The Stabilizing Effect of Sucrose upon Respiratory Syncytial Virus Infectivity (33054)

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Respiratory syncytial virus (RS) was first isolated from a chimpanzee in 1956 by Morris *et al.* (1). Since then it has been recognized as an important agent in respiratory infection in both children and adults (2). Studies of RS virus have been hampered by its lability. This report describes the stabilizing effect of hypertonic sucrose upon RS infectivity during storage at various temperatures.

Materials and Methods. Virus. The Long strain of RS virus (originally obtained from Dr. R. M. Chanock, NIH) was used throughout these studies. It was propagated 6 times in KB cells, twice in Chang liver cells, once in AV_2 cells, and 8–14 times in BS-C-1 cells.

Cell cultures. BS-C-1 cultures were grown in roller tubes and in 16-oz bottles. Bottle cultures contained confluent monolayers after 7 days and tubes after 4 days.

Growth medium. Medium 199 containing 1.12 gm/liter of sodium bicarbonate, 2% noninactivated horse serum, and appropriate antibiotics was used throughout these studies.

Maintenance and assay medium. Serum-free medium 199 containing 1.68 gm/liter of sodium bicarbonate, and appropriate antibiotics was used.

Virus production. Sixteen oz bottle cultures were inoculated each with 50 ml of maintenance medium containing 1000–10,000 $TCID_{50}$ per culture. The cultures were harvested at the peak of infectivity after about 4 days of incubation at 37°C.

Infectivity assay. Tenfold serial dilutions of virus were made in maintenance medium. One-half ml of each dilution was added to each of 10 BS-C-1 tube cultures plus 1.5 ml of maintenance medium. The cultures were incubated on a roller tube apparatus at 37°C for 7 days. Fifty percent CPE end points were determined by the method of Reed and Muench (3). A statistical analysis of 30 replicate assays performed in our laboratory established that a 0.4 log variation between assay values on a given day was significant (4). Thus, in Table I, the differences in assay values listed in the vertical columns should be significant within this 0.4 log range, while in Tables II and III, this would apply only to values listed horizontally. Assay errors greater than 0.4 logs apparently occurred from 1 day's assay to the next when different cultures and possibly different technicians were involved in the assays.

TABLE I. Stability of RS Virus for 3 Months in Various Concentrations of Hypertonic Sucrose Solutions (—70°C).

Sucrose fraction (%)	Infectivity titer		
	Initial (/0.5 ml)	Post storage (/0.5 ml)	Recovery (%)
18.5	1.63[a]	0.71	12.0
23.0	3.50	1.72	1.6
28.5	3.68	3.00	21.0
33.5	4.17	3.91	55.0
44.5	5.17	5.44	205.0
56.0	5.38	5.29	82.0

[a] Log base 10.

Sucrose. Sterile gradient solution no. 1[2] possessing a refractive index of 1.4351 to 1.4486 was used. All sucrose concentrations were calculated on a weight/weight basis.

Results. Fractions collected following isopycnic banding of RS virus in a sucrose gradient were assayed for infectivity, stored at —70°C for 3 months, and were again assayed. The results are seen in Table I. Although the initial titers of the various fractions varied, because of the distribution of virus in the gradient, it can be seen that the stability of the virus was maintained best in sucrose concentrations exceeding 30%.

To confirm the initial observation of the

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TABLE II. Stability of RS Virus in Two Concentrations of Sucrose at 4°C and at 37°C in 44.5% Sucrose.

Temp. (°C):	4				37	
Conc (%):	17		44.5		44.5	
Day	Sucrose	Control	Sucrose	Control	Sucrose	Control
0	4.24	3.63	3.79	3.42	3.57	3.65
1	3.71	≦2.50	2.83	≦2.50	3.71	2.00
2	—[a]	—	—	—	2.63	0.63
3	3.00	≦1.50	3.00	≦1.50	—	—
5	—	—	—	—	≦2.50	≦0.50
6	—	—	—	—	2.50	0
7	2.11	≦1.50	3.00	≦1.50	1.83	0
10	0.63	≦0.50	3.68	≦0.50	—	—
14	0.56	≦0.50	3.43	≦0.50	—	—
24	—	—	3.43	—	—	—
37	—	—	2.69	—	—	—

[a] — = not tested.

stabilizing effect of hypertonic sucrose, freshly harvested virus was mixed in concentrations of sucrose approximating those which appeared to be either effective (44.5%) or relatively ineffective (17%). Appropriate controls were included for each dilution of virus. Samples were stored at 4 and 37°C. Results are shown in Table II.

The virus held in 44.5% sucrose showed no significant loss in viability after 24 days of storage at 4°C whereas the virus stored in 17% sucrose showed more than a 99% loss of infectivity following 14 days of storage at the same temperature. The rate of inactivation was more rapid in the corresponding sample stored at 4°C. Infectivity was lost following 1 week of storage at this temperature. A loss of infectivity in excess of 90% was noted in all control samples within the first 24 hours of storage.

In further studies, samples of RS virus stored at 4, —20, and —70°C in 44.5% sucrose were periodically assayed during a 64-week period. Results are shown in Table III.

A significant loss of infectivity occurred between the first and second weeks at —20°C. At 4°C a significant loss was not noted until after the fourth week. At —70°C no appreciable loss occurred until after the fifteenth week, then only a gradual loss was observed during the remainder of the 64-week period. These data suggested that a temperature of —70°C was most desirable for the long-term storage of sucrose-stabilized virus and that 4°C was somewhat better than —20°C.

Wulff *et al.* (5) reported on the stability of monkey kidney grown, Long strain, RS virus. Fluids were stored at various temperatures without the addition of a stabilizing agent. Viable virus could not be detected in her samples beyond the sixth week of storage at 4, —20, and —70°C. These findings confirm the lability of RS virus in the absence of a suitable stabilizer. In contrast, the infectivity of both the Long and Simon strains of RS virus was maintained for up to

TABLE III. Effect of Temperature on Viability of RS Virus in 44.5% Sucrose.

	Temperature (°C)		
Weeks	4	—20	—70
1	3.83	3.83	3.63
2	2.71	2.56	4.17
3	3.57	1.79	3.38
4	3.68	1.63	3.56
6	3.17	0.83	4.38
8	2.71	2.24	4.00
10	2.50	1.56	3.65
12	2.38	2.00	3.29
15	1.57	≦0.50	3.35
64	≦0.50	≦0.50	2.65

TABLE IV. Long-Term Storage of RS Virus (—70°C) in 44.5% Sucrose.

Months	Strains	
	Long	Simon
0	4.56, 4.44	4.65
21		4.29, 4.38
28	4.17, 4.29	

2.5 years in our laboratory when stored in 44.5% sucrose at —70°C. These data are seen in Table IV.

Hypertonic sucrose stabilized RS virus under the conditions described above. Another important use of sucrose would be the preservation of clinical specimens until virus isolation attempts can be made. To test this, a dilute solution of RS virus was prepared which contained 7 $TCID_{50}$/0.5 ml. Cotton-tipped swabs were dipped in this solution, then stored either in medium 199 or in medium containing 44.5% sucrose at 4°C. At intervals between days 0 and 21, samples were tested for viability. The swabs were stored in vials containing 1.0 ml of diluent. In order to reduce the sucrose content to acceptable levels for tissue culture assay, 5.0 ml of medium was added to each tube just before assay. The total 6.0-ml volumes were inoculated into Falcon flask cultures of BS-C-1 cells and no additional medium was added. Cultures were refed at 4 and 7 days. The RS virus CPE was detected between 7 and 14 days. The results of this study are seen in Table V.

Swabs held in 44.5% sucrose at 4°C yielded viable virus for a period of 1 week, whereas in medium 199, virus could only be recovered during the first 24 hours of storage.

Discussion. The problem of instability of RS virus can, in part, be solved by storage of the virus in a hypertonic sucrose solution at —70°C. The retention of viability at 4°C, or even 37°C, is enhanced as compared to nonsucrose-containing control medium. We have successfully employed hypertonic sucrose solution for stabilizing RS virus seeds and standards for over 2 years in our laboratory. This has facilitated serum neutralization tests, animal inoculations, and other procedures in which a standard dose of virus is desired from 1 week to the next. Sucrose was useful, also, in stabilizing CF antigen. Sucrose provides better stabilization of the virus than does serum or other protein solutions and has the additional advantage of not being antigenic.

There are some disadvantages to the use of hypertonic sucrose solutions, but in most instances these are easily overcome. At the 44.5% level sucrose is toxic to cell cultures and produces pain upon injection in animals. A 1 in 10 dilution will reduce the concentration to levels tolerated by tissue culture cells, while a 1 in 4, or 1 in 5 dilution eliminates the difficulty upon injection. For most laboratory procedures, such dilutions can be made without serious effect on the outcome of the experiment. Attempts to separate the virus from sucrose by absorption to and elution from G-25 Sephadex columns has resulted in significant losses of virus. Ordinary dialysis was also difficult.

The use of sucrose as a stabilizer of RS virus in simulated clinical specimens produced favorable results. This may be of value to clinical virologists as a means of preserving specimens until they reach the laboratory.

Summary. The effect of various concentrations of sucrose upon retention of RS virus viability during storage at different temperatures was studied. As the concentration of sucrose was increased, the survival of virus was enhanced. A concentration of 44.5%

TABLE V. The Stabilizing Effect of Hypertonic Sucrose (4°C) upon Simulated RS Virus Clinical Specimens.

Day	Sucrose (44.5%)		Medium 199	
	Sample no. 1	Sample no. 2	Sample no. 1	Sample no. 2
0	+	+	+	0
1	+	+	+	0
2	+	+	0	0
3	+	+	0	0
7	+	+	0	0
14	0	0	0	0
21	0	0	0	0

+ = RS, CPE
0 = no CPE

sucrose with storage at —70°C appeared to be optimal for retention of RS virus infectivity. Virus was stored under these conditions for periods in excess of 2 years with no significant loss in infectivity. Storage at —20°C, even in the presence of hypertonic sucrose, was less satisfactory than at 4°C or at —70°C. Cotton swabs containing less than 10 $TCID_{50}$ retained viable virus in 44.5% sucrose at 4°C for a period of 7 days. The application of these observations in both the laboratory and clinic are discussed.

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Renin Secretion during Mannitol Diuresis and Ureteral Occlusion* (33055)

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A considerable body of evidence supports the concept that the macula densa is involved in the control of renin release (1). The present experiments were designed to distinguish between two of the hypotheses concerning the possible sensory signal to the macula densa: (i) It has been proposed that a decrease in total sodium load, independent of concentration, to the macula densa induces increased renin secretion (1); and (ii) in contrast, Thurau has proposed that an increased intratubular sodium concentration induces increased renin secretion (2).

Results of the only extensive micropuncture observations (3) in a situation known to increase renin secretion, namely reduction of renal arterial pressure, were consistent with both hypotheses, since early distal sodium load decreased whereas sodium concentration increased. Conversely, mannitol diuresis which has been shown to inhibit the renin release induced by pressure reduction (4), increased early distal sodium load and decreased sodium concentration (5–7).

In order to produce simultaneously a decrease in both early distal sodium load and sodium concentration, the present experiments employed complete ureteral occlusion during maximal mannitol diuresis. Stop-flow analysis has clearly demonstrated that, during the period of ureteral occlusion, the sodium concentration of the fluid within the loop of Henle and distal nephron either does not change or decreases (8), whereas the delivery of fluid (and, therefore, the total sodium load) to these nephron segments virtually ceases (9).

Methods. Experiments were performed on 9 mongrel dogs weighing 15–20 kg and maintained on standard dog chow (Friskies). The dogs were anesthetized with 30 mg/kg sodium pentobarbital intravenously with supplements given as required. The right ureter was catheterized, and a catheter (2.4 mm, o.d.) was manipulated, via a femoral vein and the vena cava, into the right renal vein. Arterial blood was obtained from a femoral artery catheter. Mean arterial pressure was monitored continuously using a Statham transducer and Grass polygraph. In 3 experiments total renal blood flow was monitored continuously using a Carolina square-wave electromagnetic flow meter and probe.

Experimental manipulations were not begun until at least 45 min after completion of all operative procedures. Control samples were then obtained following which the ani-

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