
64	One part 20% hydrolized gelatin plus four parts Solution #63	
65	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	Sodium glutamate	3%
66	One part 20% hydrolized gelatin plus four parts Solution #65	

67	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	4%
68	One part 20% hydrolized gelatin plus four parts Solution #67	
69	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	5%
70	One part 20% hydrolized gelatin plus four parts Solution #69	
71	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	2%
	human serum albumin	2%
72	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	3%
	human serum albumin	3%
73	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	4%
	human serum albumin	4%
74	sucrose	30%
	K_2HPO_4	0.123%
	KH_2PO_4	0.051%
	sodium glutamate	5%
	human serum albumin	5%

TABLE 5

Stabilizing Solution Number (See Table 4)	PFU/Ml (log 10) Time Held at 40°C		
	Dried		Dried 5 Days Plus 12 Hours After Rehydration
	0 Days	5 Days	
1	3.86	2.92	0
2	4.01	2.62	0
3	3.69	2.96	0
4	3.87	3.13	0
5	3.73	3.07	0
6	4.0	3.25	2.25
7	3.82	2.85	0
8	3.94	3.06	0
9	3.67	3.04	0
10	4.03	3.19	0
11	3.88	3.07	0
12	3.74	3.06	0
13	3.93	2.95	0
14	3.92	2.90	0

Assay Controls

PBS (Frozen, not dired): 3.03

PBS Dried: 0

PBS Dried, 5 days at 40°C: 0

PBS Dried, 12 hours at 40°C after Rehydration: 0

Measles Ref. Std.: 6.01

TABLE 6

Stabilizing Solution Number (See Table 4)	PFU/ml (log 10) Days Dried Virus Held at 40°C	
	0	5
15	3.77	3.00
16	3.83	2.86
17	3.77	2.89
18	3.83	2.95
19	3.87	2.98
20	4.06	3.16
21	3.79	2.49
22	4.06	3.11
23	3.76	2.91
24	3.77	3.10
25	3.57	2.33
26	3.96	2.89
27	3.46	2.71

Assay Controls

PBS (Frozen, not dried): 3.20

PBS Dried: 0

PBS Dried 5 days at 40°C: 0

Measles Ref. Std.: 6.01

TABLE 7

Stabilizing Solution Number (See Table 4)	PFU/Ml (log 10) Time Held at 40°C		
	Dried		Dried 5 Days Plus 12 Hours After Rehydration
	0 Days	5 Days	
28	3.87	2.77	0
29	3.71	2.42	0
30	3.88	2.67	0
31	3.31	1.79	0
32	3.76	2.51	0
33	3.51	2.26	0
34	3.60	2.19	0
35	3.69	2.10	0
36	3.66	1.87	0
37	3.80	2.30	0
38	3.65	2.21	0
39	3.67	2.37	0
40	3.78	2.43	0
41	3.19	1.17	0
42	3.94	2.05	0

Assay Controls

PBS (Frozen, Not Dried): 1.60

PBS Dried: 0.69

PBS Dried, 5 days at 40°C: 0

PBS Dried, 12 Hours at 40°C after Rehydration: 0

Measles Ref. Std.: 5.90

TABLE 8

Stabilizing Solution Number (See Table 4)	PFU/Ml (log 10) Time Held at 40°C		
	Dried		Dried 5 Days Plus 12 Hours After Rehydration
	0 Days	5 Days	
43	3.48	1.65	0
44	3.96	2.44	0
45	3.26	1.95	1.65
46	3.85	2.45	1.87
47	3.33	2.10	0
48	3.81	2.32	0
49	3.72	2.59	0
50	3.75	2.56	0
51	3.75	2.61	0
52	3.64	2.58	0
53	3.41	2.68	0
54	3.94	3.15	0
55	3.56	2.37	0
56	3.59	2.43	0
57	3.64	2.63	0

Assay Controls

PBS (Frozen, not dired): 1.84

PBS Dried: 0

PBS Dried, 5 days at 40°C: 0

PBS Dried, 12 hours at 40°C after Rehydration: 0

Measles Ref. Std.: 5.90

TABLE 9

Stabilizing Solution Number (see Table 4)	PFU/Ml (log 10) Time Held at 40°C		
	Dried		Dried 5 Days Plus 12 Hours After Rehydration
	0 Days	5 Days	
58	3.83	2.79	1.17
59	3.82	2.95	1.11
60	3.90	2.95	1.25
61	3.87	2.90	0
62	3.89	2.73	0
63	3.79	2.71	0
64	3.92	3.23	1.81
65	3.92	3.00	0
66	3.83	3.21	1.11
67	3.74	3.00	0
68	3.75	3.01	1.36
69	3.62	2.61	0
70	3.45	2.95	1.0
71	3.47	2.65	0
72	3.77	2.75	0

Assay Controls

PBS (Frozen, not dried): 3.31

PBS Dried: <1.0

PBS Dried, 5 days at 40°C: 0

PBS Dried, 12 hours at 40°C after Rehydration: 0

Measles Ref. Std.: 5.96

TABLE 9A

SUMMARY OF VIRUS LOSS FROM DATA IN TABLES 5-9

Stabilizing Solution (see Table 4)	PFU/ml (log 10) Loss: Dried 5 Days 40°C	PFU/ml (log 10) Loss: Dried 5 Days 40°C Plus 12 Hours 40°C after Rehydration*
1	0.94	3.86
2	1.39	4.01
3	0.73	3.69
4	0.74	3.87
5	0.66	3.73
6	0.75	1.75**
7	0.97	3.82
8	0.88	3.94
9	0.63	3.67
10	0.84	4.03
11	0.81	3.88
12	0.68	3.74
13	0.98	3.93
14	1.02	3.92
15	0.77	N.D.
16	0.97	N.D.
17	0.88	N.D.
18	0.88	N.D.
19	0.89	N.D.
20	0.90	N.D.
21	1.30	N.D.
22	0.95	N.D.
23	0.85	N.D.
24	0.67	N.D.
25	1.24	N.D.
26	1.07	N.D.
27	0.75	N.D.
28	1.10	3.87
29	1.29	3.71
30	1.21	3.88
31	1.52	3.31
32	1.25	3.76
33	1.25	3.51
34	1.41	3.60
35	1.59	3.69
36	1.79	3.66
37	1.50	3.80
38	1.44	3.65
39	1.30	3.67
40	1.35	3.78
41	2.02	3.19
42	1.89	3.94
43	1.83	3.48
44	1.52	3.92
45	1.31	1.61**
46	1.40	1.98**

TABLE 9A (cont'd)

SUMMARY OF VIRUS LOSS FROM DATA IN TABLES 5 - 9

Stabilizing Solution (see Table 4)	PFU/ml (log 10) Loss: Dried 5 Days 40°C	PFU/ml (log 10) Loss: Dried 5 Days 40°C Plus 12 Hours 40°C after Rehydration*
47	1.23	3.33
48	1.49	3.81
49	1.13	3.72
50	1.19	3.75
51	1.14	3.75
52	1.06	3.64
53	0.73	3.41
54	0.79	3.94
55	1.19	3.56
56	1.16	3.59
57	1.01	3.64
58	1.04	2.66**
59	0.87	2.71**
60	0.95	2.65**
61	0.97	3.87
62	1.16	3.89
63	1.08	3.79
64	0.69	2.11**
65	0.92	3.92
66	0.62	2.72**
67	0.74	3.74
68	0.74	2.39**
69	1.01	3.62
70	0.50	2.45**
71	0.82	3.47
72	1.02	3.77

* Titer indicated is loss of all original infectivity unless indicated otherwise

** Not all virus infectivity lost

TABLE 10

Stabilizing Solution Number (See Table 4)	PFU/Ml (log 10) Time Held at 40°C		
	Dried		
	0 Days	5 Days	12 Hours after Rehydration
73	3.79	-	1.83
74	3.77	-	2.18
3	3.05	2.41	0.69
4	3.46	2.59	1.25
5	3.11	2.84	1.36
9	3.19	2.43	0
13	3.46	2.57	1.0
14	3.07	2.61	0.90
25	3.29	2.15	0
27	3.19	2.43	0

Assay Controls

PBS (Frozen, not died): 2.46

PBS (Dried, 5 days at 40°C): 0

Measles Ref. Std.: 5.88

TABLE 10A

Stabilizing Solution (see Table 4)	PFU/ml (log 10) Loss	
	Dried 5 Days 40°C	12 Hours 40°C after Rehydration
73	N.D.	1.96
74	N.D.	1.59
3	0.64	2.36
4	0.87	2.21
5	0.27	1.75
9	0.76	3.19*
13	0.89	2.46
14	0.46	2.17
25	1.14	3.29*
27	0.76	3.19*

* No infective virus detectable

Table 11

pH of Stabilizing Solutions

Solutions Listed in Table 1		Solutions Listed in Table 4			
Solution Numbers	pH	Solution Numbers	pH	Solution Numbers	pH
1	6.8	1	7.0	38	6.8
2	6.0	2	6.0	39	6.8
3	6.8	3	6.8	40	6.8
4	6.0	4	6.0	41	6.8
5	6.4	5	6.8	42	6.0
6	6.0	6	6.0	43	7.0
7	7.0	7	6.8	44	6.0
8	6.0	8	6.0	45	7.0
9	6.8	9	7.0	46	6.0
10	6.0	10	6.0	47	7.0
11	7.0	11	6.8	48	6.0
		12	6.8	49	6.8
		13	6.4	50	6.8
		14	6.8	51	6.8
		15	6.8	52	6.8
		16	7.0	53	7.0
		17	7.0	54	6.0
		18	6.8	55	6.8
		19	6.8	56	6.4
		20	6.0	57	6.4
		21	6.4	58	6.8
		22	6.0	59	6.8
		23	6.4	60	6.8
		24	6.0	61	6.8
		25	7.0	62	6.8
		26	6.0	63	7.0
		27	6.8	64	6.0
		28	6.8	65	6.8
		29	6.4	66	6.0
		30	6.8	67	7.0
		31	7.2	68	6.8
		32	6.0	69	6.8
		33	6.8	70	6.0
		34	6.8	71	6.4
		35	7.0	72	6.8
		36	6.4	73	6.8
		37	6.4	74	6.4

TABLE 12

Stabilizing Solution Number (See Table 4)	Rehydrating Fluid	PFU/ml (log 10) Dried 0 Days 40°C	Dried 5 Days 40°C Plus 12 Hours 40°C after Rehydration	Dried 5 Days 40°C Plus 12 Hours 4°C after Rehydration
6	H_2O	3.78	0.90	2.64
	Buffer	-	1.30	2.65
8	H_2O	3.56	0.69	2.67
	Buffer	-	1.11	-
10	H_2O	3.61	1.0	2.73
	Buffer	-	1.30	2.80

Assay Controls

PBS (frozen, not dried): 2.45

Measles Ref. Std.: 5.88

TABLE 12A

Stabilizing Solution (See Table 4)	Rehydrating Fluid	PFU/Ml (log 10) Loss Held 5 Days at 40°C Dried Plus an Additional 12 Hours after Rehydration at 40°C	4°C
6	H_2O	2.88	1.14
	Buffer	2.48	1.13
8	H_2O	2.87	0.89
	Buffer	2.45	-
10	H_2O	2.61	0.88
	Buffer	2.31	0.81

TABLE 13

Stabilizing Solution Number (See Table 4)	Rehydrating Fluid	PFU/ml (log 10) Dried 0 Time at 40°C	Rehydrated virus Held 12 Hours at 40°C	4°C
2	H_2O	3.86	1.63	3.74
	Buffer	3.64	1.30	3.58
4	H_2O	3.90	1.60	3.72
	Buffer	3.75	1.81	3.56
6	H_2O	3.82	1.68	3.46
	Buffer	3.79	2.10	3.49
8	H_2O	3.66	1.0	3.58
	Buffer	3.69	1.36	3.61
10	H_2O	3.71	1.51	3.54
	Buffer	3.68	1.51	3.43
13	H_2O	3.68	0.90	3.52
	Buffer	3.68	1.0	3.55
20	H_2O	3.85	1.84	3.67
	Buffer	3.71	1.47	3.51
21	H_2O	3.71	1.51	3.61
	Buffer	3.58	1.63	3.36

Assay Control

Measles Ref. Std.: 6.03

TABLE 14

Stabilizing Solution Number (See Table 4)	Rehydrating Fluid	PFU/ml (log 10) Dried 0 Time at 40°C	Rehydrated Virus Held 12 Hours at 40°C	Rehydrated Virus Held 12 Hours at 4°C
22	H_2O	3.95	2.05	3.82
	Buffer	3.90	2.40	3.83
23	H_2O	3.70	1.54	3.66
	Buffer	3.74	1.92	3.65
24	H_2O	3.87	2.11	3.78
	Buffer	3.90	2.44	3.77
26	H_2O	3.70	1.74	3.68
	Buffer	3.69	2.07	3.65
29	H_2O	3.73	0	3.64
	Buffer	3.73	1.36	3.51
32	H_2O	3.55	1.83	3.41
	Buffer	3.47	1.69	3.42
36	H_2O	3.47	0.90	3.40
	Buffer	3.62	1.51	3.29
37	H_2O	3.45	0	3.24
	Buffer	3.50	1.51	3.23

Assay Control

Measles Ref. Std.: 5.98

TABLE 15

Stabilizing Solution Number (See Table 4)	Rehydrating Fluid	PFU/Ml (log 10) Dried 0 Time at 40°C	Rehydrated Virus Held 12 Hours at 40°C	Rehydrated Virus Held 12 Hours at 4°C
42	H_2O	3.70	1.87	3.52
	Buffer	3.82	2.30	3.60
44	H_2O	3.77	1.72	3.47
	Buffer	3.71	2.07	3.69
46	H_2O	3.51	2.09	3.60
	Buffer	3.59	2.30	3.63
48	H_2O	3.71	2.07	3.64
	Buffer	3.66	2.40	3.45
54	H_2O	3.76	2.17	3.52
	Buffer	3.68	1.92	3.62
56	H_2O	3.71	1.83	3.27
	Buffer	3.58	2.12	3.40
57	H_2O	3.73	1.60	3.22
	Buffer	3.54	2.17	3.42
64	H_2O	3.56	1.11	3.40
	Buffer	3.57	1.36	3.23

Assay Control

Measles Ref. Std.: 5.85

TABLE 15A

SUMMARY OF VIRUS LOSS FROM TABLES 13 - 15

Stabilizing Solution (see Table 4)	Rehydrating Fluid	PFU/Ml (log 10) Loss Rehydrated Virus Held 12 Hours at 40°C	4°C
2	H_2O	2.23	0.12
	Buffer	2.34	0.06
4	H_2O	2.30	0.18
	Buffer	1.94	0.19
6	H_2O	2.14	0.36
	Buffer	1.69	0.30
8	H_2O	2.66	0.08
	Buffer	2.33	0.08
10	H_2O	2.20	0.17
	Buffer	2.17	0.25
13	H_2O	2.78	0.16
	Buffer	2.68	0.13
20	H_2O	2.01	0.18
	Buffer	2.24	0.20
21	H_2O	2.20	0.10
	Buffer	1.95	0.22
22	H_2O	1.90	0.13
	Buffer	1.50	0.07
23	H_2O	2.16	0.04
	Buffer	1.82	0.09
24	H_2O	1.76	0.09
	Buffer	1.46	0.13
26	H_2O	1.96	0.02
	Buffer	1.62	0.04
29	H_2O	3.73*	0.09
	Buffer	2.37	0.22
32	H_2O	1.72	0.14
	Buffer	1.78	0.05
36	H_2O	2.57	0.07
	Buffer	2.11	0.33
37	H_2O	3.45*	0.21
	Buffer	1.99	0.27

* No detectable infective virus

TABLE 15A (cont'd)

SUMMARY OF VIRUS LOSS FROM TABLES 13-15

Stabilizing Solution (see Table 4)	Rehydrating Fluid	PFU/Ml (log 10) loss Rehydrated Virus Held 12 Hours at 40°C	4°C
42	H_2O	1.83	0.18
	Buffer	1.52	0.22
44	H_2O	2.05	0.30
	Buffer	1.64	0.02
46	H_2O	1.42	0
	Buffer	1.29	0
48	H_2O	1.64	0.07
	Buffer	1.26	0.21
54	H_2O	1.59	0.24
	Buffer	1.76	0.06
56	H_2O	1.88	0.44
	Buffer	1.46	0.18
57	H_2O	2.13	0.51
	Buffer	1.37	0.12
64	H_2O	2.45	0.16
	Buffer	2.21	0.34

TABLE 16

Stabilizing Solution Number (See Table 4)	Rehydrating Fluid	PFU/Ml (log 10) Dried 0 Time at 40°C	Dried 5 Days at 40°C	Dried 5 Days at 40°C Plus 12 Hours 40°C after Rehydration
3	H_2O	3.74	2.54	0.47
4	Buffer	3.79	2.56	0.69
5	H_2O	3.59	2.53	0
6	Buffer	3.85	2.98	1.39
9	H_2O	3.51	2.35	0
10	Buffer	3.90	2.77	0.69
12	H_2O	3.71	2.44	0
15	H_2O	3.32	2.76	0
18	H_2O	3.55	2.60	0
19	H_2O	3.50	2.40	0
20	Buffer	3.91	2.85	0.69
23	H_2O	3.54	2.23	0.47
24	Buffer	3.63	2.79	1.17
27	H_2O	3.27	2.30	0

			Dried Virus Not Held at 40°C Rehydrated Virus Held at	
			40°C	4°C
66	H_2O	3.36	1.79	2.91
	Buffer	3.35	1.11	2.85

Assay Control

Measles Ref. Std.: 6.02

TABLE 16A

Stabilizing Solution	Original pH	Rehydrating Fluid	PFU/Ml (Log 10) Loss: Dried 5 Days 40°C	PFU/Ml (Log 10) Loss: Dried - 5 Days 40°C Plus 12 Hours 40°C after Reconstitution
3	6.8	H_2O	1.20	3.27
4	6.0	Buffer	1.23	3.10
5	6.8	H_2O	1.06	3.59*
6	6.0	Buffer	0.87	2.46
9	7.0	H_2O	1.16	3.51*
10	6.0	Buffer	1.13	3.21
12	6.8	H_2O	1.27	3.71*
15	6.8	H_2O	0.56	3.32*
18	6.8	H_2O	0.95	3.55*
19	6.8	H_2O	1.10	3.50*
20	6.0	Buffer	1.06	3.22
23	6.4	H_2O	1.31	3.07
24	6.0	Buffer	0.84	2.46
27	6.8	H_2O	0.97	3.27*

			PFU/Ml (log 10) Loss after 12 Hours at 40°C	PFU/Ml (log 10) Loss after 12 Hours at 4°C
66	6.0	H_2O	1.57	0.45
66	6.0	Buffer	2.24	0.50

* No infective virus detectable

TABLE 17

Stabilizing Solution Number (See Table 4)	PFU/Ml (log 10)* Dried 0 Time at 40°C	Dried 5 Days at 40°C	Dried 5 Days at 40°C Plus 12 Hours 40°C after Rehydration	Dried 5 Days at 40°C Plus 12 Hours 4°C after Rehydration
4	3.78	2.29	0.90	1.76
8	3.70	1.86	1.17	1.76
10	3.58	2.39	1.0	1.94
20	3.59	1.94	0.69	1.77
23	3.57	1.11	Sloughed	1.74
46	3.51	1.36	0	1.25
54	3.62	2.27	1.30	2.50
64	3.44	1.87	0.47	2.44
66	3.28	2.09	0.90	2.03
70	3.44	2.18	0.47	1.94
71	3.54	1.65	Sloughed	1.63
Attenuax® Lot 1608V (Exp. date June 21, 1977)	2.75	1.89	0	Not Done

Assay Control

Measles Ref. Std.: 5.87

* All samples rehydrated with buffer except the Merck Sharp & Dohme vaccine which was rehydrated with fluid provided by Merck for this purpose

TABLE 17A

Stabilizing Solution	Original pH	PFU/Ml (log 10) Loss: Dried 5 Days at 40°C	Held 5 Days at 40°C Dried Plus an Additional 12 Hours after Rehydration at 40°C	4°C
4	6.0	1.49	2.88	2.02
8	6.0	1.84	2.53	1.94
10	6.0	1.19	2.58	1.64
20	6.0	1.65	2.90	1.82
23	6.4	2.46	-	1.83
46	6.0	2.15	3.51*	2.26
54	6.0	1.35	2.32	1.12
64	6.0	1.57	2.97	1.00
66	6.0	1.19	2.38	1.25
70	6.0	1.26	2.97	1.50
71	6.4	1.89	-	1.91
Attenuax® Lot 1608V	Not Indicated or Determined	0.86	2.75*	N.D.

* No infective virus detectable

TABLE 18

Stabilizing Solution Number (See Table 4)	Dried* 0 Time at 40°C	12 Hours at Temperature after Rehydration: Rehydrating Fluid	PFU/ml 4°C	PFU/ml 40°C
2	3.82	2	3.55	1.87
5	3.69	5	3.52	1.17**
13	3.62	13	3.52	Sloughed
14	3.70	14	3.41	Sloughed
21	3.66	21	3.43	Sloughed
45	2.94	45	2.72	0**
46	3.65	46	3.45	2.02
58	3.55	58	3.52	Sloughed
59	3.48	59	3.48	Sloughed
60	3.53	60	3.41	Sloughed
64	3.53	64	3.23	Sloughed
66	3.45	66	3.21	Sloughed
68	3.52	68	3.39	Sloughed
70	3.46	70	3.18	Sloughed
73	3.45	73	3.43	1.06**
74	3.59	74	3.55	Sloughed

Assay Control

Measles Ref. Std.: 5.94

* All samples assayed at 0 time 40°C were rehydrated with H_2O

** Cell toxicity; approximately 75% cell sheet destroyed

TABLE 19

Stabilizing Solution (See Table 4)	Proportion Stabilizing Solution to 20% Hydrolized Gelatin Adjusted to pH 7.4	pH	PFU/ml (log 10) 18 Hours at 4°C	PFU/ml (log 10) 6 Hours at 4°C plus 12 Hours at 40°C
PBS	3+1	6.8	3.26	0.90
	2+1	7.0	3.14	1.30
	1+1	7.0	3.13	0
	1+2	7.2	3.23	0
	1+3	7.2	3.10	0
High phosphate buffered solution (0.615% K_2HPO_4 + 0.255% KH_2PO_4)	3+1	6.8	2.94	2.00
	2+1	6.8	2.91	<1.00
	1+1	7.0	3.19	<1.00
	1+2	7.0	2.79	<1.00
	1+3	7.0	2.99	1.90
9	3+1	6.8	3.57	<1.00
	2+1	7.0	3.30	<1.00
	1+1	7.2	3.46	<1.00
	1+2	7.2	3.30	<1.00
	1+3	7.2	3.37	<1.00
19	3+1	6.8	3.60	2.30
	2+1	6.8	3.52	1.90
	1+1	7.0	3.49	2.36
	1+2	7.0	3.49	1.47
	1+3	7.0	3.42	1.90
21	3+1	7.0	3.50	1.90
	2+1	7.0	3.51	1.90
	1+1	7.0	3.54	1.47
	1+2	7.2	3.41	1.90
	1+3	7.2	3.33	<1.00

Assay Control

Measles Ref. Std.: 6.05

TABLE 20

Stabilizing Solution (See Table 4)	Proportion Stabilizing Solution to 20% Hydrolized Gelatin Adjusted to pH 7.4	pH	PFU/ml (log 10) 18 hours at 4°C	PFU/ml (log 10) 6 Hours at 4°C plus 12 Hours at 40°C
23	3+1	6.8	3.55	2.36
	2+1	6.8	3.50	2.30
	1+1	7.0	3.48	1.69
	1+2	7.2	3.64	1.47
	1+3	7.2	3.67	1.47
25	3+1	6.4	3.55	1.90
	2+1	6.8	3.54	1.47
	1+1	6.8	3.49	1.47
	1+2	7.0	3.40	<1.03
	1+3	7.0	3.46	<1.00
31	3+1	6.4	3.40	<1.00
	2+1	6.8	3.23	<1.00
	1+1	6.8	3.44	1.69
	1+2	7.0	3.41	<1.00
	1+3	7.2	3.41	<1.00
41	3+1	6.8	3.52	<1.00
	2+1	7.0	3.44	<1.00
	1+1	6.8	3.28	<1.00
	1+2	7.0	3.28	<1.00
	1+3	7.2	3.36	<1.00
43	3+1	6.8	3.31	<1.00
	2+1	6.8	3.45	<1.00
	1+1	7.0	3.47	1.47
	1+2	7.2	3.33	<1.00
	1+3	7.2	3.30	<1.00

Assay Control

Measles Ref. Std.: 6.02

TABLE 21

Stabilizing Solution (See Table 4)	Proportion Stabilizing Solution to 20% Hydrolized Gelatin Adjusted to pH 7.4	pH	PFU/ml (log 10) 18 Hours at 4°C	PFU/ml (log 10) 6 Hours at 4°C plus 12 Hours at 40°C
45	3+1	6.4	3.47	1.36
	2+1	6.8	3.48	1.25
	1+1	6.8	3.59	<1.00
	1+2	7.2	3.46	0.00
	1+3	7.2	3.47	0.00
47	3+1	6.4	3.35	1.54
	2+1	6.4	3.56	1.25
	1+1	6.8	3.48	1.25
	1+2	7.0	3.57	1.11
	1+3	7.2	3.57	0.47
53	3+1	6.8	3.40	0.00
	2+1	7.0	3.49	1.65
	1+1	6.8	3.27	0.00
	1+2	7.0	3.37	0.00
	1+3	7.2	3.38	0.00
63	3+1	6.4	3.43	1.39
	2+1	6.8	3.49	1.69
	1+1	7.0	3.47	0.00
	1+2	7.2	3.28	1.00
	1+3	7.0	3.31	0.47
65	3+1	6.8	3.40	1.72
	2+1	6.8	3.35	1.63
	1+1	6.8	3.46	1.00
	1+2	7.0	3.30	0.00
	1+3	7.2	3.34	0.00
67	3+1	6.4	3.39	1.65
	2+1	7.0	3.40	1.39
	1+1	6.8	3.38	0.90
	1+2	7.2	3.37	1.00
	1+3	7.2	3.44	1.00
69	3+1	6.4	3.41	1.74
	2+1	7.0	3.29	1.63
	1+1	6.8	3.35	1.44
	1+2	7.2	3.37	1.47
	1+3	7.2	3.48	1.39

Assay Control

Measles Ref. Std.: 6.03

TABLE 21A

Loss in Titer of Rehydrated Virus Held 12 Hours at 40°C
in Comparison with Rehydrated Virus Held Only at Refrigerature Temperature
(Calculated from Data Presented in Tables 19, 20 and 21)

Stabilizing Solution (See Table 4)	Proportion stabilizing solution to 20% hydrolized gelatin adjusted to pH 7.4	PFU/ml (log 10) Loss in titer of rehydrated virus held 12 hours at 40°C in comparison with refrigerated virus
PBS	3+1	2.36
	2+1	1.84
	1+1	3.13*
	1+2	3.23*
	1+3	3.10*
High phosphate buffered solution (0.615% K_2HPO_4 + 0.255% KH_2PO_4)	3+1	0.94
	2+1	2.91*
	1+1	3.19*
	1+2	2.79*
	1+3	2.09
9	3+1	3.57*
	2+1	3.30*
	1+1	3.46*
	1+2	3.30*
	1+3	3.37*
19	3+1	1.30
	2+1	1.62
	1+1	1.13
	1+2	2.02
	1+3	1.52
21	3+1	1.60
	2+1	1.61
	1+1	2.07
	1+2	1.51
	1+3	3.33*
23	3+1	1.19
	2+1	1.20
	1+1	1.79
	1+2	2.17
	1+3	2.20
25	3+1	1.65
	2+1	2.07
	1+1	2.02
	1+2	3.40*
	1+3	3.46*

TABLE 21A
(cont'd)

Stabilizing Solution (See Table 4)	Proportion stabilizing solution to 20% hydrolized gelatin adjusted to pH 7.4	PFU/ml (log 10) Loss in titer of rehydrated virus held 12 hours at 40°C in comparison with refrigerated virus
31	3+1	3.40*
	2+1	3.23*
	1+1	1.75
	1+2	3.41*
	1+3	3.41*
41	3+1	3.52*
	2+1	3.44*
	1+1	3.28*
	1+2	3.28*
	1+3	3.36*
43	3+1	3.31*
	2+1	3.45*
	1+1	2.00
	1+2	3.33*
	1+3	3.30*
45	3+1	2.11
	2+1	2.23
	1+1	3.59*
	1+2	3.46*
	1+3	3.47*
47	3+1	1.81
	2+1	2.31
	1+1	2.23
	1+2	2.46
	1+3	3.10
53	3+1	3.40*
	2+1	1.84
	1+1	3.27*
	1+2	3.37*
	1+3	3.38*
63	3+1	2.04
	2+1	1.80
	1+1	3.47*
	1+2	2.28
	1+3	2.84
65	3+1	1.78
	2+1	1.72
	1+1	2.46
	1+2	3.30*
	1+3	3.34*

TABLE 21A
(cont'd)

Stabilizing Solution (See Table 4)	Proportion stabilizing solution to 20% hydrolized gelatin adjusted to pH 7.4	PFU/ml (log 10) Loss in titer of rehydrated virus held 12 hours at 40°C in comparison with refrigerated virus
67	3+1	1.74
	2+1	2.01
	1+1	2.48
	1+2	2.37
	1+3	2.44
69	3+1	1.67
	2+1	1.66
	1+1	1.91
	1+2	1.90
	1+3	2.09

* No infective virus detectable

TABLE 22

Stabilizing Solutions
(All solutions contain 0.31% K_2HPO_4 and 0.129% KH_2PO_4)

Solution No.	Sucrose (Percent)	Sodium Glutamate (Percent)	20% Hydrolized Gelatin (Proportion of Sucrose - Sodium Glutamate - PO_4 Buffered Solution to Gelatin)
1	50	1	0
2	50	2	0
3	50	3	0
4	50	4	0
5	50	5	0
6	50	1	4+1
7	50	2	4+1
8	50	3	4+1
9	50	4	4+1
10	50	5	4+1
11	50	1	3+1
12	50	2	3+1
13	50	3	3+1
14	50	4	3+1
15	50	5	3+1
16	50	1	2+1
17	50	2	2+1
18	50	3	2+1
19	50	4	2+1
20	50	5	2+1
21	50	1	1+1
22	50	2	1+1
23	50	3	1+1
24	50	4	1+1
25	50	5	1+1
26	50	1	1+2
27	50	2	1+2
28	50	3	1+2
29	50	4	1+2
30	50	5	1+2
31	50	1	1+3
32	50	2	1+3
33	50	3	1+3
34	50	4	1+3
35	50	5	1+3
36	50	1	1+4
37	50	2	1+4
38	50	3	1+4
39	50	4	1+4
40	50	5	1+4
41	40	1	0
42	40	2	0
43	40	3	0
44	40	4	0
45	40	5	0

Solution No.	Sucrose (Percent)	Sodium Glutamate (Percent)	20% Hydrolized Gelatin (Proportion of Sucrose - Sodium Glutamate - PO_4 Buffered Solution to Gelatin)
46	40	1	4+1
47	40	2	4+1
48	40	3	4+1
49	40	4	4+1
50	40	5	4+1
51	40	1	3+1
52	40	2	3+1
53	40	3	3+1
54	40	4	3+1
55	40	5	3+1
56	40	1	2+1
57	40	2	2+1
58	40	3	2+1
59	40	4	2+1
60	40	5	2+1
61	40	1	1+1
62	40	2	1+1
63	40	3	1+1
64	40	4	1+1
65	40	5	1+1
66	40	1	1+2
67	40	2	1+2
68	40	3	1+2
69	40	4	1+2
70	40	5	1+2
71	40	1	1+3
72	40	2	1+3
73	40	3	1+3
74	40	4	1+3
75	40	5	1+3
76	40	1	1+4
77	40	2	1+4
78	40	3	1+4
79	40	4	1+4
80	40	5	1+4
81	30	1	0
82	30	2	0
83	30	3	0
84	30	4	0
85	30	5	0
86	30	1	4+1
87	30	2	4+1
88	30	3	4+1
89	30	4	4+1
90	30	5	4+1

Solution No.	Sucrose (Percent)	Sodium Glutamate (Percent)	20% Hydrolized Gelatin (Proportion of Sucrose - Sodium Glutamate - PO_4 Buffered Solution to Gelatin)
91	30	1	3+1
92	30	2	3+1
93	30	3	3+1
94	30	4	3+1
95	30	5	3+1
96	30	1	2+1
97	30	2	2+1
98	30	3	2+1
99	30	4	2+1
100	30	5	2+1
101	30	1	1+1
102	30	2	1+1
103	30	3	1+1
104	30	4	1+1
105	30	5	1+1
106	30	1	1+2
107	30	2	1+2
108	30	3	1+2
109	30	4	1+2
110	30	5	1+2
111	30	1	1+3
112	30	2	1+3
113	30	3	1+3
114	30	4	1+3
115	30	5	1+3
116	30	1	1+4
117	30	2	1+4
118	30	3	1+4
119	30	4	1+4
120	30	5	1+4
121	20	1	0
122	20	2	0
123	20	3	0
124	20	4	0
125	20	5	0
126	20	1	4+1
127	20	2	4+1
128	20	3	4+1
129	20	4	4+1
130	20	5	4+1
131	20	1	3+1
132	20	2	3+1
133	20	3	3+1
134	20	4	3+1
135	20	5	3+1
136	20	1	2+1
137	20	2	2+1
138	20	3	2+1
139	20	5	2+1
140	20	5	2+1

Solution No.	Sucrose (Percent)	Sodium Glutamate (Percent)	20% Hydrolized Gelatin (Proportion of Sucrose - Sodium Glutamate - PO_4 Buffered Solution to Gelatin)
141	20	1	1+1
142	20	2	1+1
143	20	3	1+1
144	20	4	1+1
145	20	5	1+1
146	20	1	1+2
147	20	2	1+2
148	20	3	1+2
149	20	4	1+2
150	20	5	1+2
151	20	1	1+3
152	20	2	1+3
153	20	3	1+3
154	20	4	1+3
155	20	5	1+3
156	20	1	1+4
157	20	2	1+4
158	20	3	1+4
159	20	4	1+4
160	20	5	1+4
161	10	1	0
162	10	2	0
163	10	3	0
164	10	4	0
165	10	5	0
166	10	1	4+1
167	10	2	4+1
168	10	3	4+1
169	10	4	4+1
170	10	5	4+1
171	10	1	3+1
172	10	2	3+1
173	10	3	3+1
174	10	4	3+1
175	10	5	3+1
176	10	1	2+1
177	10	2	2+1
178	10	3	2+1
179	10	4	2+1
180	10	5	2+1
181	10	1	1+1
182	10	2	1+1
183	10	3	1+1
184	10	4	1+1
185	10	5	1+1
186	10	1	1+2
187	10	2	1+2
188	10	3	1+2
189	10	4	1+2
190	10	5	1+2

Solution No.	Sucrose (Percent)	Sodium Glutamate (Percent)	20% Hydrolized Gelatin (Proportion of Sucrose - Sodium Glutamate - PO_4 Buffered Solution to Gelatin)
191	10	1	1+3
192	10	2	1+3
193	10	3	1+3
194	10	4	1+3
195	10	5	1+3
196	10	1	1+4
197	10	2	1+4
198	10	3	1+4
199	10	4	1+4
200	10	5	1+4

TABLE 23

Stabilizing Solution (See Table 22)	PFU/Ml (log 10) Time Held at 40°C	
	0 Days (Dried)	5 Days (Dried) Plus 12 Hours after Rehydration
1	0.00	0.00
2	2.39	0.00
3	2.65	0.00
4	2.81	0.00
5	3.06	0.00
21	2.57	0.00
22	2.51	0.90
23	2.89	0.00
24	2.95	0.00
25	3.01	0.00
26	3.06	0.00
27	2.86	0.00
28	2.97	0.00
29	2.92	0.00
30	2.44	0.00
31	2.39	0.00
32	2.79	0.47
33	2.92	0.00
34	3.76	0.00
35	2.95	0.00
36	2.51	0.00
37	2.44	0.00
38	2.63	0.00
39	2.77	0.00
40	2.47	0.00

Assay Control

Measles Ref. Std.: 5.51

TABLE 24

Stabilizing Solution (See Table 22)	PFU/Ml (log 10) Time Held at 40°C			
	0 Days (Dried)	12 Hours after Rehydration with Water	12 Hours after Rehydration with S.M. 199	5 Days (Dried) Plus 12 Hours after Rehydration with Water
81	3.06	0.47	0.47	0.00
82	3.34			0.00
83	3.35			0.00
84	3.28			1.36
85	3.18	1.30	0.00	0.00
101	3.30			0.90
102	3.50			0.69
103	3.17			1.39
104	3.48			0.47
105	3.18	0.47	1.00	0.47
106	3.27	1.44	0.69	0.00
107	3.38			1.36
108	3.32			0.69
109	3.23			0.69
110	3.32	0.00	1.36	0.00
111	3.03	0.47	1.39	0.00
112	3.07			0.00
113	3.19			0.00
114	3.13			0.00
115	2.72			0.00
116	3.27	0.90	1.30	0.00
117	3.16			0.00
118	3.20			0.00
119	2.65			0.00
120	2.90			0.00

Assay Control

Measles Reference Standard: 5.92

TABLE 25

Stabilizing Solution (See Table 22)	PFU/Ml (log 10) Time Held at 40°C	
	0 Days (Dried)	5 Days (Dried) Plus 12 Hours after Rehydration
41	3.23	0.00
42	3.01	0.00
43	3.19	0.00
44	2.90	0.00
45	3.28	0.00
61	3.48	1.17
62	3.49	0.90
63	3.07	0.47
64	3.29	0.47
65	3.16	1.25
66	3.19	0.00
67	3.27	0.47
68	3.32	0.69
69	2.97	0.00
70	3.27	0.00
71	3.13	0.69
72	3.31	0.00
73	3.34	0.00
74	3.06	0.00
75	3.09	0.69
76	3.32	0.00
77	3.30	0.00
78	3.29	0.00
79	2.92	0.00
80	3.02	0.00
101	N.D.	1.44*
101	N.D.	1.36**

Assay Control

Measles Ref. Std.: 5.94

* rehydrated with Synthetic Mixture 199
** rehydrated with water

TABLE 26

Stabilizing Solution (See Table 22)	PFU/Ml (log 10) Time Held at 40°C		
	Dried		Dried 5 Days Plus 12 Hours after Rehydration
	0 Days	5 Days	
6	3.50	2.00	0.00
7	3.41	2.60	0.90
8	3.60	2.39	0.00
9	3.56	2.57	0.47
10	3.77	2.81	1.17
11	3.58	2.83	0.69
12	3.69	3.03	0.00
13	3.55	2.74	1.39
14	3.69	2.90	1.39
15	3.61	2.69	1.00

Assay Control

Measles Reference Standard: 5.90

TABLE 27

Stabilizing Solution (See Table 22)	PFU/Ml (log 10) Time Held at 40°C		
	Dried		Dried 5 Days Plus 12 Hours after Rehydration
	0 Days	5 Days	
16	3.44	2.72	1.17
17	3.32	2.74	1.36
18	3.38	2.74	1.36
19	3.66	2.83	0.69
20	3.35	2.94	0.00
46	3.48	2.69	0.00
47	3.32	2.36	0.90
48	3.38	2.14	1.00
49	3.42	2.65	0.00
50	3.33	2.46	1.17
51	3.38	2.54	0.00
52	3.33	2.79	1.00
53	3.16	2.63	1.30
54	3.22	2.51	0.00
55	3.29	2.44	1.30
56	3.22	2.74	1.11
57	3.28	2.51	0.00

Assay Control

Measles Reference Standard: 5.85

TABLE 28

Stabilizing Solution (See Table 22)	PFU/Ml (log 10) Time Held at 40°C		
	Dried		Dried 5 Days Plus 12 Hours after Rehydration
	0 Days	5 Days	
58	3.64	3.04	1.39
59	3.49	2.60	0.00
60	3.70	3.07	1.39
86	3.63	2.87	1.39
87	3.70	2.69	1.00
88	3.66	2.91	1.00
89	3.61	2.94	0.47
90	3.65	3.03	1.00
91	3.29	2.68	1.00
92	3.39	3.11	0.00
93	3.67	2.89	1.11
94	3.69	3.06	0.90
95	3.43	3.04	0.00
96	3.38	2.44	0.00
97	3.52	2.25	0.90
98	3.51	2.90	1.11

Assay Control

Measles Reference Standard: 5.90

TABLE 29

Stabilizing Solution (See Table 22)	PFU/Ml (log 10) Time Held at 40°C		
	Dried		Dried 5 Days Plus 12 Hours after Rehydration
	0 Days	5 Days	
99	3.40	2.86	1.11
100	3.64	2.83	0.00
126	3.41	3.06	0.47
127	3.32	2.99	0.90
128	3.37	2.39	0.69
129	3.50	2.81	1.00
130	3.41	2.51	0.90
131	3.41	2.68	0.47
132	3.38	2.47	0.69
133	3.18	2.74	1.17
134	3.45	2.76	1.25
135	3.38	2.90	1.00
136	3.30	2.30	0.00
137	3.26	2.47	0.47
138	3.13	1.47	0.00
139	3.23	2.17	0.00
140	3.08	2.47	0.69

Assay Control

Measles Reference Standard: 5.82

TABLE 30

Stabilizing Solution (See Table 22)	PFU/Ml (log 10) Time Held at 40°C		
	Dried		Dried 5 Days Plus 12 Hours after Rehydration
	0 Days	5 Days	
166	3.47	2.00	0.00
167	3.45	2.11	0.00
168	3.41	1.69	0.00
169	3.38	1.69	0.00
170	3.45	2.17	0.00
171	3.39	2.00	0.00
172	3.15	2.39	0.00
173	3.26	<1.00	0.90
174	3.30	<1.00	0.00
175	3.25	2.11	0.00
176	3.23	1.47	0.00
177	2.94	1.47	0.00
178	2.90	<1.00	0.00
179	2.74	1.69	0.00
180	3.00	<1.00	0.00

Assay Control

Measles Reference Standard: 6.27

TABLE 31

Stabilizing Solution (See Table 22)	PFU/Ml (log 10) 0 Days (Dried)	12 Hours at 4°C after Rehydration	12 Hours at 40°C after Rehydration
121	3.06		0.00
122	<1.00		0.00
123	3.29		0.00
124	3.35		0.00
125	2.30		Toxic
141	3.23		0.90
142	3.27		1.11
143	3.35		1.11
144	3.23		0.00
145	3.07		1.11
146	2.83		0.69
147	3.07		0.90
148	2.92		1.47
149	3.04		0.90
150	3.26		0.69
151	3.27		1.11
152	3.16		0.69
153	2.96		1.00
154	2.97		0.90
155	2.81		1.25
9*	3.21	3.07	0.00
9*	3.03**	3.03**	2.30**

Assay Control

Measles Reference Standard: 6.73

* See Table 4 for description of stabilizing solution
** Dried virus reconstituted with peanut oil

TABLE 32

Stabilizing Solution (See Table 22)	PFU/Ml (log 10) 0 Days (Dried)	PFU/Ml (log 10) 12 Hours at 40°C after Rehydration
6	3.58	0.00
7	3.58	0.00
8	3.60	1.25
9	3.70	1.11
10	3.70	1.17
11	3.71	1.11
12	3.65	0.00
13	3.66	0.47
14	3.70	1.44
15	3.69	1.00
16	3.64	0.00
17	3.60	1.39
18	3.73	1.00
19	3.64	1.17
20	3.71	1.47
46	3.64	0.69
47	3.65	0.00
48	3.66	0.47
49	3.69	0.00
50	3.71	0.47
51	3.62	0.00
52	3.54	0.90
53	3.58	0.90
54	3.64	0.47
55	3.34	0.90
196	3.18	0.47
197	3.26	0.00
198	3.06	0.00
199	1.69	0.69
200	3.29	1.17

Assay Control

Measles Ref. Std.: 5.92

TABLE 33

Stabilizing Solution (See Table 22)	PFU/Ml (log 10) 0 Days (Dried)	12 Hours at 40°C after Rehydration
56	3.62	2.42
57	3.73	2.37
58	3.77	2.27
59	3.66	1.99
60	3.72	2.37
86	3.86	2.19
87	3.70	2.20
88	3.69	2.30
89	3.70	2.21
90	3.64	1.91
91	3.59	2.01
92	3.60	2.06
93	3.43	2.07
94	3.47	1.83
95	3.49	2.06
96	3.44	1.74
97	3.46	1.63
98	3.59	1.81
99	3.60	2.30
100	3.73	1.69
126	3.55	1.06
127	3.48	1.51
128	3.59	1.57
129	3.59	1.77
130	3.57	1.99
131	3.41	1.74
132	3.55	1.39
133	3.63	1.57
134	3.59	1.57
135	3.54	1.81
136	3.52	1.44
137	3.35	1.39
138	3.01	0.69
139	3.42	1.39
140	3.23	1.25
166	3.47	0.69
167	3.37	0.69
168	3.47	0.90

Assay Control

Measles Ref. Std.: 5.96

TABLE 34

Stabilizing Solution (See Table 22)	PFU/Ml (log 10) 0 Days (Dried)	12 Hours at 40°C after Rehydration
156	3.32	1.30
157	3.30	1.11
158	2.99	0.00
159	3.10	1.36
160	3.07	1.00
161	3.48	0.47
162	3.41	0.00
163	3.30	0.47
164	3.50	1.44
165	3.59	1.17
181	3.12	0.00
182	3.13	1.44
183	3.09	1.17
184	3.18	1.00
185	2.96	1.11
186	3.27	1.00
187	3.22	1.63
188	3.11	1.00
189	3.12	1.36
190	3.07	1.00
191	3.01	0.90
192	2.92	0.69
193	2.97	0.00
194	2.77	0.90
195	2.72	0.00

Assay Control

Measles Ref. Std.: 5.95

TABLE 35

Stabilizing Solution (See Table 22)	PFU/Ml (log 10) 0 Days (Dried)	12 Hours at 40°C after Rehydration
169	3.54	1.39
170	3.31	1.90
171	3.35	1.36
172	3.36	1.00
173	3.30	1.36
174	3.44	1.17
175	3.49	1.77
176	3.33	1.0
177	3.24	1.25
178	3.12	1.39
179	3.24	1.39
180	3.01	0.00

Assay Control

Measles Ref. Std.: 5.96

TABLE 36

Summary of Data Presented In Tables 23 - 35
Calculated as Virus Infectivity Loss

Solution No. (See Table 22)	PFU/ml (log 10) Loss: Held 5 days at 40°C dried	Held 5 days at 40°C dried plus 12 hours at 40°C after rehydration	Not held at 40°C dried - held 12 hours at 40°C after rehydration
1		No infection virus after drying	
2		2.39*	
3		2.65*	
4		2.81*	
5		3.06*	
6	1.50	3.50*	3.58*
7	0.81	2.51	3.58*
8	1.21	3.60*	2.35
9	0.99	3.09	2.59
10	0.96	2.60	2.53
11	0.75	2.89	2.60
12	0.66	3.69*	3.65*
13	0.81	2.16	3.19
14	0.79	2.30	2.26
15	0.92	2.61	2.69
16	0.72	2.27	3.64*
17	0.58	1.96	2.21
18	0.64	2.02	2.73
19	0.83	2.97	2.47
20	0.41	3.35	2.24
21		2.57*	
22		1.61	
23		2.89*	
24		2.95*	
25		3.01*	
26		3.06*	
27		2.86*	
28		2.97*	
29		2.92*	
30		2.44*	
31		2.39*	
32		2.32	
33		2.92*	
34		3.76*	
35		2.95*	
36		2.51*	
37		2.44*	
38		2.63*	
39		2.77*	
40		2.47*	
41		3.23*	
42		3.01*	
43		3.19*	
44		2.90*	
45		3.28*	

TABLE 36
(cont'd)

Solution No. (See Table 22)	PFU/ml (log 10) Loss: Held 5 days at 40°C dried	Held 5 days at 40°C dried plus 12 hours at 40°C after rehydration	Not held at 40°C dried - held 12 hours at 40°C after rehydration
46	0.79	3.48*	2.95
47	0.96	2.42	3.65*
48	1.24	2.38	3.19
49	0.77	3.42*	3.69*
50	0.87	2.16	3.24
51	0.84	3.38	3.62*
52	0.54	2.33	2.64
53	0.53	1.86	2.68
54	0.71	3.22*	3.17
55	0.85	1.98	2.44
56	0.48	2.11	1.20
57	0.77	3.28*	1.36
58	0.60	2.25	1.50
59	0.89	3.49*	1.67
60	0.63	2.31	1.35
61		2.31	
62		2.59	
63		2.60	
64		2.82	
65		1.91	
66		3.19*	
67		2.80	
68		2.63	
69		2.97	
70		3.27	
71		2.44	
72		3.31*	
73		3.34*	
74		3.06*	
75		2.40	
76		3.32	
77		3.30	
78		3.29	
79		2.92	
80		3.02*	
81		3.06*	
82		3.34*	
83		3.35*	
84		1.92	
85		3.18*	
86	0.76	2.24	1.67
87	1.01	2.70	1.50
88	0.75	2.66	1.39
89	0.67	3.14	1.49
90	0.62	2.65	1.73
91	0.61	2.29	1.58

TABLE 36
(cont'd)

Solution No. (See Table 22)	PFU/ml (log 10) Loss: Held 5 days at 40°C dried	Held 5 days at 40°C dried plus 12 hours at 40°C after rehydration	Not held at 40°C dried - held 12 hours at 40°C after rehydration
92	0.28 ←	3.39*	1.54
93	0.78	2.56	1.36
94	0.63	3.39	1.64
95	0.39	3.43*	1.43
96	0.94	3.38*	1.70
97	1.27 ←	2.62	1.83
98	0.61	2.40	1.78
99	0.54	2.29	1.30
100	0.81	3.64*	2.04
101		2.40	
102		2.81	
103		1.78 ←	
104		3.01	
105		2.71	
106		3.27*	
107		2.02	
108		2.63	
109		2.54	
110		3.32*	
111		3.03*	
112		3.07*	
113		3.19*	
114		3.13*	
115		2.72*	
116		3.27*	
117		3.16*	
118		3.20*	
119		2.65*	
120		2.90*	
121			3.06*
122		No infectious virus after drying	
123			3.29*
124			3.35*
125		Toxic	
126	0.35	2.94	2.49
127	0.33	2.42	1.97
128	0.98	2.68	2.02
129	0.69	2.50	1.82
130	0.90	2.51	1.58
131	0.73	2.94	1.67
132	0.91	2.69	1.98
133	0.44	2.01	2.06
134	0.69	2.20	2.02
135	0.48	2.28	1.73
136	1.00	3.30*	2.08
137	0.79	2.79	1.96

TABLE 36
(cont'd)

Solution No. (See Table 22)	PFU/ml (log 10) Loss: Held 5 days at 40°C dried	Held 5 days at 40°C dried plus 12 hours at 40°C after rehydration	Not held at 40°C dried - held 12 hours at 40°C after rehydration
138	1.66	3.13*	2.32
139	1.06	3.23*	2.03
140	0.61←	2.39	1.98
141			2.33
142			2.16
143			2.34
144			3.23*
145			1.96
146			2.14
147			2.17
148			1.45
149			2.14
150			2.57
151			2.16
152			2.47
153			1.96
154			2.07
155			1.56
156			2.02
157			2.19
158			2.99*
159			1.74
160			2.07
161			3.01
162			3.41*
163			2.83
164			2.06
165			2.42
166	1.47	3.47*	2.78
167	1.34	3.45*	2.68
168	1.72	3.41*	2.57
169	1.69	3.38*	2.15
170	1.28	3.45*	1.41
171	1.39	3.39*	1.99
172	0.76	3.15*	2.36
173	>2.26	2.36	1.94
174	>2.30	3.30*	2.27
175	1.14	3.25*	1.72
176	1.76	3.23*	2.33
177	1.47	2.94*	1.99
178	>1.90	2.90*	1.73
179	1.05	2.74*	1.85
180	>2.00	3.00*	3.01*
181			3.12*
182			1.69
183			1.92

TABLE 36
(cont'd)

Solution No. (See Table 22)	PFU/ml (log 10) Loss: Held 5 days at 40°C dried	Held 5 days at 40°C dried plus 12 hours at 40°C after rehydration	Not held at 40°C dried - held 12 hours at 40°C after rehydration
184			2.18
185			1.85
186			2.27
187			1.59
188			2.11
189			1.76
190			2.07
191			2.11
192			2.23
193			2.97*
194			1.87
195			2.72*
196			2.71
197			3.26*
198			3.06*
199			1.00
200			2.12

* No infective virus detectable

TABLE 37

Loss in Titer of Dried Virus after 5 Days at 40°C
(all concentrations of sodium glutamate grouped together - titers of each of the 5 sodium glutamate groups combined)

Concentration Sucrose	Proportion stabilizing solution to 20% hydrolyzed gelatin solution			Total Loss
	4+1	3+1	2+1	
50%	5.47	3.93	3.18	12.58
40%	4.63	3.47	3.37	11.47
30%	3.47	2.69	4.17	10.67
20%	3.25	3.25	5.12	11.62
10%	5.12	>7.85	>8.18	>23.53
Total loss	21.94	>18.19	>24.02	

TABLE 38

Loss in Titer of Dried Virus after 5 Days at 40°C
(Listed by both gelatin and glutamate concentration)

Concentration Sucrose	Concentration Sodium Glutamate 1%	2%	3%	4½	5%
			Gelatin Proportion 4+1		
50%	1.50	0.81	1.21	0.99	0.96
40%	0.79	0.96	1.24	0.77	0.87
30%	0.76	1.01	0.75	0.67	0.62
20%	0.35	0.33	0.98	0.69	0.90
10%	1.47	1.34	1.72	1.69	1.28
Total loss	4.87	4.45	5.9	4.81	4.63
			Gelatin Proportion 3+1		
50%	0.75	0.66	0.81	0.79	0.92
40%	0.84	0.54	0.53	0.71	0.85
30%	0.61	0.28	0.78	0.63	0.39
20%	0.73	0.91	0.44	0.69	0.48
10%	1.39	0.76	>2.26	>2.30	1.14
Total loss	4.32	3.15	4.82	5.12	3.78
			Gelatin Proportion 2+1		
50%	0.72	0.58	0.64	0.83	0.41
40%	0.48	0.77	0.60	0.89	0.63
30%	0.94	1.27	0.61	0.54	0.81
20%	1.00	0.79	1.66	1.06	0.61
10%	1.76	1.47	>1.90	1.05	>2.00
Total loss	4.90	4.88	>5.41	4.37	4.46

TABLE 39

Loss in Titer of Dried Virus held 5 Days at 40°C
Plus 12 Hours at 40°C after Rehydration
(All concentrations of sodium glutamate grouped together - titers of each of the 5 sodium glutamate groups combined)

Proportion Stabilizing Solution to 20% Hydrolized Gelatin Solution	Concentration Sucrose 50%	40%	30%	20%	10%	Total Loss
1+0 (No gelatin)	10.91*	15.61	14.85			
4+1	15.30	13.86	12.96	13.05	17.16	72.33
3+1	13.65	12.77	15.06	12.12	15.45	69.05
2+1	12.57	13.44	14.33	13.84	14.81	68.99
1+1	13.03	12.23	12.71			
1+2	14.25	14.86	13.78			
1+3	14.35	14.55	15.14			
1+4	12.82	15.85	15.18			
Total loss	106.88	113.17	114.01			

* One sample had no infectious virus after drying;
all other 4 samples in this series lost all infectivity after drying

TABLE 40

Loss in Titer of Dried Virus held 5 Days at 40°C
Plus 12 Hours at 40°C after Rehydration
(Listed by both gelatin and glutamate concentration)

Concentration Sucrose	Concentration Sodium Glutamate 1%	2%	3%	4%	5%
			Gelatin Proportion 1+0 (None)		
50%	**	2.39*	2.65*	2.81*	3.06*
40%	3.23*	3.01*	3.19*	2.90*	3.28*
30%	3.06*	3.34*	3.35*	1.92	3.18*
20%					
10%					
Total loss					
			Gelatin Proportion 4+1		
50%	3.50*	2.51	3.60*	3.09	2.60
40%	3.48*	2.42	2.38	3.42*	2.16
30%	2.40	2.70	2.66	3.14	2.65
20%	2.94	2.42	2.68	2.50	2.51
10%	3.47*	3.45*	3.41*	3.28*	3.45*
Total loss	15.79	13.50	14.71	15.53	13.37
			Gelatin Proportion 3+1		
50%	2.89	3.69*	2.16	2.30	2.61
40%	3.38	2.33	1.86	3.22*	1.98
30%	2.29	3.39*	2.56	3.39	3.43*
20%	2.94	2.69	2.01	2.20	2.28
10%	3.39*	3.15*	2.36	3.30*	3.25*
Total loss	14.89	15.25	10.95	14.41	13.55
			Gelatin Proportion 2+1		
50%	2.27	1.96	2.02	2.97	3.35
40%	2.11	3.28*	2.25	3.49*	2.31
30%	3.38*	2.62	2.40	2.29	3.64*
20%	3.30*	2.79	3.13*	3.23*	2.39
10%	3.23*	2.94*	2.90*	2.74*	3.00*
Total loss	14.29	13.59	12.70	14.72	13.69

TABLE 40
(cont'd)

Concentration Sucrose	Concentration Sodium Glutamate 1%	2%	3%	4%	5%
		Gelatin Proportion 1+1			
50%	2.57*	1.61	2.89*	2.95*	3.01*
40%	2.31	2.59	2.60	2.82	1.91
30%	2.40	2.81	1.78	3.01	2.71
20%					
10%					
Total loss					
		Gelatin Proportion 1+2			
50%	3.06*	2.86*	2.97*	2.92*	2.44*
40%	3.19*	2.80	2.63	2.97	3.27
30%	3.27*	2.02	2.63	2.54	3.32*
20%					
10%					
Total loss					
		Gelatin Proportion 1+3			
50%	2.39*	2.32	2.92*	3.76*	2.95*
40%	2.44	3.31*	3.34*	3.06*	2.40
30%	3.03*	3.07*	3.19*	3.13*	2.72*
20%					
10%					
Total loss					
		Gelatin Proportion 1+4			
50%	2.51*	2.44*	2.63*	2.77*	2.47*
40%	3.32	3.30	3.29	2.92	3.02*
30%	3.27*	3.16*	3.20*	2.65*	2.90*
20%					
10%					
Total loss					

* No infective virus detectable
** No infectivity after drying

TABLE 41

Loss in Titer of Dried Virus Not Held at 40°C
but Held 12 Hours at 40°C after Rehydration
(All concentrations of sodium glutamate grouped together -
titers of each of the 5 sodium glutamate groups combined)

Proportion Stabilizing Solution to 20% Hydrolized Gelatin Solution	Concentration Sucrose				
	50%	40%	30%	20%	10%
1+0 (None)				9.7*	13.73
4+1	14.63	16.72	7.78	9.88	11.59
3+1	14.39	14.55	7.55	9.46	10.28
2+1	13.29	7.08	8.65	10.37	10.91
1+1				12.02	10.76
1+2				10.47	9.80
1+3				10.02	11.90
1+4				11.01	12.15

* One sample had no infectious virus after drying; another was toxic to the tissue culture and there was no evidence of infectivity with the remaining three samples in this series.

TABLE 42

Loss in Titer of Dried Virus not Held at 40°C
but Held 12 Hours at 40°C after Rehydration
(listed by both gelatin and glutamate concentration)

Concentration Sucrose	Concentration Sodium Glutamate 1%	2%	3%	4%	5%
	Gelatin Proportion 1+0 (None)				
50%					
40%					
30%					
20%	3.06*	**	3.29	3.35*	toxic
10%	3.01	3.41*	2.83	2.06	2.42
Total loss					
	Gelatin Proportion 4+1				
50%	3.58*	3.58*	2.35	2.59	2.53
40%	2.95	3.65*	3.19	3.69*	3.24
30%	1.67	1.50	1.39	1.49	1.73
20%	2.49	1.97	2.02	1.82	1.58
10%	2.78	2.68	2.57	2.15	1.41
Total loss					
	Gelatin Proportion 3+1				
50%	2.60	3.65*	3.19	2.26	2.69
40%	3.62*	2.64	2.68	3.17	2.44
30%	1.58	1.54	1.36	1.64	1.43
20%	1.67	1.98	2.06	2.02	1.73
10%	1.99	2.36	1.94	2.27	1.72
Total loss					
	Gelatin Proportion 2+1				
50%	3.64*	2.21	2.73	2.47	2.24
40%	1.20	1.36	1.50	1.67	1.35
30%	1.70	1.83	1.78	1.30	2.04
20%	2.08	1.96	2.32	2.03	1.98
10%	2.33	1.99	1.73	1.85	3.01*
Total loss					
	Gelatin Proportion 1+1				
50%					
40%					
30%					
20%	2.33	2.16	2.34	3.23*	1.96
10%	3.12*	1.69	1.92	2.18	1.85
Total loss					

TABLE 42
(cont'd)

Concentration Sucrose	Concentration Sodium Glutamate 1%	2%	3%	4%	5%
		Gelatin Proportion 1+2			
50%					
40%					
30%					
20%	2.14	2.17	1.45	2.14	2.57
10%	2.27	1.59	2.11	1.76	2.07
Total loss					
		Gelatin Proportion 1+3			
50%					
40%					
30%					
20%	2.16	2.47	1.76	2.07	1.56
10%	2.11	2.23	2.97	1.87	2.72
Total loss					
		Gelatin Proportion 1+4			
50%					
40%					
30%					
20%					
20%	2.02	2.19	2.99*	1.74	2.07
10%	2.71	3.26*	3.06*	1.00	2.12
Total loss					

* No infective virus detectable
** No infectivity after drying

TABLE 43

Stabilizing Solution (See Table 22)	Rehydrating Fluid	PFU/Ml (log 10) 0 Days (Dried)*	12 Hours at 4°C after Rehydration	12 Hours at 40°C after Rehydration
84	H_2O	3.37	3.11	0.90
84	M $MgSO_4$	3.45	2.51	0.47
84	M Na_2SO_4	3.23	2.54	Toxic
85	H_2O	3.17	2.68	1.05
85	M $MgSO_4$	3.23	2.76	0.69
85	M Na_2SO_4	3.22	2.68	Toxic
103	H_2O	3.33	3.06	1.17
103	M $MgSO_4$	2.97	2.69	0.00
103	M Na_2SO_4	3.00	1.90**	Toxic
105	H_2O	3.24	2.91	0.90
105	M $MgSO_4$	3.09	<1.00	0.00
105	M Na_2SO_4	3.29	1.47**	Toxic
106	H_2O	3.27	2.89	0.69
106	M $MgSO_4$	3.25	2.39	0.47
106	M Na_2SO_4	3.13	2.69**	Toxic

Assay Control

Measles Reference Standard: 5.96

* All 0 day samples rehydrated with water
** Dried sample did not dissolve

TABLE 44

Stabilizing Solution (See Table 22)	Rehydrating Fluid	PFU/Ml (log 10) 0 Days (Dried)	12 Hours at 4°C After Rehydration	12 Hours at 40°C After Rehydration
86	H_2O	3.80	3.36	0.90
86	1.0 M $MgSO_4$		3.40	0.69
86	0.5 M $MgSO_4$			0.00
86	0.25 M $MgSO_4$			0.47
86	0.125 M $MgSO_4$			0.47
86	0.06 M $MgSO_4$			1.05
86	1.0 M Na_2SO_4		2.60	Toxic
86	0.5 M Na_2SO_4			Toxic
86	0.25 M Na_2SO_4			1.39
86	0.125 M Na_2SO_4			1.05
86	0.06 M Na_2SO_4			1.00

Assay Control

Measles Reference Standard: 5.92

TABLE 45

Solution No.	Concentration	Sucrose 30%	Potassium Phosphate*	1% Na Glutamate	20% Hydrolized Gelatin (Proportion of 4 parts of Solution plus 1 part of Gelatin)
1	1 M $MgSO_4$	No	No	No	No
2	1 M $MgSO_4$	Yes	No	No	No
3	1 M $MgSO_4$	Yes	Yes	No	No
4	1 M $MgSO_4$	Yes	No	Yes	No
5	1 M $MgSO_4$	Yes	Yes	Yes	No
6	1 M $MgSO_4$	No	No	No	Yes
7	1 M $MgSO_4$	Yes	No	No	Yes
8	1 M $MgSO_4$	Yes	Yes	No	Yes
9	1 M $MGSO_4$	Yes	No	Yes	Yes
10	1 M $MgSO_4$	Yes	Yes	Yes	Yes
11	1 M Na_2SO_4	No	No	No	No
12	1 M Na_2SO_4	Yes	No	No	No
13	1 M Na_2SO_4	Yes	No	No	No
14	1 M Na_2SO_4	Yes	No	Yes	No
15	1 M Na_2SO_4	Yes	Yes	Yes	No
16	1 M Na_2SO_4	No	No	No	Yes
17	1 M Na_2SO_4	Yes	No	No	Yes
18	1 M Na_2SO_4	Yes	Yes	No	Yes
19	1 M Na_2SO_4	Yes	No	Yes	Yes
20	1 M Na_2SO_4	Yes	Yes	Yes	Yes
21	H_2O only	No	No	No	No
22	H_2O only	Yes	No	No	No
23	H_2O only	Yes	Yes	No	No
24	H_2O only	Yes	No	Yes	No
25	H_2O only	Yes	Yes	Yes	No
26	H_2O only	No	No	No	Yes
27	H_2O only	Yes	No	No	Yes
28	H_2O only	Yes	Yes	No	Yes
29	H_2O only	Yes	No	Yes	Yes
30	H_2O only	Yes	Yes	Yes	Yes

* 0.123% K_2HPO_4
0.051% KH_2PO_4

TABLE 46

Stabilizing Solution (See Table 45)	PFU/Ml (log 10) 0 Days*	12 hours at 4°C*	12 Hours at 40°C*
1 (Frozen control)	<1.00	<1.00	0.00
1 (Dried)	<1.00	<1.00	0.00
11 (Frozen control)	<1.00	<1.00	0.00
11 (Dried)	<1.00	<1.00	0.00
21 (Frozen control)	<1.00	<1.00	0.00
21 (Dried	<1.00	<1.00	0.00
10 Dried	3.43	3.11	0.00
10 Dried	3.36**	3.19**	0.00**

Assay Control

Measles Ref. Std.: 5.96

* Dried samples rehydrated with water if not otherwise indicated; frozen samples thawed and held at temperature

** Rehydrated with 0.01 M EDTA

TABLE 47

Stabilizing Solution (See Table 45)	Description	PFU/Ml (log 10) 0 Days*	12 Hours at 4°C*	12 Hours at 40°C*
2	Frozen control	3.62	3.45	0.00
2	Dried	3.05	2.47	0.00
3	Frozen control	3.61	3.35	0.47
3	Dried	3.27	1.47	0.00
4	Frozen control	3.63	3.29	2.12
4	Dried	3.42	1.90	0.00
5	Frozen control	3.76	3.69	1.68
5	Dried	3.34	2.74	0.69
6	Frozen control	3.32	3.22	0.00
6	Dried	1.17	0.00	0.00
12	Frozen control	3.54	3.62	Toxic
12	Dried	0.00	Toxic	Toxic
13	Frozen control	3.35	3.43	Toxic
13	Dried	2.25	0.00	Toxic
14	Frozen control	3.65	3.45	Toxic
14	Dried	0.00	0.00	0.00
15	Frozen Control	3.75	2.54	Toxic
15	Dried	1.00	<1.00	Toxic
16	Frozen control	3.52	3.55	1.17
16	Dried	1.00	1.47	0.00
22	Frozen control	3.69	3.33	2.07
22	Dried	2.75	2.30	0.69
23	Frozen control	3.54	3.55	1.30
23	Dried	3.11	2.65	0.00
24	Frozen control	3.62	3.55	1.68
24	Dried	3.77	3.36	1.68
25	Frozen control	3.70	3.76	1.86
25	Dried	3.63	3.16	1.36
26	Frozen control	3.63	3.46	1.36
26	Dried	3.18	2.54	1.11
20	Dried	2.90	2.30	0.00
20	Dried	2.84**	2.36**	Toxic**

Assay control

Measles Ref. Std.: 5.96

* Dried samples rehydrated with water if not otherwise indicated; frozen samples thawed and held at temperature

** Rehydrated with 0.01 M EDTA

TABLE 48

Stabilizing Solution (See Table 45)	Description	PFU/Ml (log 10) 0 Days*	12 Hours at 4°C	12 Hours at 40°C
1	Dried	<1.00	<1.00	0.00
2	Dried	3.19	1.90	0.00
3	Dried	3.42	1.47	0.00
4	Dried	3.34	1.47	0.00
5	Dried	3.25	<1.00	0.00
6	Dried	2.36	<1.00	0.00
7	Dried	3.38	2.92	0.00
7	Frozen control	3.74	3.56	1.79
7	Dried	3.45	3.13	0.00
8	Frozen control	3.70	3.56	1.86
8	Dried	3.52	3.26	1.44
9	Frozen control	3.64	3.56	1.54
9	Dried	3.35	2.87	1.00
10	Frozen control	3.69	3.75	1.97
10	Dried	3.43	2.95	0.90
17	Frozen control	3.58	3.60	Toxic
17	Dried	2.89	2.47	Toxic
18	Frozen control	3.51	3.36	Toxic
18	Dried	3.04	2.36	Toxic
19	Frozen control	3.62	3.42	Toxic
19	Dried	2.81	2.39	Toxic
20	Frozen control	3.94	3.75	1.69
20	Dried	3.15	2.76	0.00
27	Frozen control	3.74	3.61	2.02
27	Dried	3.55	3.30	2.18
28	Frozen control	3.73	3.70	2.08
28	Dried	3.58	3.34	2.02
29	Frozen control	3.71	3.45	2.00
29	Dried	3.35	3.00	0.69
30	Frozen control	3.90	3.80	2.14
30	Dried	3.72	3.55	0.90
30	Dried	3.65	3.53	1.96
30	Dried	3.70**	3.58**	1.84**

Assay control

Measles Ref. Std.: 6.01

* Dried Samples rehydrated with water if not otherwise indicated; frozen samples thawed and held at temperature

** Rehydrated with 0.01 M EDTA

TABLE 49

Stabilizing Solution (See Table 45)	PFU/Ml (log 10) Time Held at 40°C		
	Dried		Dried 5 Days Plus 12 Hours after Rehydration
	0 Days	5 Days	
25	3.39	2.17	0.00
26	3.01	0.00	0.00
27	3.58	2.91	0.69
28	3.74	2.81	0.90
29	3.61	3.05	1.17
30	3.67	2.95	1.05

Assay Control

Measles Reference Standard: 5.90

TABLE 50

Stabilizing Solution (See Table 45)	PFU/Ml (log 10) Time Held at 40°C		
	Dried		Dried 5 Days Plus 12 Hours after Rehydration
	0 Days	5 Days	
8	3.34	2.68	0
9	3.17	2.57	0
10	3.13	2.36	0.69
11	<1.00	<1.00	Toxic
12	<1.00	<1.00	Toxic
13	<1.00	<1.00	Toxic
14	<1.00	<1.00	Toxic
15	<1.00	1.69	Toxic
16	1.47	<1.00	0
17	2.36	1.90	Toxic
18	2.47	2.00	Toxic
19	2.68	2.00	0
20	2.36	1.90	0.69
21	1.00	1.69	0.47
22	2.97	1.90	0
23	3.02	1.69	0
24	3.28	2.11	0

Assay Control

Measles Reference Standard: 6.06

TABLE 51

Summary of Data Presented In Tables 46-50
Calculated as Virus Infectivity Loss

Solution No. (See Table 45)	PFU/Ml (log 10) Loss: Held 5 Days at 40°C Dried	Held 5 Days at 40°C Dried Plus 12 Hours at 40°C after Rehydration	Not Held at 40°C Dried Held 12 Hours at 40°C after Rehydration
1	No infectivity after drying		
2			3.19*, 3.05*
3			3.42*, 3.27*
4			3.34*, 3.42*
5			3.25*, 2.65
6			2.36*, 1.17*
7			3.45*
8	0.66	3.34*	2.08
9	0.60	3.17*	2.25
10	0.77	2.44	2.53
11	No infectivity after drying		
12	No infectivity after drying		Toxic
13	No infectivity after drying		Toxic
14	No infectivity after drying		
15	No infectivity after drying		Toxic
16	1.47	1.47*	1.00*
17	0.46	Toxic	Toxic
18	0.47	Toxic	Toxic
19	0.68	2.68*	Toxic
20	0.46	1.67	3.15*
21	No infectivity after drying		
21	0.00	0.53	
22	1.07	2.97*	2.06
23	1.33	3.02*	3.11*
24	1.17	3.28*	2.09
25	1.22	3.39*	2.27
26	3.01*	3.01*	2.07
27	0.67	2.89	1.37
28	0.93	2.84	1.56
29	0.56	2.44	2.66
30	0.72	2.62	2.82, 1.69

* No infective virus detectable

TABLE 52

Comparison of Stability of Dried and Not Dried Virus
Held at 4°C and 40°C for 12 Hours
(Summary of Data Presented in Tables 46-48
Calculated as Virus Infectivity Loss)

Stabilizing Solution (See Table 45)	Description	PFU/Ml (log 10) Loss Held 12 Hours at 4°C after Thawing or Rehydration	PFU/Ml (log 10) Loss Held 12 Hours at 40°C after Thawing or Rehydration
1	Not dried	No infective virus at 0 time	
	Dried	No infective virus at 0 time	
2	Not dried	0.17	3.62*
	Dried	0.78	3.05*
3	Not dried	0.26	3.14
	Dried	1.80	3.27*
4	Not dried	0.34	1.51
	Dried	1.52	3.42*
5	Not dried	0.07	2.08
	Dried	0.60	2.65
6	Not dried	0.10	3.32*
	Dried	1.17*	1.17*
7	Not dried	0.18	1.95
	Dried	0.32	3.45*
8	Not dried	0.14	1.84
	Dried	0.26	2.08
9	Not dried	0.08	2.10
	Dried	0.48	2.35
10	Not dried	+0.06	1.72
	Dried	0.48	2.53
11	Not dried	No infective virus at 0 time	
	Dried	No infective virus at 0 time	
12	Not dried	+0.08	Toxic
	Dried	Toxic	Toxic
13	Not dried	+0.08	Toxic
	Dried	2.25*	Toxic
14	Not dried	0.20	Toxic
	Dried	No infective virus at 0 time	
15	Not dried	1.21	Toxic
	Dried	1.00*	Toxic

TABLE 52
(cont'd)

Stabilizing Solution (See Table 45)	Description	PFU/Ml (log 10) Loss Held 12 Hours at 4°C after Thawing	PFU/Ml (log 10) Loss Held 12 Hours at 40°C or Rehydration
16	Not dried	+0.03	2.35
	Dried	+0.47	1.00*
17	Not dried	+0.02	Toxic
	Dried	0.42	Toxic
18	Not dried	0.15	Toxic
	Dried	0.88	Toxic
19	Not Dried	0.20	Toxic
	Dried	0.42	Toxic
20	Not dried	0.19	2.25
	Dried	0.39	3.15*
21	Not dried	No infectious virus at 0 time	
	Dried	No infectious virus at 0 time	
22	Not dried	0.36	1.62
	Dried	0.45	2.06
23	Not dried	+0.01	2.24
	Dried	0.46	3.11*
24	Not dried	0.07	1.94
	Dried	0.41	2.09
25	Not dried	+0.06	1.84
	Dried	0.47	2.27
26	Not dried	0.17	2.27
	Dried	0.64	2.07
27	Not dried	0.13	1.72
	Dried	0.25	1.37
28	Not dried	0.03	1.65
	Dried	0.24	1.56
29	Not dried	0.26	1.71
	Dried	0.35	2.66
30	Not dried	0.10	1.76
	Dried	0.17	2.82

*No infective virus detectable