STUDIES ON PERSISTENT INFECTIONS OF TISSUE CULTURES*

V. The Initial Stages of Infection of L(MCN) Cells by Newcastle Disease Virus

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Cultures of Earle's strain L cells or a subline of these cells, L(MCN),[1] readily sustain persistent infections following inoculation of Newcastle disease (NDV), mumps, or parainfluenza viruses (1–3). Previous reports of this series (1, 4–6) were concerned mainly with an analysis of the established carrier state and restricted largely to NDV infections. The results in brief were as follows.

Infectious virus was recoverable at all times from carrier cultures in concentrations ranging from 10^2 to 10^6 infectious units. Most of the virus was cell-associated and generally less than 10 per cent of it was found free in the media. Although on the average no more than one in 50 cells was infected, *i.e.* capable of yielding infectious progeny, the remaining cells were highly resistant to infection with vesicular stomatitis virus (VSV) and other viruses (1). On plating of intact or sonically disrupted cells similar numbers of plaques were obtained on monolayers of chick embryo fibroblasts. This indicated that each infected cell contained no more than one plaque-forming unit (pfu) of NDV at any given time and that production of infectious virus proceeded at a very slow rate (5). Indeed, upon transfer of a few infected cells among many non-infected ones to fresh L(MCN) cultures, the infection so introduced led merely to doubling of infected cells every 6 to 8 hours. At the time when 1 to 2 per cent of the cell population had become infected, the cultures were totally resistant to superinfection with VSV (5).

Addition of anti-NDV serum to carrier populations rapidly decreased the titers of virus in medium and cells as well as the resistance of the culture to superinfection with VSV (1). However, when the antiserum was omitted from the media after periods as long as 2 to 3 months virus often became again detectable. Cured cultures behaved like normal cell populations in every respect tested, as did virus-free clones derived from carrier cultures in the presence of anti-NDV serum. None of these was resistant to VSV and all became again persistently infected upon reexposure to NDV (1). Thus, no selection of cells had occurred.

The resistance of carrier cultures to superinfection by VSV as well as to the carried virus presented all the characteristics of classical interference. Resistant cells adsorbed challenge

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[1] The MCN cells thought to be derived from the bone marrow of a leukemic patient (7) turned out to be of murine origin and identical with Earle's strain L cells (8). They are designated now as L(MCN) cells.

virus to the same extent as did normal cells (4). After adsorption, the challenge virus went into eclipse; only a small fraction of the added virus could be recovered from the cells at the end of the exposure period. It was not clear, however, how interference was established. The amount of infectious virus in the persistently infected cultures was small at all times. Although the production of non-infectious, hemagglutinating virus particles following primary infection of L cells with NDV had been reported (9), such components, if present in carrier cultures, failed to reach detectable levels. With the discovery of interferon (10), it seemed possible that such a substance might account for the resistance observed in the persistently infected cultures. Indeed, interferon was found in the medium of carrier cultures, but only in very low concentrations (6).

It was of interest to determine to what extent interferon and non-infectious viral components contribute to the maintenance of the persistent infection. Because of the low level of the infectious process and the relative insensitivity of hemagglutinin and interferon assays, it seemed unlikely that analysis of the equilibrium stage would readily furnish an answer. Study of the early phases of infection leading to the establishment of the carrier state was thought more likely to provide the desired information, since cytopathic effects observed after the initial exposure of the cultures to adequate concentrations of NDV indicated a relatively high rate of cellular infection and viral replication (1, 9). The results of these efforts are reported below.

Materials and Methods

Cells.—L(MCN) cells were grown in a medium consisting of 30 per cent Parker's solution 199, 60 per cent Scherer's maintenance solution, 10 per cent horse serum inactivated at 56°C for 40 minutes, and penicillin and streptomycin (100 units and μg per ml, respectively). The pH of the medium was adjusted with sodium bicarbonate. Stock cultures were maintained in Blake bottles seeded with 5×10^6 cells in 25 ml of medium at pH 7.0. Cells were refed 4 days after seeding with medium at pH 7.4 and passed or used for experimental purposes after 1 week of total incubation at 37°C. Test tube cultures, with or without coverslips, were prepared by seeding 1×10^5 cells in 1 ml volumes. For plaque assays, 4×10^6 cells in 5 ml medium were transferred to plastic flasks (Falcon Plastic Company, Los Angeles) and inoculated 16 hours later.

Viruses.—The Victoria strain of Newcastle disease virus was used after reisolation from L(MCN) carrier cultures and 2 to 4 allantoic passages in chick embryos. The virus was sedimented from the allantoic fluid by centrifugation at 29,000 RPM for 1 hour, resuspended in culture medium, and distributed in 1-ml amounts into ampuls, which, after shell freezing in a dry-ice alcohol bath, were stored at −65°C until used. Occasionally, allantoic fluid seeds were employed. The infectivity titers ranged from 4.0 to 7.9×10^9 pfu per ml, the hemagglutinin (HA) titers from 1.3 to 2.6×10^8 units per ml, and the pfu/HA ratios from 2.7 to 3.1×10^6. This virus will be referred to as standard NDV or NDV_{ST}. Other types of NDV seed will be described in the text.

The Indiana strain of VSV was obtained from the American Type Culture Collection and seed lots were prepared either from infected L(MCN) or chick embryo fibroblast cultures after 2 or 3 passages in the respective systems. The titers ranged from 4.0×10^7 to 1.8×10^8 pfu per ml when tested on monolayers of L(MCN) cells.

Infectivity Assays.—NDV titers were determined by plaque assays in monolayers of chick embryo fibroblasts, prepared as described (1) except that plastic flasks were used instead of

Petri plates. The cultures were inoculated with 0.4 ml amounts of appropriate virus dilutions, incubated for 1 hour at 37°C, and then overlayed with 8 ml of medium consisting of 30 per cent Parker's solution 199, 50 per cent Scherer's maintenance solution, 20 per cent inactivated horse serum, 1.2 per cent Difco agar, neutral red 1:40,000 (final concentration), and penicillin and streptomycin. The pH was adjusted to 7.4 by addition of sodium bicarbonate. Plaques were counted after incubation at 37°C for 3 and 7 days and the results expressed as the number of pfu per milliter on the 7th day.

VSV titers were determined by plaque assays on monolayers of L(MCN) cells prepared as described above. The overlay employed after inoculation was the same as used for chick embryo fibroblasts except that the pH was adjusted to 7.6. Plaques were counted 2 and 4 days after inoculation and the number of pfu per milliter was calculated from the 4th day readings.

Hemagglutinin Titrations.—These were carried out as described (1).

Interferon Assays.—Materials for interferon assays were dialyzed overnight at 4°C against 40 volumes of 0.05 M phthalate buffer at pH 2.2. The samples were then dialyzed twice against 40 volumes each of 0.01 M phosphate-buffered saline at pH 7.2 for 8 and 16 hours, respectively. The samples were stored at −20°C until assayed, usually within 2 weeks.

Serial 2-fold dilutions of the specimens were made in culture medium and 0.5 ml volumes of given dilutions were transferred into 3 to 4 plastic flasks each. After addition of 4.5 ml of an L(MCN) cell suspension (9.0×10^5 cells per ml) and thorough mixing, the cultures were incubated for 16 hours when the media were removed. Following inoculation of about 40 pfu of VSV in 0.4 ml of Hanks' solution, the monolayers were overlayed and further handled as described above. The highest dilution of interferon reducing the number of plaques by at least 50 per cent was taken as the end-point.

Anti-NDV Serum.—Rabbits were injected 5 times at weekly intervals with 1 ml amounts of a suspension of NDV in Hanks' solution containing 3.1 to 6.0×10^9 pfu per ml. Beginning 1 week after the last dose and for several weeks thereafter blood was withdrawn by cardiac puncture and the sera were separated. The antibody titers obtained ranged from 1.0 to 2.0×10^4 50 per cent plaque-inhibiting units per ml when tested against 20 to 30 pfu of NDV on chick embryo monolayers.

Fluorescent Antibody Staining.—The gamma globulin fraction of rabbit anti-NDV serum was separated by ammonium sulfate precipitation and coupled to fluorescein isothiocyanate by conventional means (11). The labeled gamma globulin was adsorbed twice with mouse liver powder before use. Coverslips removed from culture tubes were washed in phosphate-buffered saline solution of pH 7.2, thoroughly dried at 37°C, fixed in acetone, and stored at −20°C. Staining with fluorescent conjugate proceeded at 37°C for 1 hour and after thorough washing and 2 final dips into distilled water the coverslips were mounted on slides with a semipermanent mounting medium (12). The percentage of cells showing virus-specific immunofluorescence was determined by counting cells to a total of 1000.

Other techniques are described in the Experimental section.

EXPERIMENTAL

Factors Influencing Adsorption of NDV and Infection of L(MCN) Cells.—The contemplated studies required observations on many cultures infected in parallel for evaluation of the course and extent of infection by virus-specific immunofluorescence, yields of viral progeny and interferon, and cellular growth rates. In order to provide for greatest possible uniformity of the infected cultures it seemed best to expose suspended cells in bulk to the virus and then to prepare with them the appropriate number of cultures. This plan necessitated

preliminary experiments to evolve standard, reproducible procedures for infection.

Suspensions of counted L(MCN) cells, usually 25-ml volumes, were exposed to NDV_{ST} at known input multiplicities and incubated with agitation at temperatures ranging from 24–37°C for 1 to 2 hours. As controls, virus was inoculated into corresponding volumes of cell-free medium. At the end of the adsorption period the preparations were centrifuged at 800 RPM for 10 minutes. Samples of the supernates were stored at −20°C for virus assay to be performed usually within 1 week. The sedimented cells were washed three times, the second time with the addition of anti-NDV serum 1:25 in order to remove or neutralize remaining extracellular virus. The cells were then resuspended in medium, counted, adjusted to 1×10^5 per ml, and seeded into culture tubes containing coverslips. The coverslips were harvested from 12 to 48 hours later and stained with labeled anti-NDV rabbit gamma globulin. The degree of adsorption of virus was calculated by subtracting the number of pfu found at the end of the adsorption period in the supernate of the cell suspensions from that noted in the cell-free controls. No inactivation of virus became evident in the virus-medium mixtures during incubation at 37°C for 2 hours. From these data were computed the adsorption multiplicity, the efficiency of adsorption, and the percentage of cells expected to be infected according to Poisson's formula. The percentage of actually infected cells was determined by counting the number of immunofluorescent cells among a total of 1000 cells.

The results of various experiments may be summarized as follows. Adsorption of virus was of the same order whether the suspended cells were exposed at 24°, 30°, or 37°C. Infection of the cells, as determined by virus-specific immunofluorescence 24 hours later, was markedly influenced, however, by the temperature maintained during exposure. Penetration of virus into the cells occurs readily only at 37°C (13) and thus, at the lower temperatures, cell-attached virus remained accessible to the viral antibodies employed during washing of the cells prior to seeding of the cultures. The efficiency of adsorption depended upon the relative concentration of the cells. It increased from <10 to about 50 per cent as the number of cells was raised from 1 to 5×10^5 per ml. Further increases failed to improve significantly the efficiency of adsorption. Maximal attachment of virus was evident usually within 1 hour. The initial reproductive cycle of NDV_{ST} in L(MCN) cells extended over 24 hours and secondary spread of the infection became noticeable only thereafter. These results led to the establishment of a standard procedure for all experiments to be reported. Suspensions of cells at a concentration of about 5×10^5 per ml were exposed at 37°C for 1 hour to NDV at an input multiplicity double that of the desired adsorption multiplicity. All other steps remained as described above. As shown in Table I this technique yielded results which were reproducible and predictable within a narrow range. It is seen that the number of cells calculated to be infected from the adsorption data matched reasonably well the results of fluorescent antibody staining at 24 hours; *i.e.*, at the end of the initial infectious cycle. It became also evident that under appropriate conditions all cells could be induced to synthesize viral antigen.

The Initial Stages of Infection.—Several experiments were carried out to

determine cellular growth rates, viral replication, and interferon production in relation to the multiplicity of infection by NDV_{ST}. Of these experiments, two will be presented in detail.

In the first experiment, cells were infected by the standard procedure to achieve actual multiplicities of infection of 0.1, 1.0, and 10.0. Uninfected cells were carried in parallel. Adequate numbers of cultures with and without coverslips were prepared from each pool of cells and these were harvested at 6 (for cell count only), 24, 48, 72, and 168 hours. At each time

TABLE I

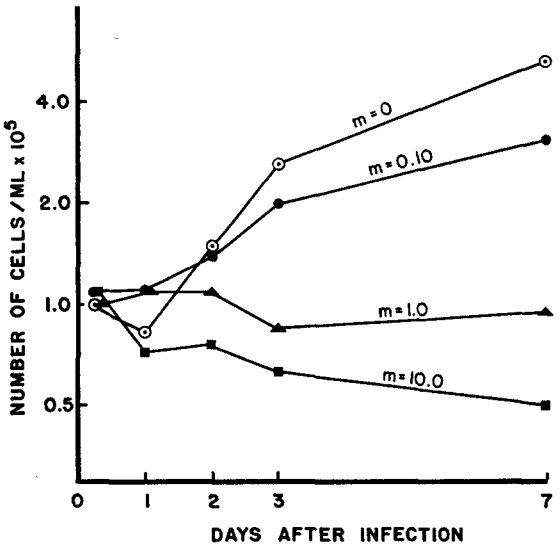
Adsorption of NDV_{ST} and Infection of L(MCN) Cells under Standard Conditions of Exposure

Input multiplicity	Absorption multiplicity	Efficiency of adsorption	Cells infected (from adsorption)	Cells infected (from 24 hr. staining)
		per cent	*per cent*	*per cent*
20.0	12.0	60	100	98
18.0	12.0	67	100	97
10.0	4.7	47	99	99
8.4	4.7	56	99	99
7.2	2.8	39	94	94
5.1	3.8	75	98	90
3.6	1.2	33	70	61
3.6	1.5	42	78	73
2.0	0.8	40	55	66
1.8	0.9	50	59	48
1.8	0.9	50	59	52
1.4	0.7	50	50	43
1.3	0.6	46	45	45
0.3	0.1	33	9	17
0.2	0.1	50	9	7
0.2	0.1	50	9	10

coverslips were removed from 4 tubes for immunofluorescent staining. After removal of the coverslips the tubes were refed with medium at pH 7.4 containing about 100 pfu of VSV to ascertain the degree of interference established by the time of harvest. The cells of 10 cultures without coverslips were scraped into the medium, pooled, and counted. Samples of the pool were frozen and thawed 4 times and stored at −20°C for plaque titrations on chick embryo fibroblast monolayers and interferon assays on monolayers of L(MCN) cells.

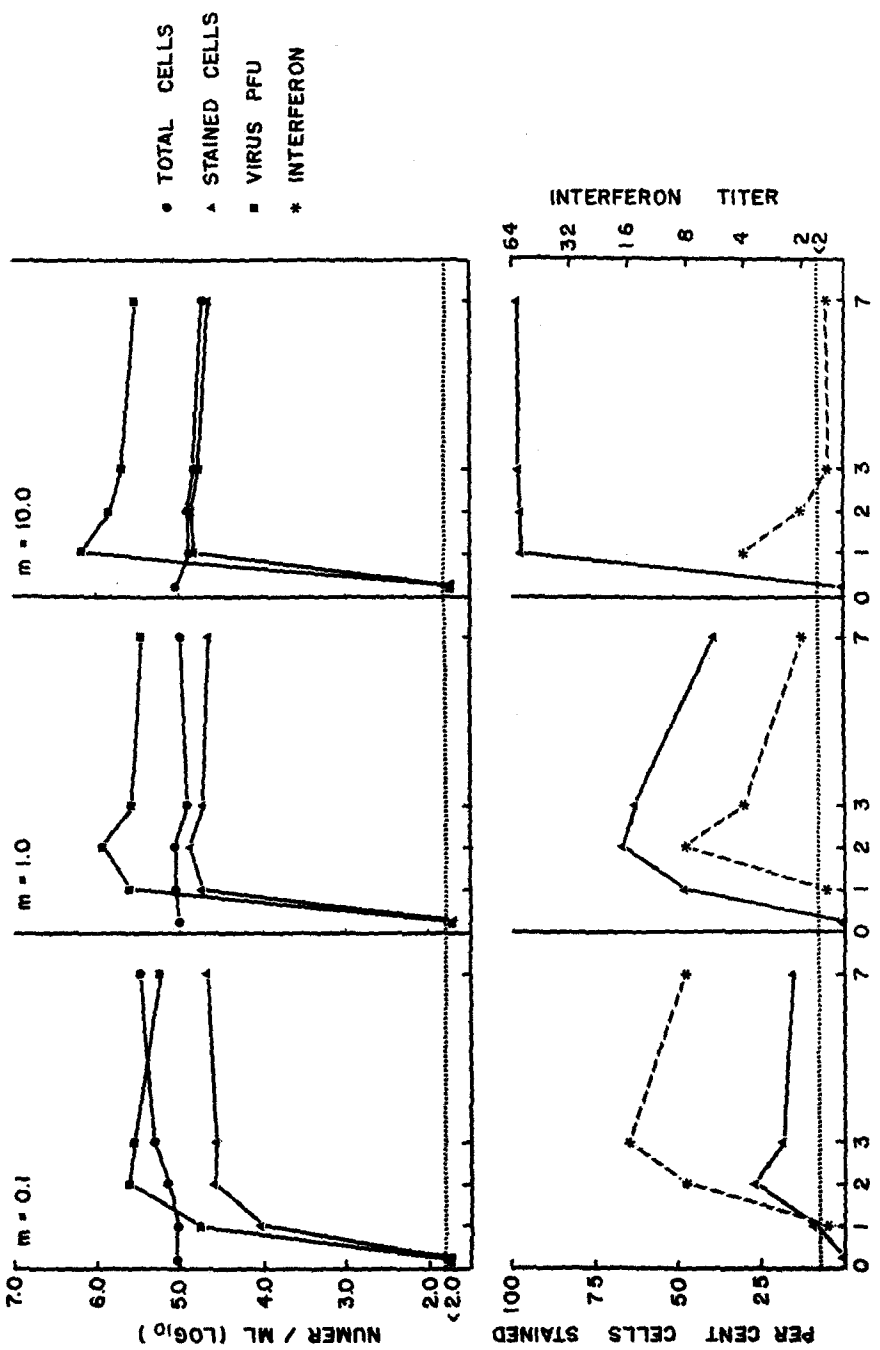
The results of cell counts are presented in Text-fig. 1. It is seen that the control cultures, presumably because of the extensive handling, remained stationary for 24 hours but then began to grow rapidly. The growth rates of the infected cell populations were reduced in agreement with earlier observations (1) and they were the more affected the higher the multiplicity of infection. At a multiplicity of 10 many of the cells remained attached to the glass, but their number gradually declined over a period of 7 days and the cultures eventually died. At the lower multiplicities the cultures survived, showing persistent viral infections.

Text-Fig. 2 shows the number and per cent of immunofluorescent cells, viral growth curves, and interferon production in the 3 sets of infected cultures. At the lower multiplicities of infection the total number of stained cells reached maximal levels 2 days after exposure and these were maintained essentially unchanged for the remainder of the experimental period. At the highest multiplicity, 97 per cent of the cells stained in 24 hours, and no significant change in the percentage occurred during 7 days of observation although the actual number of stained cells declined gradually in parallel with the total cell population. The attaining of the peaks in staining coincided in each of the 3 sets with



TEXT-FIG. 1. Cellular growth rates of L(MCN) cell cultures infected with NDV_{ST} at varying multiplicities.

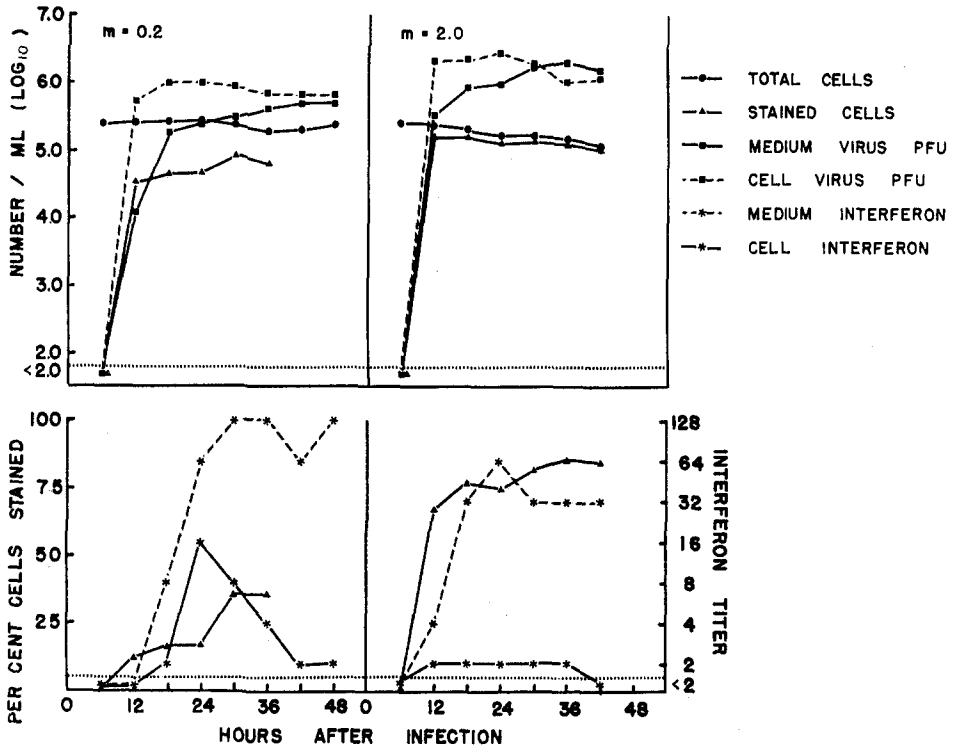
the peaks of infectious virus recoverable from the cultures. The maximal infectivity titers tended to be higher with an increase in the multiplicity of infection. The titers declined slightly in the course of further incubation. Interferon became detectable and reached peak titers with increasing delay as the multiplicity of infection decreased. Furthermore, the maximal amounts produced were higher with lower degrees of infection. Tests for interference, not shown in the text-figure, revealed that uninfected control cultures challenged with 100 pfu of VSV at 1, 2, 3, and 7 days were completely destroyed within 48 hours. In contrast, none of the infected cultures challenged at the same time showed cytopathic effects characteristic of VSV even when observed for 7 days. Thus, NDV_{ST} infection at a multiplicity as low as 0.1 induced significant resistance in the cultures to VSV in 24 hours.



TEXT-FIG. 2. Growth curves of NDVgr in L(MCN) cell cultures infected at varying multiplicities.

The second experiment to be described differed from the first in several details.

Cells were exposed to NDV_{ST} to attain actual multiplicities of infection of 0.2 and 2.0. The final cell suspensions were adjusted to contain 2.5×10^5 cells per ml instead of 1×10^5 as employed before, and they were seeded into coverslip tubes (1 ml) and 250-ml bottles



TEXT-FIG. 3. Growth curves of NDV_{ST} in cells and media after infection of L(MCN) cell cultures.

(10 ml). Harvests were made at 6-hour intervals for 48 hours and media and cells were kept separate for assays of infectious virus and interferon.

The results are summarized in Text-fig. 3. The immunofluorescence data were in line with those presented earlier. The first plateaus of stained cells (18 to 24 hours) reached 17 and 72 per cent which agreed reasonably well with the percentages of infected cells calculated from the adsorption data; *i.e.*, 18 and 84 per cent, respectively. The second plateaus were established by 30 hours. In both sets of cultures the amount of infectious virus found associated with the cells exceeded the virus in the medium for a considerable length of time

but eventually as much virus was found in the fluid as in the disintegrated cell suspension. Evidently, infectious NDV progeny produced by L(MCN) cells was released only slowly into the medium. In the culture infected at a multiplicity of 0.2 interferon became first detectable in the medium in 18 hours and reached a plateau in 30 hours. The titers in the medium exceeded at all times those in the cells. Cell-associated interferon revealed a rather sharp peak at 24 hours and then rapidly declined to low levels. At the higher multiplicity of infection, interferon appeared in the media in 12 hours and rose to maximal titer in 24 hours, but the level attained was significantly lower than in the

TABLE II

PFU Produced Per Stained Cell in Relation to Adsorption Multiplicity of NDV

Virus	Adsorption multiplicity	Time after exposure			
		Day 1	Day 2	Day 3	Day 7
NDV_{ST}	<0.1	18	21	—	—
	0.1	5	11	10	4
	0.1	36	37	13	12
	0.1	12	20	18	5
	0.2	27	—	—	—
	0.6	17	16	18	3
	0.9	7	13	7	8
	1.8	30	—	—	—
	12.0	21	9	8	7
$NDV_{L(MCN)}$	0.1	23	13	—	—
	0.2	13	6	5	2
	2.0	15	8	10	3
	12.5	2	1	—	—
	19.0	3.5	3	2	1.5

cultures infected with one-tenth the amount of virus. Cell-associated interferon was barely detectable in this set of cultures between 12 and 36 hours.

From these two experiments, as well as several others, it was evident that the number of immunofluorescent cells reached a maximal level in 1 to 2 days which remained essentially unchanged for the periods of observation of usually 7 days. The increases in the percentages of stained cells from the 1st to 2nd days after exposure undoubtedly were due to secondary cycles of infection by viral progeny derived from the initially infected cells. There was no evidence of tertiary cycles. The decline in the percentages of stained cells after the 2nd day may largely be ascribed to multiplication of uninfected cells.

The yields of infectious virus per stainable cell were generally low, not exceeding 40 pfu at any given harvest. As shown in the upper part of Table II,

cultures exposed to different multiplicities of NDV_{ST} gave similar yields per infected cell. Higher values were observed in the first 2 or 3 days but then the PFU to stainable cell ratios declined toward the low levels observed in established carrier cultures (5).

The increasing delay in interferon production and the improved yields with a decrease in the multiplicity of infection from 10 to 0.1 suggested that the cells infected during the exposure period yielded little if any interferon and rather that those which escaped infection initially were subsequently induced to synthesize interferon on exposure to viral components released in the first infectious cycle. In line with these considerations, a correlation of maximal

TABLE III

Ratio of Unstained Cells to Interferon Titer in NDV_{ST}-Infected L(MCN) Cell Cultures

Adsorption multiplicity	Unstained cells 24 hrs.	Maximal interferon titer	Unstained cells per interferon unit
0.1	99,400	16	6220
0.1	102,000	32	3190
0.1	68,900	32	2150
0.2	232,000	128	1810
0.6	44,600	16	2780
0.8	34,000	16	2120
0.9	57,300	8	7170
1.8	47,600	32	1486
12.0	2000	2	1000
Mean			3103

interferon titers to the number of stainable cells yielded widely discrepant results. In contrast, as shown in Table III, a correlation of non-stainable cells 24 hours after infection of the cultures to the amounts of interferon detectable at 48 hours gave values in a relatively close range. About 3000 non-stainable cells on the average produce one interferon unit. This unit is defined as the amount of interferon which protects half of the 4×10^6 cells in monolayer cultures against infection by VSV.

Demonstration of a Non-Infectious Component in the Viral Progeny of L(MCN) Cells Which Induces Interferon Synthesis.—In order to test the hypothesis put forth in the preceding section the viral progeny of L(MCN) cells collected 24 hours after a saturation infection with NDV_{ST} was used for exposure of fresh cell populations.

Suspended cells were exposed to NDV_{ST} by the standard procedure at an actual multiplicity of 10. The final suspension was adjusted to 6×10^5 cells per ml and each of 10 Blake bottles was seeded with 30 ml, and tubes containing coverslips were seeded with 1 ml volumes. After

incubation for 24 hours 97 per cent of the cells revealed antigen. The cells in the Blake bottles were scraped into the medium and pooled. The suspension was freed of cells at 1800 RPM for 10 minutes at 4°C and then subjected to centrifugation at 29,000 RPM for 1 hour to separate any interferon that might have been present from the viral components. The sedimented viral particles were resuspended in 12 ml of medium. This preparation, referred to as $NDV_{L(MCN)5}$ contained 2.0×10^8 pfu and 6.4×10^2 HA units (HAU) per ml. Thus, the pfu/HAU ratio of 3.1×10, was about 10 times lower than that of NDV_{ST} seeds, which denoted the presence of noninfectious, hemagglutinating components in confirmation of the report of Wilcox (9).

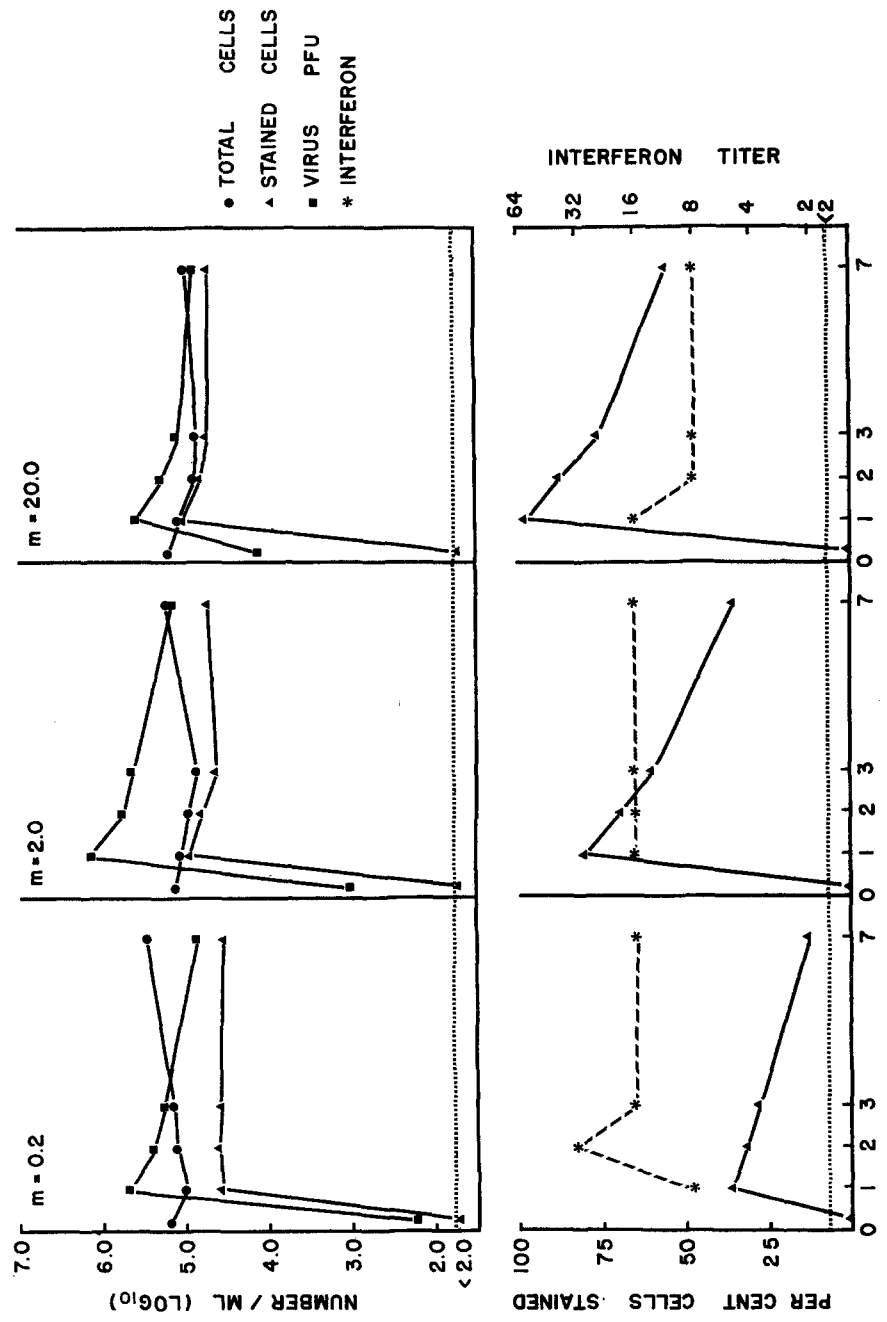
The $NDV_{L(MCN)}$ preparation was used to expose fresh cell populations by the standard technique at input multiplicities of 0.37, 3.7, and 37, based upon the infectious virus present in the seeds. Test tubes, with and without coverslips, were seeded with 1×10^6 cells and the cultures were processed as in the experiments employing NDV_{ST}.

Slightly more than 50 per cent of the infectious input virus was adsorbed within 1 hour at 37°C, leading to actual multiplicities of infection of about 0.2, 2, and 20. Comparison of the percentages of cells calculated to be infected from the adsorption data and those actually infected according to immunofluorescence elicited 24 hours later revealed close agreement. $NDV_{L(MCN)}$ and NDV_{ST} behaved alike in these respects, but there the similarities ended.

First of all, the quality of fluorescent antibody staining was different when cells infected with NDV_{ST} are compared with those infected with $NDV_{L(MCN)}$. As shown in Fig. 1, brilliant staining was obtained with standard virus. The cells were filled with antigen which often revealed needle like structures. Infection with the viral progeny of L(MCN) cultures produced much less stainable antigen in the cells and this was aggregated into small globular configurations. This type of staining is also predominant in infected cells of established carrier cultures.

The results of total and immunofluorescent cell counts and of assays for total virus and interferon are summarized in Text-fig. 4. Cellular division resumed more rapidly than in cultures exposed to NDV_{ST} at similar multiplicities of infection (m). Even with the largest dose of $NDV_{L(MCN)}$ ($m = 20$) the cultures survived and became viral carriers.

The number and percentages of immunofluorescent cells as well as the yields of infectious virus attained maximal levels in all 3 sets of cultures by 24 hours. There was no evidence of a secondary spread of the infection. The total numbers of stainable cells remained constant thereafter or showed slight declines during the 7 days of observation, whereas the percentages of immunofluorescent cells steadily decreased during this period. The infectivity titers did not increase in proportion to the infectious virus in the inocula as observed with NDV_{ST}, and the number of pfu per stained cell declined to <4 (Table II) at multiplicities of infection in excess of 10. The 6 hour harvest showed successively higher levels of infectious virus as the multiplicity of infection increased. The presence of non-infectious viral components in the inocula apparently reduced the neutralizing efficiency of the anti-NDV serum employed for washing of the cells.



TEXT-FIG. 4. Growth curves of $NDV_{L(MCN)}$ in L(MCN) cell cultures infected at varying multiplicities.

The interferon assays failed to disclose marked differences in the levels attained at the 3 multiplicities of infection with $NDV_{L(MCN)}$, again in contrast with the results obtained with NDV_{ST}. Furthermore, maximal or near maximal titers were observed in 24 hours with little change thereafter. One may conclude that the non-infectious component in the inocula was responsible for the early synthesis of interferon at the low multiplicity of infection and for the relatively high titers at the high multiplicity. On the basis of the pfu/HA ratio of the inoculum about 10 non-infectious virus particles were added to the cell suspensions for every pfu, and the multiplicities of the former were, therefore, about 2, 20, and 200. In line with these calculations one might not expect to observe maximal interferon titers in 24 hours with the first of these multiplicities, and indeed a slight increase was noted by 48 hours.

The Effect of Anti-NDV Serum on Interferon Production When Added 2 Hours after Exposure.—According to the interpretation of the data presented in the preceding sections it was postulated that anti-NDV serum added after exposure of cells to NDV_{ST} should prevent the production of interferon in the cultures, whereas antiserum would have no effect on the yields of interferon from cell populations exposed to $NDV_{L(MCN)}$. This was indeed observed.

Suspended cells were exposed to either NDV_{ST} or $NDV_{L(MCN)}$ at various multiplicities of infectious virus. After seeding of the cells one half of the cultures received 0.2 ml of inactivated normal rabbit serum, the other half an equal volume of anti-NDV serum. Harvests were made at 6 hours (for cell count only) and at 24 and 48 hours, and processed as described before.

The results of 2 experiments are summarized in Table IV. It is evident from experiment 1 that rabbit serum, whether normal or immune, in a final dilution of 1:6, was toxic for L(MCN) cells since about 60 per cent of the cells were lost by 24 hours. At that time surviving cells started to divide. In the 2nd experiment, a higher dilution of serum (1:20) was employed which resulted in less toxicity.

The results of fluorescent antibody staining at 24 hours were in line with the multiplicity of infection both for NDV_{ST} and $NDV_{L(MCN)}$ regardless of the type of serum used. Evidence for secondary spread was absent in the groups receiving anti-NDV serum. Cultures in which all cells became immunofluorescent at 24 hours showed similar staining at 48 hours in the case of NDV_{ST} and a decline in the percentage of stainable cells as noted earlier in the case of $NDV_{L(MCN)}$. The yields of pfu per immunoflourescent cell, measurable only in the normal serum groups, were likewise in agreement with the previous experiments. As seen in the last two columns of the table, interferon production was undectectable in the cultures infected with NDV_{ST} and treated with antiserum whether the multiplicity of infection was 0.2 or 10. In the controls treated with normal rabbit serum titers of interferon were obtained at the expected times. As

predicted, anti-NDV serum did not reduce the 24-hour yields of interferon of cells exposed to $NDV_{L(MCN)}$.

The Nature of the Interferon-Inducing Component.—The foregoing experiments have shown that cells initially infected with NDV_{ST} yield a mixed progeny of

TABLE IV

Effect of Anti-NDV Serum on the Production of Interferon by L(MCN) Cells Infected with NDV_{ST} and $NDV_{L(MCN)}$

Exper. No.	Inoculum	Input multiplicity	Serum added*	Cells/ml × 10^4			Cells stained		PFU/stained cell		Interferon	
				6 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24. hrs	48 hrs.
							per cent	*per cent*				
1	NDV_{ST}	0.2	IS	7.0	2.9	5.0	4	5	—	—	<2	<2
		0.2	NS	7.0	3.5	5.2	4	36	18	21	<2	8
	$NDV_{L(MCN)}$	0.2	IS	7.1	3.8	4.5	11	12	—	—	8	8
		0.2	NS	7.1	3.6	4.4	12	20	23	13	8	16
	$NDV_{L(MCN)}$	12.5	IS	6.0	3.0	3.7	100	76	—	—	16	16
		12.5	NS	6.0	1.9	3.2	100	84	2	1	16	16
2	NDV_{ST}	1.0	IS	21.0	14.0	16.0	53	56	—	—	<2	<2
		1.0	NS	21.0	12.0	13.0	60	81	N.D.‡	N.D.	4	16
	NDV_{ST}	10.0	IS	20.0	10.0	10.0	97	98	—	—	<2	<2
		10.0	NS	21.0	11.0	10.0	98	96	N.D.	N.D.	4	2
	$NDV_{L(MCN)}$	1.0	IS	20.0	16.0	19.0	49	48	—	—	32	32
		1.0	NS	20.0	18.0	18.0	60	33	N.D.	N.D.	16	32
	$NDV_{L(MCN)}$	10.0	IS	21.0	16.0	16.0	94	84	—	—	32	16
		10.0	NS	20.0	14.0	17.0	97	80	N.D.	N.D.	16	16

* IS, rabbit anti-NDV serum; NS, normal rabbit serum, added 2 hours after infection. The sera were diluted 1:6 in the first, and 1:20 in the second experiment.

‡ Not done.

infectious and non-infectious virus particles but little, if any, interferon. The non-infectious component so obtained induces the synthesis of interferon in remaining cells whether or not they adsorb infectious virus in addition. While further work is needed to characterize this component in a definitive manner experiments were carried out to exclude the possibility that it represents merely thermally inactivated standard virus and to compare its activities with those of ultraviolet-inactivated NDV_{ST}.

Thermal inactivation rates of NDV_{ST}: In preliminary experiments the inactiva-

tion rates *in vitro* at 37°C were determined for NDV_{ST} suspended in allantoic fluid and culture medium, respectively.

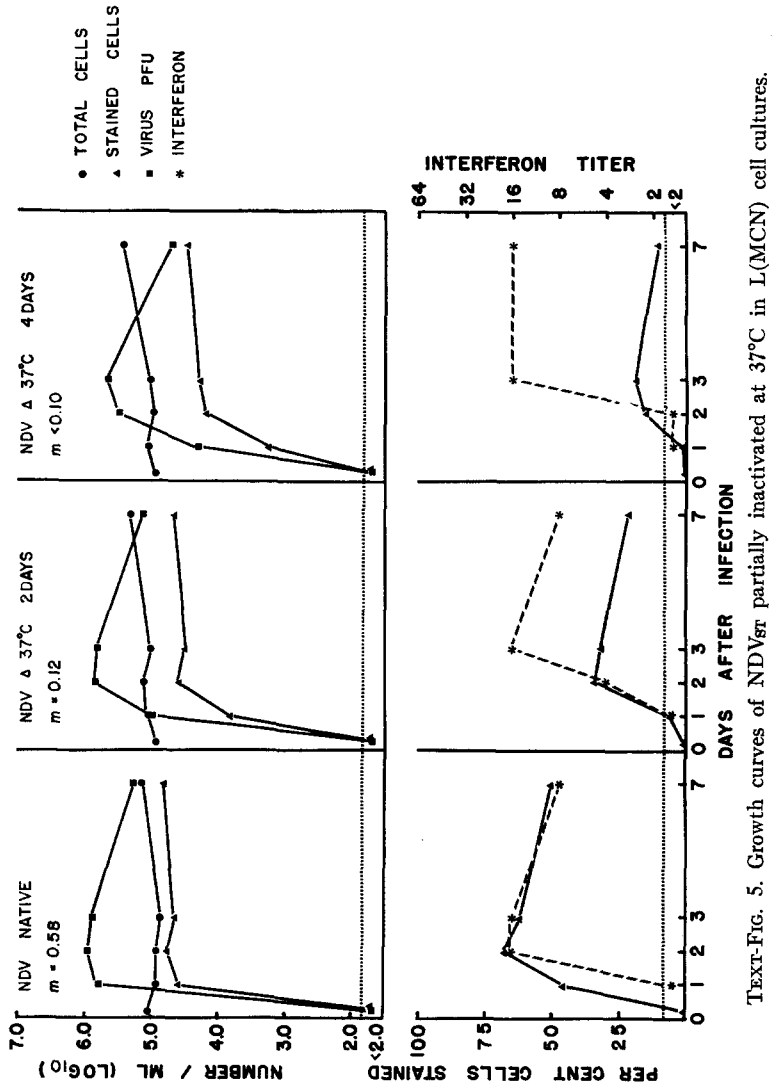
Virus was sedimented from infected allantoic fluids at 29,000 RPM for 1 hour and resuspended either in normal allantoic fluid derived from 12-day-old chick embryos or in culture medium. One ml samples of each preparation were placed in ampuls, which were then sealed and incubated in a water bath at 37°C. At the start and at daily intervals thereafter ampuls were transferred to −20°C. When all preparations were at hand, surviving virus was measured in plaque assays.

The results showed that inactivation proceeded at linear rates and that these depended on the medium in which the virus was suspended. In allantoic fluid the half life was approximately 8 hours, whereas in culture medium it was 23 hours. Thus, thermally inactivated virus was more likely to be found in significant amounts in the NDV_{ST} seed preparations from chick embryos than in virus materials derived from infected L(MCN) cultures.

Exposure of L(MCN) cells to thermally inactivated NDV_{ST}: In the first experiment cells were exposed by the standard procedure to NDV_{ST} in allantoic fluid which had been incubated *in vitro* at 37°C until no plaques were obtained in dilutions 10^{-1} to 10^{-3}. The hemagglutinin titer was unaffected (3.2×10^2 HA units per ml). The input multiplicities were 16 and 160 based on the number of pfu prior to inactivation. There was no impairment of the cellular growth rates; none of the cells showed immunofluorescence during the 7 days of observation; no infectious virus was found; and most important, no interferon became detectable. In additional experiments cells were exposed to partially heat-inactivated virus preparations.

NDV_{ST} suspended in culture medium was incubated at 37°C *in vitro* to reduce the infectivity titer by about one and two $\log_{10}$ units, respectively. The original and 2 heated preparations were used to expose L(MCN) at two input multiplicities (1.2 and 0.3) based upon the residual infectivity titers rather than on total virus present. All further steps followed the established procedures. Since both sets yielded principally similar results only one will be presented.

As seen in Text-fig. 5, the efficiency of adsorption of infectious virus was markedly reduced by the presence of thermally inactivated virus in the inocula. This is evident from the actual multiplicities of infection attained. The percentage of cells revealing virus-specific immunofluorescence at 24 hours conformed to expectations based on the adsorption data. The quality of staining at this time was throughout of the type noted upon infection with NDV_{ST} (see Fig. 1). The number of immunofluorescent cells and the titers of infectious virus increased substantially between 24 and up to 72 hours and the yields of pfu per infected cell were comparable to those obtained with NDV_{ST} (see Table II). Thus, the thermally inactivated virus in the inocula, in contrast to the non-infectious component in $NDV_{L(MCN)}$ seeds, did not prevent secondary spread of the infection, nor did it induce early interferon synthesis. Interferon



TEXT-FIG. 5. Growth curves of NDV_{BT} partially inactivated at 37°C in L(MCN) cell cultures.

became detectable, however, in due course but the time of its appearance clearly depended upon the extent of the infectious process resulting in the production and release of non-infectious virus from infected cells. In agreement with this interpretation, calculation of the number of unstained cells required to yield one interferon unit yielded values between 2000 and 6700 in line with the data presented in Table III.

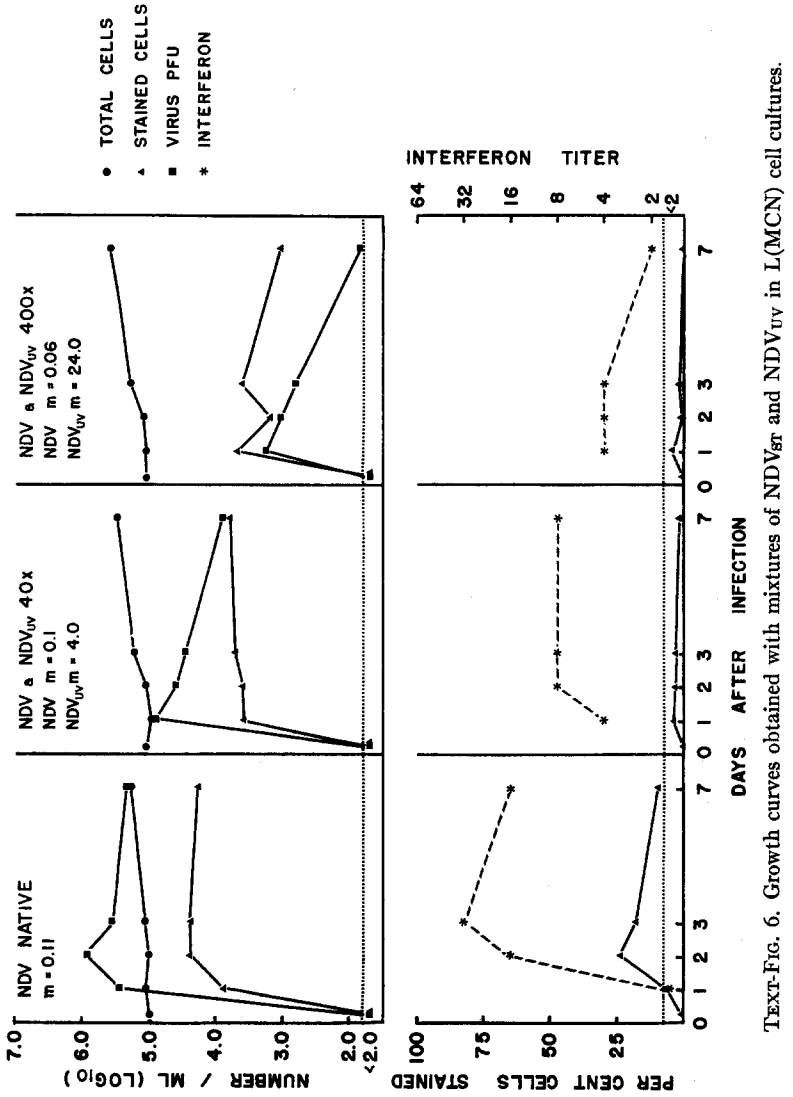
These experiments showed that thermally inactivated virus reduced the efficiency of adsorption of infectious virus and failed to cause interference or synthesis of interferon. It thus differed in at least these 3 respects from the non-infectious component found in the viral progency derived from L(MCN) cells infected with NDV_{ST}. The latter, therefore, cannot be considered to be thermally inactivated virus and may represent an incomplete or deficient virus particle.

Exposure of L(MCN) cells to ultraviolet-inactivated NDV_{ST}: It has been shown previously that ultraviolet-inactivated virus (NDV_{UV}) induces interference in L and L(MCN) cells against superinfection with VSV and synthesis of interferon (6, 14). It was of interest to compare NDV_{UV} with the incomplete viral component derived from L(MCN) cultures.

In a preliminary experiment L(MCN) cells were exposed to NDV_{UV} by the standard procedure using input multiplicities increasing in 10-fold steps from 0.2 to 200, taking one HA unit as equivalent to 10^6 virus particles. All other procedures were as described before. None of the cultures revealed immunofluorescent cells nor infectious virus during a 7 day period. Those exposed to input multiplicities of 20 and 200 of MDV_{UV} produced detectable interferon in 24 hours but only to titers of 1:4 in both cases. No further production was measurable and these levels persisted for 7 days. It was evident that with NDV_{UV} at the two high multiplicities, 30,000 cells were required to yield one interferon unit, a number 5 to 10 times greater than that observed in cultures infected with NDV_{ST} at appropriate multiplicities. Thus, NDV_{UV} appeared to be a relatively inefficient inducer of interferon synthesis. This was further established in the following experiment designed to determine additional effects of NDV_{UV}.

NDV_{ST} was diluted in a preparation of NDV_{UV} so as to obtain virus suspensions containing 40 or 400 UV-inactivated particles per pfu. Lots of L(MCN) cells were exposed to these two mixtures or only NDV_{ST} at the same input multiplicities of infectious virus (0.2). The remainder of the experiment followed the established procedures.

The efficiency of adsorption of infectious virus was hardly affected by the presence of NDV_{UV}. Only with the larger dose was the adsorption possibly reduced. The percentage of cells calculated to be infected agreed well with the percentage of immunofluorescent cells at 24 hours. The cells exposed to the mixtures of NDV_{UV} and NDV_{ST} grew somewhat better than the controls inoculated with NDV_{ST} only. The data presented in Text-fig. 6 show that the presence of



TEXT-FIG. 6. Growth curves obtained with mixtures of NDV_{St} and NDV_{UV} in L(MCN) cell cultures.

NDV_{UV} in the inoculum held the infectious process to one cycle since there was neither a significant increase in immunofluorescent cells nor in total infectious virus in these two groups between 24 and 48 hours. On the contrary, the virus titers decreased steadily after the 1st day peak. Thus, interference was early established in the cells escaping infection during the exposure period. Furthermore, the inactivated virus affected even the initial cycle of viral replication as evident from comparison of the virus titers at 24 hours. In the cultures exposed to NDV_{UV} at estimated multiplicities of 4 and 24 the titers amounted to 30 and less than 1 per cent of the value obtained on infection with only NDV_{ST}. This was reflected also in the yields of pfu per stainable cell at 24 to 48 hours which decreased from 36 to 22 to <1 in the groups exposed to NDV_{ST} alone, or NDV_{ST} plus 40 times, or plus 400 times the number of NDV_{UV} particles respectively. The interferon curve for the NDV_{ST} series followed the expected pattern. When NDV_{UV} was present in the inoculum interferon became detectable in 24 instead of 48 hours. The relatively low titers obtained at that time represented most likely interferon production induced by the inactivated virus. With the larger dose of NDV_{UV} no further increase in interferon was noted whereas with the smaller amount the titer rose 2-fold, indicative of some interferon synthesis in consequence of the infectious process. Yet, the maximal level amounted to only 25 per cent of that produced by the cultures infected with NDV_{ST} alone.

From these experiments it is evident that NDV_{UV} is comparable in most respects to the incomplete viral component derived from NDV-infected L(MCN) cells. The major point of difference is that NDV_{UV} was found a less efficient inducer of interferon synthesis.

DISCUSSION

The experiments presented above afford insight into the initial phases of infection of L(MCN) cell populations by Newcastle disease virus and provide an explanation for the survival of the cultures. The protective mechanisms involved in these early stages are likely to be operative also in established carrier cultures and restrict in them at all times the infectious process to a limited number of cells.

The *adsorption of virus and actual infection of the suspended cells* during the initial exposure period increased up to a point with an increase in the cell concentration in confirmation of an earlier report (15), but with suspensions containing 5×10^5 or more cells per ml the percentage of virus which was adsorbed was of a similar order, that is, 50 per cent of the input on the average. Presence of thermally inactivated virus in the inoculum reduced the efficiency of adsorption at 37°C. This effect could not be ascribed to enzymatic destruction of cell receptors because (*a*) thermal inactivation at 37°C is expected, if anything, to diminish the receptor-destroying enzyme activity of the virus; and (*b*) ultra-

violet-inactivated standard virus or the non-infectious viral component released from infected L(MCN) cells failed to affect the adsorption of infectious virus. It is conceivable that the thermally inactivated virus blocks cell receptors presumably on account of its reduced enzyme activity.

With the standard procedure for exposure of cell populations practically all cells could be infected if enough virus were added, and thus few, if any, of the cells were found to be intrinsically resistant. The percentage of infected cells calculated from the adsorption data on the basis of Poisson's formula matched closely the percentage of immunofluorescent cells 24 hours after exposure. The correlation between the 2 values was not altered by the presence in the inoculum of non-infectious viral components, be they thermally or ultraviolet-inactivated standard virus or incomplete particles derived from infected L(MCN) cells.

With regard to cellular multiplication, infection at a multiplicity of 5 or greater resulted in the ultimate loss of the cultures as observed also by Wilcox (9). While no evidence was obtained to indicate that infected cells were capable of dividing, the possibility has not been excluded that they underwent one division, as has been reported by Wheelock and Tamm for NDV-infected HeLa cells (16). The loss of L(MCN) cultures could be prevented if the cells adsorbed either incomplete or ultraviolet-inactivated standard virus particles in addition to infectious virus. With a reduction in the multiplicity of infection from 5.0 to less than 0.1, successively more of the cells remained unaffected, cellular growth resumed with increasing speed and the cultures survived. The growth rates ultimately attained in these persistently infected populations were always reduced in comparison to those of uninfected cultures, in line with earlier observations (1). The generation time of L cells may be slowed down also after exposure to NDV_{UV} or interferon (17). The decrease in cellular growth rates is apparently reflected in a significant increase in aerobic glycolysis (18). While exposure of cells to crude interferon preparations has been reported to cause similar changes in cellular metabolism (19), highly purified interferon has failed to increase aerobic glycolysis (20). Yet, the factor causing depression of the rate of cellular division could not be differentiated from interferon on the basis of the criteria presently employed for its characterization (17).

Both *fluorescent antibody staining and infectivity titrations* revealed that the initial cycle of NDV_{ST} infection in L(MCN) cultures is surprisingly long in that secondary spread of the virus became evident only after 24 hours. There was no indication of tertiary cycles if the multiplicity of infection was as low as 0.1. The size of the secondary step was always low in spite of the fact that enough infectious progeny became available from the initially infected cells to infect all the remaining cells in the cultures. It is concluded that some of the cells had acquired resistance by the time they were exposed to first cycle progeny. In line with this conclusion, the cultures resisted superinfection at 24 hours with 1000 pfu of vesicular stomatitis virus. This result confirmed that many, but not

necessarily all cells were resistant at this time since the dose of VSV employed provided an immediate challenge for only a fraction of the population. The toxic effects of large doses of VSV (14) prevented simultaneous challenge of all cells. Secondary spread of infection was entirely absent when NDV inocula were employed which contained appreciable quantities of incomplete or ultraviolet-inactivated standard virus in addition to infectious virus. Thermally inactivated virus had no such effect.

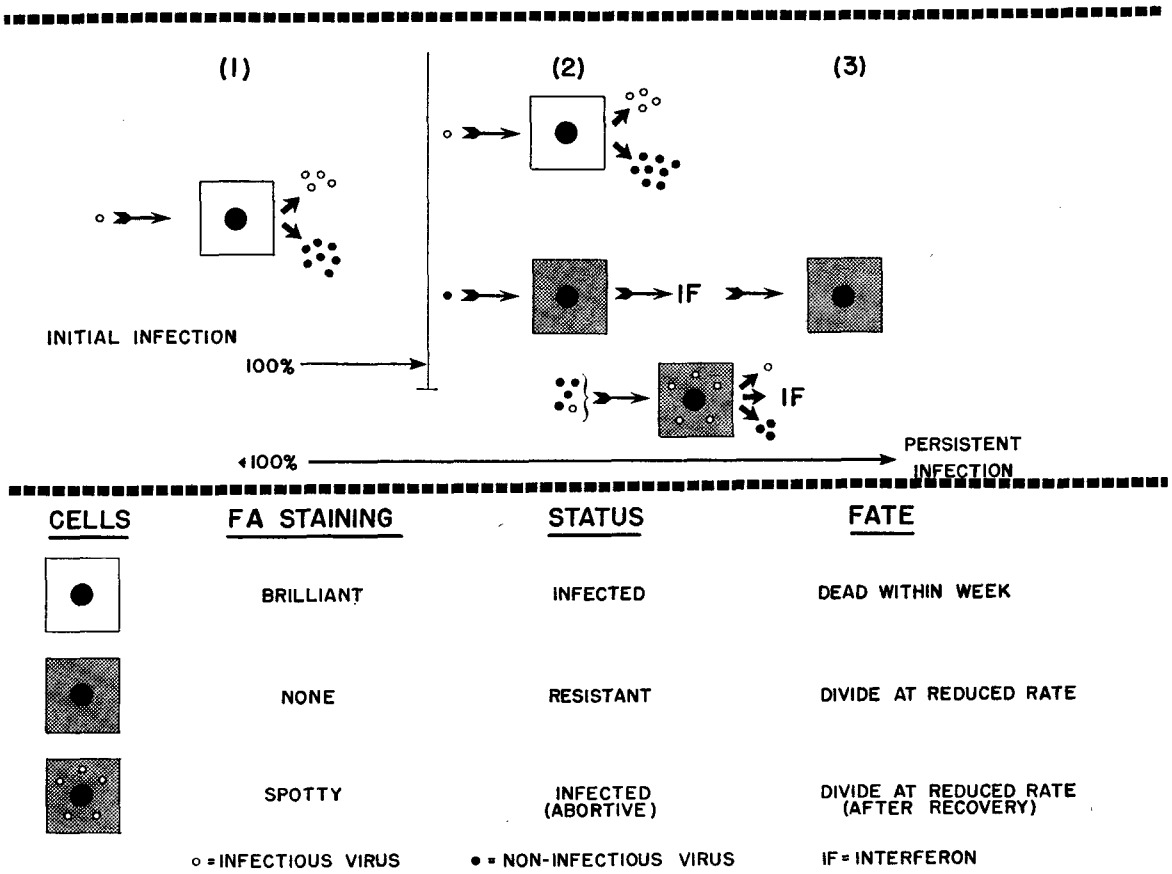
During the 7 day period of observation the titers of cell-associated virus exceeded those of extracellular virus except for the 48 to 72 hour interval when both titers were about equal. The number of pfu found per immunofluorescent cell was relatively high in the first few days (up to 40) but then it declined toward the level (about 1) usually found in carrier cultures (4). No plating for infectious centers was carried out but it is likely that all antigen-containing cells produced some infectious virus since earlier unpublished experiments with carrier populations revealed a close correlation between the percentages of stained cells and of cells inducing plaques on monolayers. The yields of pfu per infected cells were drastically reduced if cells adsorbed incomplete or UV-inactivated virus in addition to infectious virus. This was reflected also in reduced virus-specific immunofluorescence in the infected cells which was restricted to a few globules of antigen. Under such conditions of abortive infection the virus-cell equilibrium characteristic of the carrier state was established within a few days. Thermally inactivated virus again failed to show such effects. It is evident that adsorption of an excess of appropriate kinds of non-infectious virus particles along with infectious virus interfered with the full development of virus replication. There is no doubt that this occurred to some extent already during the secondary cycles of infection with NDV_{ST} and was the rule in carrier cultures.

The production of a partly non-infectious NDV progeny in L cells has been demonstrated previously by Wilcox (3, 9) but in carrier cultures or freshly infected populations hemagglutinating activity could not be detected under the conditions employed. However, following centrifugal concentration of the 24 hour viral progeny of L(MCN) cultures after a saturation inoculum of NDV_{ST} hemagglutination became measurable and the pfu/HA ratios of such preparations were about 10 to 20 times lower than those of standard virus. Thus, one infected cell was capable of producing on the average between 100 and 200 total virus particles within 24 hours after infection. The non-infectious portion of the progeny provides the key to the early development of the cellular resistance discussed above and the production of interferon.

The role of the non-infectious virus particles as sole interferon producers was established by the following points: (*a*) when all cells were infected initially by NDV_{ST} little or no interferon was produced; (*b*) at lower multiplicities of infection with NDV_{ST} an inverse relationship was found to exist between the

percentage of immunofluorescent cells and the maximal titers of interferon obtained. Furthermore, the peaks were reached with increasing delay as the dose of seed virus was reduced, again indicating that infected cells did not yield interferon but rather those which adsorbed non-infectious virus in secondary or later cycles; correspondingly, (*c*) correlation of stainable cells to interferon titers gave widely discrepant results whereas a similar correlation on the basis of unstained cells yielded values within a reasonably narrow range. From 1000 to 7000 unstained cells (3000 on the average) produced one interferon unit. The variation from experiment to experiment may be due in part to inherent inaccuracies of total and immunofluorescent cell counts, differences in the sensitivity of cultures used for interferon titrations, and harvests of interferon at suboptimal times. Since the interferon unit is arbitrarily defined as the amount of interferon capable of rendering half of the 4×10^6 cells in a monolayer resistant to VSV, it follows that one cell may produce enough interferon to protect between 300 and 2000 cells, or 666 on the average; (*d*) the partially non-infectious progeny of L(MCN) cells collected 24 hours after a saturation infection produced high and similar titers of interferon within 24 hours whether the multiplicity of infectious virus was 20.0 or 0.2, and the percentage of stained cells was near 100 or about 10. However, practically all cells adsorbed non-infectious virus since the estimated minimal multiplicity of this component was between 2.0 and 4.0. Production in a cell of some infectious virus *per se* did not prevent interferon from being synthesized, but as discussed, only an abortive infectious process was noted under these conditions; finally (*e*) addition of anti-NDV serum 2 hours after infection of L(MCN) cells with NDV_{ST} prevented the subsequent synthesis of interferon because the incomplete virus produced in the initial cycle was intercepted by viral antibody. In contrast, antiserum given after exposure to the partially non-infectious NDV progeny from L(MCN) cells failed to affect the yields of interferon because the incomplete particles were adsorbed by the cells prior to addition of antibody. These findings proved that interferon production in this system is a 2 stage process; first, incomplete virus is produced which in turn induces interferon production in uninfected cells.

The *properties of the non-infectious virus component* found in the NDV progeny of L(MCN) cells have not been characterized in detail. The component was sedimentable by high speed centrifugation; it could be neutralized by specific antiviral serum; it had hemagglutinating activity; it caused interference as evident both from prevention of secondary cycles of infection and early resistance of exposed cultures to VSV. Finally the component did not represent thermally inactivated virus which might accumulate during the incubation period. The last conclusion is based on the facts that the inactivation rate of NDV in culture medium was very low, with a half life of 18 hours, and that NDV_{ST} partially or totally inactivated *in vitro* at 37°C did not cause interference nor did it induce interferon production.



TEXT-FIG. 7. Scheme of the initial stages of infection of L(MCN) cell cultures with NDV.

The incomplete NDV particles were found to be more efficient inducers of interferon production than ultraviolet-inactivated standard virus particles since the latter generally produced at most only one-fourth as much interferon from the same number of cells. It is likely that during irradiation most of the standard virus particles sustain too many hits of ultraviolet before the last infectious unit is inactivated, so that their interfering activity is reduced or even abolished. It would seem that the incomplete particle offers a better means to secure high titered interferon. The non-infectious component is probably a deficient or incomplete product of NDV_{ST} replication in L(MCN) cells. It is likely to contain some ribonucleic acid, since it has been shown that incomplete forms of influenza virus cause interference only as long as the virus particles contain a threshold amount of RNA or type-specific, soluble complement-fixing antigen (21).

A *scheme of the events* occurring upon initial infection of L(MCN) cells by NDV_{ST} may be devised from the data presented. This scheme is shown in Text-fig. 7. A cell which adsorbs an infectious virus particle will produce infectious virus as well as about 10 times that amount of non-infectious particles. Such a cell will reveal brilliant, virus-specific immunofluorescence in 24 hours. If all cells are so infected, the culture will die in 7 days or slightly later. If only a fraction of the cells is infected initially, their viral progeny will spread to uninfected cells. The infectious particles will give again the results described. Non-infectious particles will render cells resistant; no viral antigen will be produced and thus no immunofluorescence will be elicited in 24 hours in the resistant cells, but they will produce interferon. The interferon so obtained will protect additional cells. In cells that adsorb infectious and non-infectious particles simultaneously or in short succession, (which, depending on the extent of the primary infection, may occur already during the second cycle), the infectious process will be aborted; little infectious and non-infectious virus will be produced, as evident from limited immunofluorescence, but considerable amounts of interferon will be released. The cells protected either by incomplete virus or interferon, or cured after an abortive infection will be capable of dividing and thus maintaining the culture alive. The descendants of once protected cells will regain at least partial susceptibility (22) and will become available for superinfection by the infectious virus present in the culture. Such effective contacts will not have to be frequent because infected cells survive for as long as 7 days.

SUMMARY

The initial stages of infection of L(MCN) cell populations with standard Newcastle disease virus (NDV_{ST}) were analyzed in an effort to elucidate the steps leading to survival of the cultures and to the indefinite persistence of the infectious process at a low level. Cells were exposed in suspension to NDV at varying multiplicities and the monolayer cultures derived from such cells

assayed at intervals for cellular growth rates, percentage of infected cells as determined by immunofluorescence, yields of viral progeny and of interferon, and, on occasion, resistance to superinfection with vesicular stomatitis virus.

The percentage of cells calculated to be initially infected on the basis of adsorption data was found to match closely the percentage of immunofluorescent cells resulting from the first infectious cycle (up to 24 hours). Cells initially infected with NDV_{ST} produced a mixed progeny of infectious virus (from 15 to 40 pfu/cell) and about 10 times as many non-infectious particles in 24 hours [$NDV_{L(MCN)}$], but little or no interferon. If all cells were infected the cultures ultimately died. At multiplicities of infection (m) of 2 or less the cultures survived with increasing ease as the percentage of infected cells was reduced. The number of pfu per infected cell was of the above order during the first 3 days; it declined thereafter. Limited secondary spread of the infection was noted by 48 hours and no further cycling was noted thereafter. As m decreased from 2.0 to 0.1 there was an increase in the yields of interferon and the time at which peak titers were reached. Addition of anti-NDV serum 2 hours after infection prevented measurable production of interferon. In contrast, following exposure of cells to $NDV_{L(MCN)}$ at multiplicities ranging from 20.0 to 0.2 (based on infectious virus) all cultures survived, no secondary spread was noted, the number of pfu per infected cells was reduced at the higher multiplicities, and the yields of interferon were similar and maximal by 24 hours and not affected by anti-NDV serum added after an adsorption period of 2 hours.

It is concluded that the non-infectious virus particles in the progeny released from NDV_{ST}-infected cells induce resistance in remaining cells or, if adsorbed simultaneously with infectious virus, abort the intracellular infectious process. In both instances interferon is produced which may then render additional cells resistant.

The non-infectious component is considered an incomplete or defective product of viral replication and not merely thermally inactivated virus. NDV_{ST} partially or completely inactivated at 37°C induced neither cellular resistance nor synthesis of interferon. The incomplete viral component behaved in all respects like ultraviolet-inactivated NDV_{ST} except that it was significantly more efficient in inducing interferon synthesis.

On the basis of the presented data a scheme has been devised and discussed which appears to explain satisfactorily the events which take place on initial infection of L(MCN) cells with NDV and which lead to the persistence of the infectious process.

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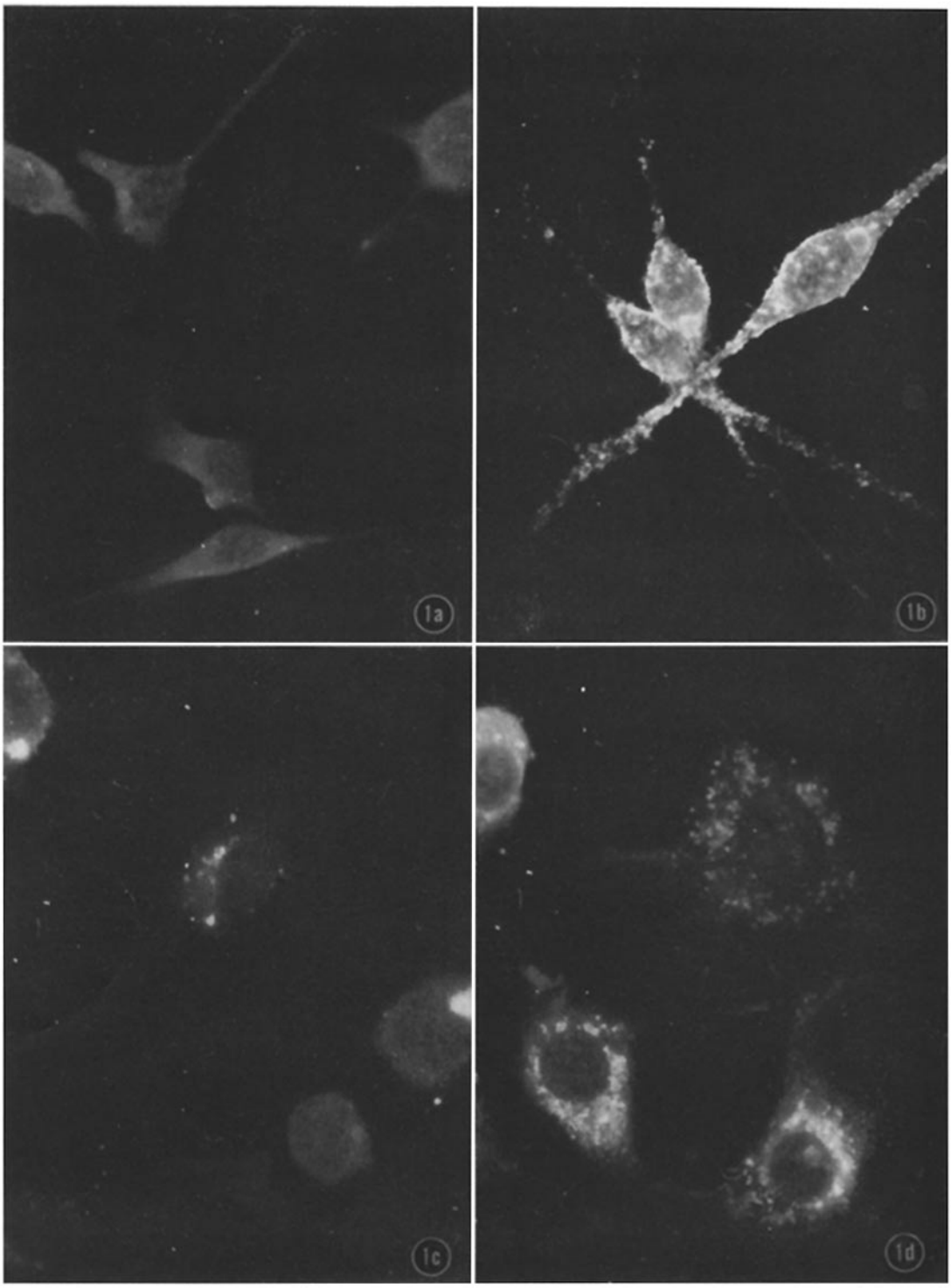
EXPLANATION OF PLATE 92

FIGS. 1 *a* to 1 *d*. Results of staining with fluorescein-conjugated anti-NDV gamma globulin. Fig. 1 *a*, non-infected cells; Fig. 1 *b*, cells infected 24 hours previously with NDV_{ST} at a high multiplicity; Fig. 1 *c*, cells infected 24 hours previously with $NDV_{L(MCN)}$ at a high multiplicity; Fig. 1 *d*, cells infected 48 hours previously with NDV_{ST} at a low multiplicity. × 1360.



(Rodriguez and Henle: Persistent infections of tissue culture)