STUDIES ON PERSISTENT INFECTIONS OF TISSUE CULTURES*

II. Nature of the Resistance to Vesicular Stomatitis Virus

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It has been shown in the preceding paper (1) that inapparent, persistent infection of MCN or Lung-To cultures with Newcastle disease (NDV), 6-6, or mumps viruses is readily detected by resistance of the cells to certain cytopathogenic viruses, especially the virus of vesicular stomatitis (VSV). Efforts were made to determine the nature of this resistance. These experiments were largely restricted to study of MCN cultures infected with NDV (referred to as MCN_{NDV}) and only few tests involved also cultures infected with 6-6 or mumps viruses (MCN_{6-6} or MCN_{Mps}). The results are compatible with the suggestion that the resistance induced by persistent infection of the cultures represents an example of viral interference.

Methods and Materials

All methods and materials employed for preparation and maintenance of the tissue cultures as well as for production of stock viruses and viral assays have been described in detail in the preceding paper (1). For *inactivation of NDV by ultraviolet irradiation* the following technic was used. Infected allantoic fluids were dialyzed in cellophane casing against 20 volumes of M/100 phosphate buffered saline (pH 7.2) in the cold room for 16 to 24 hours. Aliquots of 15 to 20 ml. of the dialyzed material were transferred to Petri dishes. These were placed opened on a rocking platform (90 one inch excursions per minute) and exposed to a General Electric germicidal lamp at a distance of 7 inches for 90 seconds. Thereafter the materials were transferred to fresh Petri plates and irradiated again for 90 seconds. The irradiated preparations were then tested for the absence of infectious virus by allantoic inoculation of 6 10-day-old chick embryos each with 0.2 ml. of undiluted or 10- and 100-fold diluted material. The allantoic fluids were harvested after 72 hours and tested for hemagglutinating activity (HA). The diluted inocula regularly failed to yield HA. Following inoculation of the undiluted materials HA activity was always found which represented unadsorbed seed HA. These materials were passed once more without dilution to assure complete absence of active virus.

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The irradiated preparations were kept at 4°C. and used for experimental purposes within 7 to 10 days.

EXPERIMENTAL

Adsorption of VSV on Cells Derived from Persistently Infected MCN Cultures

The resistance to VSV observed in persistently infected MCN cultures could reflect a failure of adsorption of the challenge virus. In order to evaluate this possibility the following experiments were carried out.

In the first experiment aliquots of trypsinized and washed suspensions of MCN, MCN_{NDV}, and MCN_{6-6} cells (2×10^5/ml.) were inoculated with VSV to provide $10^{2.5}$, $10^{4.5}$, and $10^{6.5}$ TCD_{50}/ml., respectively. The mixtures were maintained at room temperature for 60 minutes while being agitated by magnetic stirrers and then centrifuged at 2000 R.P.M. for 30 minutes. The supernates were saved and the cells resuspended in the original volume of fresh medium after being washed twice in Hanks's solution. The various samples were titrated in MCN cultures, using 0.5_{log} steps of dilution, and the 50 per cent infectivity end points were calculated according to the method of Reed and Muench. In another experiment the initial steps were similar but adsorption of VSV was permitted to proceed for 1 or 2 hours and the various samples obtained were assayed by the plaque method on monolayers of MCN cells, using 3 plates each for one of several 10-fold dilutions of the preparations.

The results are summarized in Table I. They failed to show significant differences in the adsorption of VSV by MCN, MCN_{NDV}, or MCN_{6-6} cells. In the first experiment, using the less accurate titration method, from 68 to 96 per cent (average 83 per cent) of the virus was calculated to be adsorbed by subtracting the amounts found in the supernates from the initial virus concentrations. Low and high values were found among both normal and persistently infected cells. The variations may be ascribed to the inaccuracies inherent in the titration method used. In the second experiment employing the plaque technic the results were more uniform. In one hour about 77 and 30 per cent of the virus was absorbed when 1.5×10^5 and 1.78×10^4 plaque-forming units (pfu) were added to the cells, respectively. Somewhat more VSV attached to the cells when the adsorption period was extended over 2 hours.

The virus calculated to be adsorbed could not be recovered to full extent in the cell fraction. In the first experiment the cells had been frozen and thawed, in the second they were disrupted by sonic oscillation. In either case, less than 3 per cent of the inoculum was detected in the cells as infectious virus. This suggests an eclipse of VSV after adsorption onto the cells from normal as well as persistently infected cultures.

Since the viruses involved in the persistent infections belong to the myxogroup, which are capable of destroying cell receptors, additional experiments were carried out with the receptor-destroying enzyme of *Vibrio cholerae* (RDE). Addition of as many as 60 units of this enzyme per ml. of medium 3 times at 4-day intervals prior to challenge did not alter the susceptibility of MCN cultures to VSV.

The Degree and Duration of Resistance to VSV.—In several experiments the degree of resistance of persistently infected cultures to VSV and its duration were analyzed. For this purpose growth curve technics were employed.

Adequate numbers of MCN and MCN_{NDV} or MCN_{Mps} cultures were inoculated with VSV and 4 to 5 of these were then removed from the incubator at each given time interval for assay of VSV in MCN cells.

TABLE I

Adsorption of VSV onto MCN, MCN_{NDV}, and MCN_{5-6} Cells

Exp. No.	Assay method	Initial virus concentration	Multiplicity of Infection	Adsorption period	Amount of virus adsorbed (initial titer − titer in supernate)			Amount of virus found in cells	
					MNC	MCN_{NDV}	MCN_{6-6}	MCN	MCN_{NDV}
				hrs.					
1.	TCD_{50}*	3.2×10^5	15	1	2.77×10^5 86%	2.50×10^5 78%	2.87×10^5 90%	5.6×10^4 1.7%	1.0×10^5 3.1%
		3.2×10^4	0.15	1	2.44×10^4 76%	2.68×10^4 84%	2.68×10^4 84%	1.0×10^3 3.1%	3.2×10^2 1.0%
		3.2×10^2	0.0015	1	2.20×10^2 69%	2.20×10^2 69%	2.64×10^2 82%	n.d.	n.d.
2.	pfu‡*	1.5×10^5	1.0	1	1.16×10^5 77%	1.16×10^5 77%	n.d.§	$<1.0 \times 10^3$ <0.7%	$<1.0 \times 10^3$ <0.7%
				2	1.25×10^5 83%	1.17×10^5 78%	n.d.	1.5×10^0 0.001%	1.5×10^0 0.001%
		1.78×10^4	0.15	1	5.30×10^3 30%	6.20×10^3 35%	n.d.	1.5×10^1 0.08%	$<1.0 \times 10^1$ <0.06%
				2	1.10×10^4 62%	1.06×10^4 60%	n.d.	1.5×10^0 0.008%	9.2×10^0 0.05%

* VSV titrated in MCN tube cultures using 0.5 log steps in dilution and 4 cultures per dilution.

‡ VSV titrated on monolayers of MCN cells and determination of the average number of plaque-forming units (pfu) on 3 plates per dilution.

§ n.d., not done.

The results of a representative experiment are shown in Fig. 1. Following inoculation of 10^4 TCD_{50} of VSV the virus titer in MCN cultures rose rapidly and maximal yields were obtained in 48 hours ($10^{7.2}$ TCD_{50}), at which time the cells were completely destroyed. Thereafter the titers fell at a steady rate of 0.5 $\log_{10}$ units per day, which corresponds to the inactivation rate of VSV at 37°C. In the MCN_{NDV} cells there was no increase for 24 hours. Thereafter, the titer rose to an initial peak in 3 days, which, however, reached only 1 per cent of the maximum seen in the control MCN cultures. After a temporary decline, presumably due to inactivation at 37°C., the titers rose again at the 5th or 6th day reaching levels of 10^6 TCD_{50}/ml. in 10 days. Yet, at this stage no cytopathic effects were noted. At the 14th day the cultures were so crowded that the cells

were reseeded, using one tube to make three, and the observations were continued. Two days after the reseeding the cultures degenerated, the lesions being characteristic of VSV. Another experiment with an inoculum of 10^5 TCD_{50} of VSV gave essentially similar results. VSV growth curve experiments with MCN_{Mps} cells, likewise, were in principal agreement. After inoculation of 10^2 TCD_{50}, the peak of VSV titers in the controls was reached again in 2 to 3 days and thereafter the VSV activity declined at the rate of about 0.5 $\log_{10}$ units per day. In the MCN_{Mps} cells, after a slight primary peak, the titers decreased up

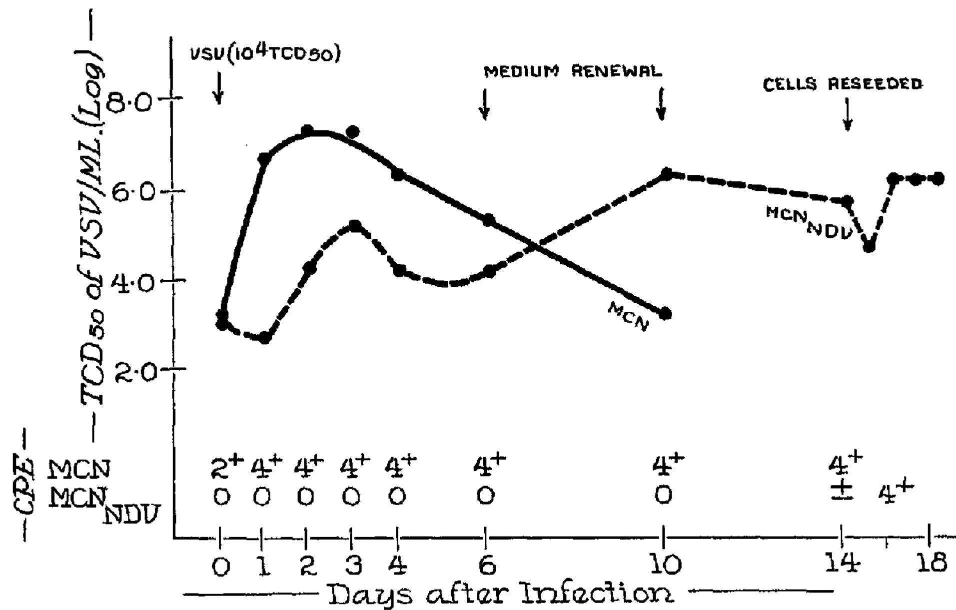

FIG. 1. Growth curve of VSV in MCN and MCN_{NDV} cultures

to the 9th day but then rose again reaching maximal levels by the 13th day with the development of 3 to 4+ cytopathic effects. These results show that resistance to VSV is overcome in time.

The Fate of the Carried Virus.—While the above experiments measured only the results obtained with the challenge virus (VSV) it was of equal interest to determine the fate of the carried viruses. The challenge virus could readily be assayed in MCN cells which are highly susceptible to VSV, and the relatively small amounts of NDV, mumps, or 6-6 viruses present in the challenged carrier cultures did not interfere with the titrations. The reverse, the determination of the carried viruses depended upon the availability of a potent anti-VSV serum since these agents propagated readily only in host systems which also supported the challenge virus (HeLa cells, chick embryos). Antisera to VSV were

produced in guinea pigs by the technic described (1). In experiments with MCN_{NDV} cells and an inoculum of 10^3 TCD_{50} of VSV the following results were obtained. The VSV titers, determined between the 6th and 27th day, were in principal agreement with those shown in Fig. 1, except that with the dose of VSV employed here, which was smaller than in the previous tests, nearly 4 weeks were required for the final destruction of the cell sheets by this agent. During the whole period of observation the NDV titers remained nearly constant, ranging from $10^{3.8}$ to $10^{4.5}$. Just before the cultures were finally destroyed some evidence was obtained of a significant decrease in NDV, although at the day of termination of the experiment the virus could still be detected in appreciable quantities ($10^{2.2}$ EID_{50}/ml.). It is possible that this ultimate decrease was caused by inactivation of NDV at 37°C. during the last 24 or 48 hours of the experiment, after all cells had been destroyed by VSV.

The Effect of pH on VSV Infection.—During the course of the work with VSV in MCN and Lung-To cells irregular results were obtained on occasion. For instance, in the preparation of seed lots of VSV in MCN bottle cultures it was noted that cellular destruction may fail to materialize, or that repeated titrations of the same seed lot, kept at −70°C. until used, yielded titers in MCN cells varying over a 100-fold or greater range. In analyzing these variable data it was realized that the following factors played a role in the reproducibility of the results. Young MCN cultures yielded usually higher VSV titers than those having been kept for many days. If medium renewals were widely spaced (8 to 14 days), the virus activity was reduced as compared to that in cultures which were fed at more frequent intervals (3 to 5 days). Infection of cultures immediately after medium renewal gave higher virus titers than inoculation of cells which had last been fed 6 or more days previously. If young cells were supplied with medium which had been removed from more than 8-day-old cultures, they yielded less virus than when fresh medium was added. These various observations suggested that either the media were depleted in time of essential nutrients required for VSV replication or that a lowering of the pH in the media, the inevitable result in actively metabolizing cultures, had an adverse effect on VSV. The latter possibility could readily be tested and the results support the validity of this alternative.

An adequate number of test tube cultures of MCN cells was prepared in the usual way by seeding approximately 10^5 cells per tube. On the 6th day of incubation the cultures were divided into 4 groups and refed with media, the pH of which had been adjusted by sodium bicarbonate to about 6.6, 6.9, 7.2, and 7.5 respectively. Immediately thereafter the tubes were inoculated with VSV diluted from 10^{-2} to 10^{-8} using 6 cultures per dilution and leaving a few cultures of each group as uninoculated controls. The tubes were observed daily for cytopathic effects. On the 3rd day after infection 2 tubes of each virus dilution of the 4 groups were removed and stored at −20°C. The pH was then adjusted in half of the remaining cultures to about 7.6 by the addition of bicarbonate and the observations were continued to the 5th day, when all cultures were placed in the freezer until titrations for VSV were carried out.

The results are presented in the upper part of Table II. At a starting pH of 6.6 no cytopathic effects were seen after 3 or 5 days of incubation, even when as many as 10^6 TCD_{50} of VSV were inoculated. If the pH of the medium was adjusted to 7.6 on the 3rd day, lesions rapidly developed. The concentrations

TABLE II

Effect of pH of the Medium upon Infection of MCN and MCN_{NDV} Cells with VSV

Cells	Inoculum	pH of medium at time of inoculation														
	Dilution of VSV	6.6					6.9					7.2				
		3rd day			5th day		3rd day			5th day		3rd day			5th day	
		CPE*	VSV‡	pH+ adjusted	CPE	VSV	CPE	VSV	pH adjusted	CPE	VSV	CPE	VSV	pH adjusted	CPE	VSV
			TCD_{50}/ml. log			TCD_{50}/ml. log		TCD_{50}/ml. log			TCD_{50}/ml. log		TCD_{50}/ml. log			TCD_{50}/ml. log
MCN	10^{-2}	0		−	0		4		−	4		4		−	4	
				+	4				+	4				+	4	
	10^{-3}	0	5.7	−	0	3.7	4		−	4		4	7.2	−	4	6.7
				+	4	7.7			+	4				+	4	
	10^{-4}	0	3.7	−	0	3.7	2–3		−	3		4	7.2	−	4	7.2
				+	4	7.7			+	4				+	4	
	10^{-5}	0	2.7	−	0	3.2	±	7.2	−	±	7.2	4	6.7	−	4	6.2
				+	4	7.2			+	4	7.2			+	4	
	10^{-6}	0	1.7	−	0	0.7	0	6.7	−	0	5.7	4	7.2	−	4	6.7
				+	4	8.2			+	4	6.7			+	4	6.2
	10^{-7}	0	1.2	−	0	<0.2	0	5.2	−	0	4.7	4	8.2	−	4	7.7
				+	4/0	7.2			+	4	7.2			+	4	
	10^{-8}	0		−	0		0		−	0		0 or 4		−	4	
				+	0				+	0/4				+§	0	
MCN_{NDV}	10^{-3}	0		−	0		0		−	0		0		−	0	
				+	(±)				+	±–1				+	3	
	10^{-4}	0	<2.2	−	0	<2.2	0	<2.2	−	0	<2.2	0	3.7	−	0	3.7
				+	(±)	<2.2			+	±	4.2			+	1–2	4.2
	10^{-5}	0	<2.2	−	0	<2.2	0		−	0		0		−	0	
				+	(±)	<2.2			+	±				+	±	
	10^{-6}	0	<1.2	−	0	<1.2	0	<1.2	−	0	<1.2	0	2.2	−	0	<1.7
				+	0	<1.2			+	0	3.2			+	0	2.7
	10^{-7}	0		−	0		0		−	0		0		−	0	
				+	0				+	0				+	0	

\+ pH adjusted on 3rd day in half of the tubes to 7.4 by addition of sodium bicarbonate.

* CPE, cytopathogenic effect; 0–4 varying degrees of cellular destruction.

‡ Number of 50 per cent infectivity doses (log/ml.) as assayed in MCN cultures.

§ Only culture which showed no lesions on 3rd day.

of VSV measured in the various cultures at the 3rd and 5th day were in agreement with the microscopic data. The titers determined after 3 days were low and indicated only slight degrees of viral propagation. In the absence of pH adjustment the titers remained of the same order, or decreased to some extent in accordance to the inactivation rate of VSV at 37°C. However, when the pH was raised on the 3rd day the virus propagated to high titer in the ensuing 48 hours in all cultures except those inoculated with the 10^{-8} dilution of VSV. At an initial pH 6.9 inhibition of the development of VSV lesions was evident only when the inoculum was small and, correspondingly, the virus titers found after 3 and 5 days were markedly higher. Wherever inhibition was apparent it was overcome by the raise in pH on the 3rd day. Since infection at pH 7.2 and 7.5 gave closely similar results only the former series is presented in the table. Complete cellular destruction was noted within 3 days in all cultures except for a few inoculated with the 10^{-8} dilution of VSV. The virus titers were high and in part probably past their peaks (see Fig. 1). On further incubation, with or without adjustment of pH, the titers showed on the whole slight declines. The cultures in the 10^{-8} group showing no lesions on the 3rd day remained healthy also after raising of the pH. It is apparent then that the pH of the medium exerts a profound effect on the propagation of VSV.

These results were of obvious importance with respect to the challenge with VSV of persistently infected cultures. As pointed out in the preceding paper (1), medium renewal prior to challenge with VSV was introduced as a routine procedure in order to infect the culture at an optimal pH. The fact remains, however, that the carrier cultures exhibit increased aerobic glycolysis (2) which might provide an explanation of the resistance to VSV. The experiment just recorded was extended, therefore, to include VSV infection of MCN_{NDV} cells under the same experimental conditions and the results are presented in the lower part of Table II. It is evident that the pH dependence of VSV infection may add to the degree of resistance of MCN_{NDV} cells but it does not seem to account entirely for this phenomenon. In the pH 6.6 group no cytopathogenicity was noted in 5 days as expected. Adjustment of the pH after 72 hours produced 2 days later at most insignificant lesions and titrations on the 5th day failed to reveal VSV in the lowest dilutions tested. In the pH 6.9 series the results were similar except that some VSV multiplication could be discerned following adjustment of the pH of the medium. Finally, the cultures inoculated at pH 7.2 or 7.5 remained apparently healthy for 5 days. However, on adjustment of the pH at the 3rd day the larger concentrations of VSV produced partial though significant cytopathic effects. Yet, the VSV titers remained of a low order.

In further experiments, conducted by Dr. Green, lactic acid or lactate was added to the media of uninfected MCN cultures in concentrations higher than those found, as a rule, in carrier cultures. These additions did not affect the susceptibility of the cells to VSV as long as the pH remained above neutrality.

Resistance Induced in MCN Cells by Inactivated NDV

The above experiment showed that the VSV activity was highly dependent on the pH of the media. Yet, the resistance of MCN_{NDV} cultures to VSV could not be ascribed entirely to the increased lactic acid production of the persistently infected preparations. The observations that VSV is adsorbed onto MCN_{NDV} cells (Table I) and that resistance is transitory (Fig. 1.) are consistent with the suggestion that viral interference is involved in this phenomenon. If correctly interpreted, addition of inactivated NDV in sufficient quantities to MCN cultures likewise should result in protection against VSV. This was indeed the case, as will be shown below.

Allantoic fluid preparations of the Victoria strain of NDV were inactivated by ultraviolet light as described in the section on materials and methods. This preparation was free of active virus and gave a high degree of protection in chick embryos when challenged with 10^4 EID_{50} 24 hours after the interfering dose, both injections being given by the allantoic route. Subsequently, 3 groups of 40 MCN cultures each containing between 5×10^5 and 1×10^6 cells were inoculated with the irradiated material so as to provide the equivalent of 10^8, 10^6, and 10^4 EID_{50} per tube, respectively. Ten of the cultures in each group were then challenged with 100 TCD_{50} of VSV after 1, 3, and 7 days of incubation. The remaining 10 cultures in each group were observed daily for absence of cytopathic effects due to the interfering virus preparation. At each time of challenge 10 previously uninoculated MCN cultures served as VSV controls.

The results of this experiment are shown in Table III. Cultures showing less than 2+ VSV degeneration were considered resistant and the rate of protection given in the table is based upon these criteria. In order to evaluate, in addition, the degree of protection, the lesions from $\pm$ to 4 were added in each group and this lesion score is also shown in the table. If the interfering dose contained the equivalent of 10^8 EID_{50} of inactivated virus, resistance was complete in 24 hours and no change occurred up to the 7th day. With an equivalent dose of 10^6 EID_{50} of NDV all cultures challenged in 24 hours still revealed evidence of VSV infection but the lesions developed more slowly than in the controls and some of the cells survived. In 3 days protection was complete. The smallest dose of interfering virus (10^4 EID_{50}) was without effect. These data showed that solid resistance to VSV can be induced by ultraviolet-inactivated NDV. Since interference was observed only when the equivalent of 10^6 or more EID_{50} of inactivated virus was added and since the cultures contained a nearly equal number of cells, it follows that the equivalent of at least one EID_{50} was required per cell to obtain this result.

It was noted in the above experiment that the cells continued to multiply. Since part of the interfering dose was not adsorbed, inactivated virus remained available for the cell progeny so that interference could be induced in them in turn. It was of interest, therefore, to remove or neutralize the excess inactivated virus after given incubation periods.

Adequate numbers of MCN cultures were inoculated with an irradiated NDV preparation providing the equivalent of 10 EID_{50} per cell. Group 1 was not handled any further. The cultures of groups 2 and 3 were washed after 2 and 24 hours, respectively, and fed with fresh medium. To the tubes of groups 4 and 5 anti-NDV serum was added after 2 and 24 hours, respectively, in sufficient quantity to neutralize all non-adsorbed interfering virus. The 6th group received the inactivated virus mixed with antiserum. Ten cultures each of the various groups were then challenged with 1000 TCD_{50} of VSV 1, 3, 7, and 14 days after the interfering dose.

TABLE III

Induction of Resistance in MCN Cultures by Ultraviolet-Inactivated NDV

Dose of inactivated NDV in equivalents of EID_{50}	Resistance of MCN cultures to 100 TCD_{50} of VSV					
	Days after interfering dose					
	1		3		7	
	No. resistant	Lesion score*	No. resistant	Lesion score	No. resistant	Lesion score
10^8	10/10‡	0	10/10	0	10/10	0
10^6	0/10	40§	10/10	0	10/10	3
10^4	0/10	40	0/10	40	0/10	40
None	0/10	40	0/10	40	0/10	40

* Sum of VSV lesions in the 10 cultures of each group.

‡ 10 out of 10 cultures protected; *i.e.* $<2+$VSV lesions in 5 days after challenge.

§ Delay in appearance of VSV lesions.

The results are shown in Table IV. It is seen that if the residual-free interfering virus was permitted to remain in the cultures, protection was solid for at least 7 days, but a slight breakthrough of VSV was noted in the group challenged at 2 weeks. If the cultures were washed or antiserum was added 2 hours after the interfering dose, protection was highest on challenge in 24 hours but decreased rapidly thereafter. Washing or addition of antiserum 24 hours after the inactivated virus gave somewhat better protection for a slightly longer period of time. The mixture of the inactivated virus and antiserum failed to induce any resistance.

These results showed that contact with interfering virus for a short period of time induced only a temporary protection of the cultures. The data do not imply, however, that resistance in any given cell is of only short duration. The cell counts obtained from representative groups 1, 3, 7, and 11 days after the interfering dose showed that cellular division was not inhibited. With a generation time of 48 hours new cells become available at a sufficient rate to account possibly for the degree of cellular degeneration, if at least part of the daughter cells are no longer resistant to VSV. This intriguing problem deserves further study. It would be essential to explore (*a*) the duration of interference in the

absence of cellular multiplication as possibly achieved by maintenance of the cultures in a deficient medium; and (*b*) the effect of multiplicity of adsorption of inactivated NDV on the carry-over of resistance to daughter cells. Such studies are now in progress.

TABLE IV

The Effect of Washing or Antibodies upon the Resistance to VSV Induced in MCN Cultures by Inactivated NDV

Treatment of MCN cultures after addition of inactivated NDV (equivalent of 10 EID_{50}/cell)	Resistance of MCN cultures to 1000 TCD_{50} of VSV							
	Days after interfering dose							
	1		3		7		14	
	No. resistant	Score*	No. resistant	Score	No. resistant	Score	No. resistant	Score
None	10/10‡	0	10/10	0	10/10	0	10/10	15
Washed after 2 hrs.	10/10	11.5	2/10	24	0/10	40	0/10	40
Washed after 24 hrs.	10/10	0	10/10	4.5	0/10	25	0/10	40
Immune serum after 2 hrs.	10/10	10	3/10	18.5	0/10	34.5	0/10	40
Immune serum after 24 hrs.	10/10	0	10/10	3	10/10	14	0/10	40
Immune serum mixed with interfering dose	0/10	40	0/10	40	0/10	40	0/10	40
Uninoculated control cultures	0/10	40	0/10	40	0/10	40	0/10	40

* Sum of lesions in the 10 cultures of each group.

‡ 10 out of 10 cultures protected; *i.e.* <2+VSV lesions 5 days after challenge.

DISCUSSION

The data presented are concerned with the striking resistance to the virus of vesicular stomatitis (VSV) exhibited by MCN cultures persistently infected with Newcastle disease, mumps or 6-6 viruses (1). It was found that this resistance is not caused merely by failure of adsorption of VSV. Cells derived from uninfected or carrier cultures adsorbed similar quantities of the challenge agent as determined by the difference in the total amount added and that remaining in the supernate after removal of the cells at the end of the adsorption period. Furthermore, only a fraction of the VSV units, calculated to be adsorbed, was recovered from the cells, regardless of their origin. This indicates that an eclipse occurs with VSV in cells, whether derived from normal or persistently infected cultures. These results deny the possibility that the receptor-destroying activity of the myxoviruses present in the infected cultures prevents the attachment of VSV. This conclusion is supported also by the failure of the receptor-destroying enzyme of *V. cholerae* (RDE) to render MCN cells resistant to VSV.

The increased lactic acid formation in the persistently infected cultures (2), likewise failed to provide a satisfactory explanation for the resistance to VSV. Although it was found that the propagation of VSV in normal MCN cells is highly pH-dependent in that below neutrality the development of virus and cytopathic effects is greatly reduced or even completely inhibited, maintenance of MCN_{NDV} cultures at suitably high pH levels did not abolish the resistance to VSV. Thus the increased aerobic glycolysis may possibly contribute to the resistance but does not appear to be the only or even major cause of it.

The resistance to VSV of persistently infected MCN cultures is not absolute. It was seen in the growth curve experiments that apparently a few cells are capable of supporting VSV as evident from the low peaks in VSV titers obtained by about the 3rd day following challenge (Fig. 1.). The resistance is also not permanent and the cultures ultimately succumb to the VSV superinfection in 2 to 4 weeks, depending upon the dose of challenge virus. During this whole period, the carried viruses remain detectable in unchanged concentrations until shortly before final destruction of the cultures by VSV.

Resistance to VSV can be induced in MCN cells also by addition of NDV inactivated by ultraviolet light. This was achieved as long as the equivalent of at least 1 EID_{50} of inactivated virus was provided per cell. Under these minimal conditions resistance is not solidly established in 24 hours but certainly by the 3rd day. The cells rendered resistant by irradiated NDV continue to divide as do those present in persistently infected cultures (1). The new generations of cells, if not in part resistant, become insusceptible by adsorption of residual-free inactivated virus particles present in the media. If the non-adsorbed irradiated virus is removed after incubation periods of 2 to 24 hours, the cultures gradually regain susceptibility to VSV. This may be explained on the basis that resistance in individual cells is transitory and/or that the daughter cells derived from resistant cells are in part or all susceptible to VSV. The breakthrough of VSV long after inoculation into persistently infected cultures may be explained on the same basis, gradual loss of resistance with time or addition of new susceptible cells by division of resistant ones. In competition for the developing susceptible cells VSV gains gradually the upper hand to the detriment of the carried virus. The transitory nature of resistance is evident also from the results of cloning experiments with cells from MCN_{NDV} cultures (1). None of the cloned lines were found to be resistant to VSV.

All these results are compatible with the conclusion that the resistance of persistently infected MCN cultures to certain cytopathogenic viruses represents an example of viral interference. (*a*) The resistance is non-specific, in that it extends to at least 3 viruses, VSV, herpes simplex and influenza A (1); (*b*) it is transitory; (*c*) it is induced by inactivated virus; (*d*) it requires time for full establishment; (*e*) it is not related to failure of adsorption of the challenge virus; and (*f*) it appears to be due to an intracellular block since the challenge virus

is adsorbed and enters into an eclipse. These various facts match similar observations made in well documented examples of interference such as that between influenza viruses in the chick embryo (see reference 3). The possible role of interference in persistent infection of Earle's L cells with equine encephalomyelitis virus has been suggested by Chambers (4).

SUMMARY

Efforts were made to elucidate the nature of the resistance to vesicular stomatitis virus (VSV) observed in MCN cultures persistently infected with Newcastle disease, mumps, or 6-6 viruses (MCN_{NDV}, MCN_{Mps}, and MCN_{6-6}, respectively).

Cells derived from persistently infected cultures adsorbed VSV to the same extent as their uninfected counterparts. Only a fraction of the adsorbed virus could be recovered from the cells indicating that it enters into an eclipse in all of the cell types.

While propagation of VSV in MCN cells is largely inhibited at low pH levels, the resistance of persistently infected cultures could not be ascribed to their increased lactic acid formation.

Resistance was not absolute in that a few cells in persistently infected cultures apparently supported VSV reproduction. Furthermore resistance of the cultures was found to be transitory in that the VSV infection gradually gained the upper hand after 2 to 4 weeks of incubation.

Addition of ultraviolet-inactivated NDV to MCN cultures induced resistance to VSV as long as the equivalent of at least one ID_{50} (for chick embryos) of inactivated virus was provided per cell. Establishment of resistance required some time and its duration depended upon whether or not the free inactivated NDV was removed or neutralized after given adsorption periods.

The transitory nature of resistance in persistently infected cultures, or in MCN cells following adsorption of inactivated NDV, is most likely explained by the fact that the cells continue to divide and that the daughter cells are, at least in part, susceptible to VSV.

The results are compatible with the conclusion that the resistance observed represents another example of interference between 2 viruses.

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