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Vesicular Stomatitis Virus in Tissue Cultures and Cell Suspensions. (22205)

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The utilization of cultured tissues and cells for the production and assay of viruses has achieved widespread application following the epochal work of Enders, Weller and Robbins (1). The direct use of suspensions of tissue fragments and cells for virus propagation has had, on the other hand, much less appeal even though Frenkel and van Waveren proved the practicability of the method with vaccinia virus nearly 20 years ago(2). More recently, Frenkel employed this method in the production of foot-and-mouth disease virus for use in commercial vaccines(3). A practical test for the determination of virus concentration directly in cell suspensions has been lacking until recently and this appears to have been responsible, in part, for the heretofore limited use of such suspensions in virus studies. Salk *et al.*(4), Lipton and Steigman(5) and Robertson *et al.*(6) have now shown that cell suspensions can be used for the assay of virus and antibodies. In their work poliomyelitis viruses and antibodies have been assayed in replicate suspensions of monkey kidney or Hela cells on the basis that recognizable pH differentials develop between infected and uninfected suspensions due to cell respiration differences.

The present authors have reported that vesicular stomatitis virus (VSV) multiplies and produces cytopathogenic changes in guinea pig kidney and bovine tongue tissues

TABLE I. Yields of Vesicular Stomatitis Virus, New Jersey Type, in Tissue Cultures and Cell Suspensions.

Exp. No.	Description of cultures and suspensions* Method§	Kidney	Vol, ml	Medium	Log TC ID_{50} per ml† No. hr incubation at 37°C 0	24	48	72	96	Virus yield ratio,‡ culture/suspension
1	C	Guinea	2	NF	—	*6.45*	5.07	6.20		.32
	S	pig	2	"	—	4.95	5.45	*6.95*		
2	C	"	2	"	3.70	*6.87*	4.87	3.53	2.00	2.20
	S	"	2	"	3.70	5.20	6.30	*6.53*	6.20	
3	C	"	2	"	2.53	*6.20*	5.70	3.10	2.00	8.53
	S	"	2	"	2.53	3.33	5.20	4.29	*5.33*	
	S	"	200	"	1.53	2.00	2.73	4.33	*5.20*	
4	C	"	2	"	2.53	*6.20*	4.30	2.87	2.33	2.89
	S	"	2	"	2.53	3.53	5.08	*5.53*	5.53	
	S	"	2	"	3.53	3.45	*5.87*	5.53	5.20	
	S	"	200	"	2.53	2.53	4.53	*5.87*	5.20	
	S	"	200	"	3.53	3.33	5.53	*5.70*	5.33	
5 (a)	C	"	2	"	3.47	4.98	*5.30*	4.47		.42
	S	"	200	"	3.47	3.62	4.78	5.15	*5.68*	
(b)	C	"	2	TF	3.47	4.62	*5.70*	4.62		7.95
	S	"	200	"	3.47	4.47	4.68	*4.80*	4.52	
6	S	"	200	"	2.53	2.50	2.83	*4.25*	4.17	
7	S	Bovine	200	"	3.80	4.47	4.93	*5.98*		

* NF and TF, see *Materials and methods.*

† Initial values of TC ID_{50} calculated from titers of inocula and dilutions effected. Italicized values represent maxima.

‡ Maximum TC ID_{50} for culture divided by that of the suspension. In Exp. 3 and 4, TC ID_{50} values employed for suspensions are averages of the maxima.

§ C = Culture; S = Suspension.

grown *in vitro*. It was also observed that this virus inhibited respiration in suspensions of the trypsin-dispersed kidney cells(7). This presumptive evidence for the growth of VSV in cellular suspensions has been confirmed in the present report and data have been obtained on the comparative yields of virus from surviving guinea pig and bovine kidney cells in suspension and from guinea pig kidney following its *in vitro* cultivation.

Materials and methods. Cell preparation. Young guinea pigs weighing approximately 250 g served as a source of kidney tissue. Bovine kidney obtained at an abattoir was cooled to approximately 4°C and brought to the laboratory. The kidneys were cut into small fragments and then digested at 37°C with 0.25% trypsin (1:300 Nutritional Biochemicals Corp.) contained in the phosphate buffered saline (PBS) described by Dulbecco and Vogt(8). After 10 minutes the supernatant fluid of the digest was decanted through 3 layers of gauze. Fresh trypsin solution was added to the undigested residue and the digestion and decantation processes were repeated until most of the kidney tissue was consumed. The combined cellular filtrates were centrifuged at 1,000 rpm for 5 minutes and the sedimented cells were then washed three times in PBS at 600 rpm for 2 minutes. The cells were finally suspended in nutrient fluid, 1 part packed cells to 200 parts of fluid. The nutrient fluid used most extensively consisted of Hanks' balanced salt mixture, horse serum and chick embryo extract, 5:3:2; in Table I and the text, this fluid is referred to as medium NF. A fluid consisting of Hanks' salt mixture, horse serum and hydrolyzed bovine plasma albumin (5% aq. Travamine, Baxter Laboratories), 95:3:2, herein referred to as medium TF was used in some instances. Penicillin G, streptomycin sulfate and phenol red were added to give concentrations of 100 units/ml, 0.1 mg/ml and 0.005%, respectively. *Cell suspensions.* Aliquots of the trypsin-dispersed cells in nutrient fluid were introduced into culture tubes (16 mm dia. x 150 mm) or 1 liter Erlenmeyer

flasks, 2 ml and 200 ml, respectively. After inoculation with virus the cell suspensions were incubated at 37°C. Tubes were held in roller drums rotating at 12 rph and each flask was shaken at one vibration per second in an upright position on a shaking machine having a one-inch stroke. Aliquots of the infected suspensions were assayed in tissue cultures for viral content every 24 hours over a 4-day period. The samples assayed represented pooled 0.05 ml aliquots from each of 10 tubes while those from flasks were single unpooled aliquots. *Tissue cultures for virus production.* Two-ml volumes of the trypsin-dispersed cells in nutrient fluid were introduced into culture tubes. These were slanted and incubated at 37°C. After 4 or 5 days the fluids were changed and at 7 days the cultures contained confluent outgrowths of epithelial cells and fibrocytes. Each of 10 cultures was inoculated with virus and at 24-hour intervals a 0.05 ml sample of infected fluid was removed from each tube for pooling and subsequent virus assay in tissue cultures. *Tissue cultures for virus assay.* These were similar to those used for virus production except that in their preparation 0.5 ml rather than 2.0 ml of the cell suspension was introduced into each tube. The assay cultures were used from 8 to 11 days after preparation. Fluid changes were made at 4 or 5 days and again just before use in titrations. In preparation for the bioassays serial 10-fold dilutions of the infectious fluids to be tested were prepared in PBS. One-tenth ml volumes of the viral dilutions were inoculated into the assay cultures, 5 cultures per dilution. After 48 hours incubation, infection of the cultures was read on the basis of cytopathogenicity and the cumulative 50% infectivity doses for tissue culture (TC ID_{50}) were calculated by the Reed and Muench method(9). *Virus.* Virus-containing fluids for inoculation of cell suspensions and cultures were those obtained after 35 or more consecutive passages of VSV, New Jersey (N. J.) type, in guinea pig kidney cultures. Tissue culture ID_{50} values were determined for all except the first used inoculum.

Results. The data in Table I reveal that the yields of virus from guinea pig kidney cell suspensions (Expts. 1-5) ranged from 4.80 to 6.95 log TC ID_{50}/ml for both media and that the lower limit was 5.20 for NF media only. This compares with a nearly identical range of 5.30 to 6.87 log units for virus produced in guinea pig cultures. A second trial (Expt. 6) with guinea pig cells in suspension in medium TF again resulted in a low yield of virus. On the other hand, bovine kidney cell suspensions in medium TF yielded a relatively high virus titer in Expt. 7. While the virus yield ranges obtained by both methods of production were nearly identical, the yield ratio column of Table I shows that for Expts. 3 and 5b the maximum concentration of VSV in cultures exceeded by approximately 8-fold that found in suspensions. The remaining four comparative experiments showed smaller differences in virus yields by the two methods.

Virus yields in cultures most often reached maximum values within the first 24-hour incubation period and declined precipitously (1.35 to 2.0 log TC ID_{50}/ml) within the next 24 hours. An exception is in Expt. 3 where a sharp drop was not revealed until 48 hours after the maximum yield had been recorded. Expt. 5 differs from the others in that the peak in virus concentration was not reached for 48 hours; the decline thereafter was again rapid.

In cell suspensions, the virus yields are not maximal until 72 or more hours after infection. In contrast to the rapid inactivation of virus which occurs in cultures immediately following peak concentrations, the maximum titers recorded in cell suspensions are not materially changed during the subsequent 24-hour period. Also, a 100-fold increase in suspension volumes did not alter the concentration of the virus in the harvested fluids.

Discussion. It is concluded that guinea pig kidney cultures or cellular suspensions infected with VSV, N. J. type, produce approximately equal quantities of virus when medium NF is employed. The recorded 8.53-fold greater yield of virus in culture than in suspension (Exp. 3) is moderated by the fact that the yields of virus produced in the suspensions of this experiment are at the lower

limit of the yield range for the suspension method when medium NF is employed. Furthermore, the highest yield of virus in the present studies was produced in a cell suspension (Exp. 1), exceeding that from the culture of Exp. 3 by a significant amount. For each of the remaining trials with medium NF the yield ratios do not reveal significant differences in virus production. Three-fold differences in TC ID_{50} values are within the chance range expected in the assay of serial 10-fold dilutions of VSV, 5 cultures per dilution.

In the presence of medium TF, guinea pig kidney cultures appear superior to suspensions for VSV production in the one case studied (Exp. 5b). With this medium more virus was produced in suspension when guinea pig kidney cells were replaced by those from the bovine.

It is considered that the delayed production of virus in suspensions may have been concomitant with the possible initiation of cellular growth. However, repeated counts of nuclei by the method of Sanford *et al.*(10) carried out at 24-hour intervals over 5-day periods on uninfected cell suspensions in flasks failed to reveal any appreciable increases in cell concentrations. Quantitative studies which would relate VSV yields to cell numbers for both trypsin-dispersed kidney cells and their progeny in tissue cultures have not been carried out.

Summary. Vesicular stomatitis virus, New Jersey type, was propagated in suspensions of surviving trypsin-dispersed kidney cells of guinea pigs as well as in cultivated epithelial cells and fibrocytes. Both production methods gave nearly equal virus yields, the highest being approximately $10^{6.9}$ tissue culture ID_{50}/ml. The highest virus concentration in cultures and suspensions were most frequently recorded at 24 and 72 hours following infection, respectively. Cell suspensions were increased in volume from 2 ml to 200 ml without altering the per ml virus yields. After peak virus concentrations had been reached, the rate of destruction of virus infectivity upon further incubation was significantly higher for cultures than for suspensions. Vesicular stomatitis virus was also shown to grow in suspensions of dispersed bovine kidney cells.

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