In the next paper, we shall prove that $\mathfrak{N}$ is a model for Z-F in which part 3 of Theorem 1 holds.

* The author is a fellow of the Alfred P. Sloan Foundation.

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A SPECIFIC COMPLEMENT-FIXING ANTIGEN PRESENT IN SV40 TUMOR AND TRANSFORMED CELLS

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Many experimental tumors, both carcinogen-induced[1] and virus-induced,[2–5] contain new cellular antigens, generally demonstrable by transplant rejection procedures. Huebner *et al.*[6] first demonstrated the presence of new, noninfectious, complement-fixing (CF) antigens clearly under viral genetic control, in adenovirus-induced tumors in hamsters and rats.

A new transplantation antigen(s) has been found in SV40-induced hamster tumors,[7–9] but it is not established whether its synthesis is under viral or host cell genetic control. This paper presents evidence for a new CF antigen in SV40 tumors and transformed tissue culture cells, formed from information contained within the viral genome. Preliminary results of these studies were presented by Huebner *et al.*[6]

Materials and Methods.—The CF procedure was identical with that used by Huebner *et al.*;[6] this is a Bengtson procedure done in microplates using overnight fixation at 4°C, with two exact units of complement. Tumor extracts consisted of 10% suspensions in Eagle's basal medium, clarified by centrifugation at 2500 rpm for 30 min, and stored at −60°C. The extracts were tested for antigens only if the undiluted extract was not anticomplementary (AC). The primary SV40-induced hamster tumors used in these studies are from experiments described in detail elsewhere.[10] Tumor extracts used as standard CF antigens were selected for having high titer reactivity with sera from tumorous hamsters.

Suspensions of both normal and transformed tissue culture cells of various species,[11] as well as tissue culture cells from a variety of hamster tumors, were prepared in the following manner. Cells grown in 32-oz Blake bottles were washed with phosphate-buffered saline (PBS) (pH 7.2), scraped off the glass with a rubber policeman, centrifuged at 150 *g* for 8 min, and resuspended in 9 volumes of PBS. These suspensions were stored at −60°C before use.

"Viral antigen" was prepared by inoculating SV40 strain 776[12] into a continuous tissue culture cell line (strain BSC-1) of African green monkey kidney (AGMK) at a multiplicity of about 10^{-4}, and harvesting the cells and fluid together when cytopathogenicity was maximal. The cell suspension was stored at −20°C, thawed, and used without further processing.[13]

Four types of sera were used as standard reagents; to avoid undue heterogeneity of antibodies, sera of individual animals generally were used: (1) serum from tumorous hamsters, selected for having high CF antibody titer against SV40 tumor extracts, and no reaction with viral antigen; (2) serum from similar hamsters, but having high CF antibody titers for both tumor extracts and

TABLE 1

REPRESENTATIVE PATTERNS OF CF ANTIBODY RESPONSES TO TUMOR AND VIRAL ANTIGEN

	Reciprocals of Antibody Titer Versus Undiluted Antigen and Antigen Titer Versus 1:20 Serum										
	Serum of hamster with primary tumor; #5299		Serum of hamster with transformed cell tumor from clone 3-3*; #5246		Serum of hamster with transformed cell tumor from clone 3-3; #5074		SV40 immune monkey serum #2159		Hamster hyperimmune anti-SV40 serum #6141		
Antigen	Antibody titer	Antigen titer	Antibody titer	Antigen titer	Antibody titer	Antigen titer	Antibody titer	Antigen titer	Antibody titer	Antigen titer	Virus titer ($TCID_{50}$/0.1 ml)
Primary SV-40 hamster tumor #1369	≥80	8	≥80	16	≥80	≥16	40	8	0†	0	$<10^0$
1st passage transplant of primary SV40 hamster tumor #2007	≥80	8	≥80	16	≥80	≥16	20	8	0	0	$<10^0$
Tumor from transformed hamster kidney cell clone 3-3; #1918	≥80	8	≥80	8	≥80	8	(20)‡	1	0	0	$<10^0$
SV40 virus (BSC-1 TC§)	0	0	10	≥ 4	0	0	640	4	20	4	$10^{8.0}$
Control (BSC-1 TC)			0	0			0	0	0	0	
Polyoma-induced hamster sarcoma	0	0	0	0	0	0	0	0	0	0	

* Clone 3-3 = clonal line derived from THK-1 line of SV40 transformed hamster kidney cells.[17]
† 0 = <20 serum antibody titer or <1 antigen titer.
‡ (20) = partial reaction at 1:20.
§ TC = tissue culture.

viral antigen; (3) convalescent serum (#2159) from a monkey inoculated with SV40 virus;[14] and (4) serum of weanling hamsters hyperimmunized with SV40 virus grown in AGMK cultures.

Tests for CF antigens in tumor extracts were done by testing against hamster serum diluted (generally 1:10 or 1:20) to contain 4–16 units of antitumor antibody, and against the monkey immune serum at a dilution of 1:20, which corresponded to 1–2 units of antibody reactive with tumor extracts. The same monkey serum was used at a dilution of 1:80 to test for viral antigen in AGMK harvests; this dilution of the serum contained 8 units of antiviral antibody (*vide infra*). Hamster sera were screened for CF antibody at a dilution of 1:5 or 1:10, using 8 units of tumor antigen or 4 units of viral antigen.

The methods used for virus assay and neutralization tests have been described elsewhere.[10]

Ultracentrifugal fractionation of antigens was done by the following procedure. Frozen material was thawed and reclarified by centrifugation at 2,500 rpm for 15 min. The supernatant fluid was centrifuged at 30,000 rpm (59,310 *g* av) for 2 hr in a Spinco no. 40 rotor at 4°C; the upper and lower halves of the supernatant fluid were saved separately. The pellet was rinsed with Eagle's basal medium, and resuspended to one-tenth volume in PBS.

Results.—Antigen in SV40 hamster tumors: Of 35 extracts of primary SV40-induced sarcomas, 32 (91%) reacted in CF with late sera from hamsters with primary tumors. Three transplanted primary tumors and seven tumors induced by inoculation of transformed hamster kidney or hamster embryo tissue culture cells all reacted with these sera. The majority of primary, transplanted, and transformed cell-induced tumors also reacted with the 1:20 dilution of the SV40 monkey immune serum. The quantity of antigen reacting with the hamster antiserum was not related to size of tumor; primary tumors less than 1 cm in diameter were as often positive, and in as high a titer, as medium-sized and large tumors. Likewise, there was no apparent relationship to presence or titer of extractable infectivity in the tumor.

Table 1 shows representative titration results with the various antisera. The three SV40 tumors showed complete reciprocal cross-reactions, indicating that the same antigen(s) was present in primary, transplanted, and clone-induced tumors. None of these tumors contained demonstrable infectivity. Although the tumor extracts reacted with the monkey immune serum, the antibody titer obtained was far lower than the titer of antiviral antibodies, and there was no reaction between these extracts and hyperimmune hamster SV40 antiserum.

Presence of antigen in transformed cells of various species origin: SV40 infection induces a proliferative response accompanied by alterations in morphology and growth pattern of tissue culture cells derived from a number of mammalian species.[11] The continuous cell lines of transformed cells derived from these cultures of various species origin provided a unique opportunity to approach the question of whether the new antigen detected in hamster tumors was formed in response to information contained in the host or in the viral genome. As seen in Table 2, "transformed" cells of hamster, rabbit, Swiss mouse, C3H mouse, and porcine origin reacted with the SV40 hamster tumor and monkey immune sera in a fashion similar to tumor extracts. The antigen was also present, in low titer, in a suspension of second passage cells from a fetal bovine kidney culture chronically infected with SV40, but showing no evidence of transformation; the supernatant culture fluid at this passage level contained only trace amounts of virus.

A variety of control antigens gave no significant reaction with these sera; it is particularly noteworthy that the positive hamster serum did not react with Forssmann antigen in guinea pig kidney, although the monkey serum gave a slight reaction. Most of the positive and control hamster antigens reacted with a low dilution

TABLE 2

PRESENCE OF "TUMOR" ANTIGEN IN CELLS OF VARIOUS SPECIES INFECTED WITH SV40

Species	Antigen Material	CF Antigen Titer Versus Indicated Serum: Primary SV40 hamster tumor serum #5586 1:10 (16 antibody units)	SV40 immune monkey serum #2159 1:20	Primary adeno 12 hamster tumor serum 1:10	Polyoma hamster tumor serum 1:10	SV40 negative African green monkey serum 1:20
Hamster	SV40 primary tumor	8	8	0[a]	0	
	TC[b] cells from SV40 primary tumor	2	0	0	0	0
	SV40 transformed TC cells-THK-1[c] (P-59)[d]	8	≥4	0	0	0
	SV40 transformed TC cells clone 3-3[e] (P-34)	≥8	2	0	0	0
	SV40 transformed TC cells clone 4-1[e] (P-37)	≥8	≥4	0	0	0
	SV40 transformed TC cells clone 4-2[e] (P-36)	≥8	≥4	0	0	0
	SV40 transformed TC cells[f] #3775	≥8	≥4	0	0	0
	Controls[h]	All 0	All 0			
Rabbit	SV 40 "transformed" kidney TC (P-12)	≥8	2	0	0	0
	Normal kidney TC (P-1)	0	0			
Swiss mouse	SV 40 "transformed" kidney TC (P-16)	≥8	≥4	0	0	0
	Normal kidney TC (P-1)	0	0			
C3H mouse	SV40 "transformed" kidney TC (P-4)	≥8	≥4	0	0	0
	Normal kidney TC (P-1)	0	0			
Pig	SV40 "transformed" kidney TC (P-2)	≥8	≥4	0	0	0
	Normal kidney TC (P-2)	0	0			
Cow	SV40 infected kidney TC (P-2)	2	0			
	Normal kidney TC (P-2)	0	0			
	Bovine papilloma transformed cells (P-23)[g]	0	0			
Guinea pig	Normal kidney suspension	0	1			

[a] 0 = no reaction with undiluted antigen.
[b] TC = tissue culture.
[c] THK-1 = continuous cell line of SV40 transformed hamster kidney cells.[16]
[d] P = passage level.
[e] Clonal lines derived from THK-1 line.[17]
[f] Tissue culture line of cells from tumors induced with SV40 transformed hamster embryo cells; these tumors were kindly supplied by Dr. Joseph Melnick.
[g] Continuous cell line of bovine conjunctiva transformed by bovine papilloma virus.[20]
[h] Normal carcass; normal kidney TC; primary polyoma tumor; polyoma primary tumor TC cells (Stanton); polyoma-transformed hamster embryo cells (Dulbecco); adenovirus 12 tumor; adenovirus 12 tumor TC cells P-26 (Schlesinger); adenovirus 18 tumor; Rous tumor.

of rabbit antisheep erythrocyte serum, indicating the presence of the Forssmann antigen in many of these cell lines;[15] however, all antigens of the other species had negative to partial reactions with this serum. The positively reacting antigens did not react with sera of hamsters with other virus-induced tumors or with a monkey serum negative for SV40 antibodies.

Comparison of some properties of tumor and viral antigens: Although the reaction of tumor extracts and transformed cells with the antiviral monkey serum suggested that the tumors contained antigens in common with the viral antigen preparation, the properties of the two types of antigen differed in at least two important respects. Firstly, antigens in tumor extracts were rendered nonreactive by heating at 50 or 56°C for 30 min, whereas the viral antigen was unaffected (Table 3). Secondly,

TABLE 3

EFFECT OF HEAT ON CF ANTIGEN TITERS OF TUMOR EXTRACTS AND VIRUS

Antigen	CF Antigen Titer versus Indicated Serum: Primary hamster tumor serum #5586 1:20 (8 antibody units)	Primary hamster tumor serum #2004 1:40 (≥4 antibody units)	SV40 immune monkey serum #2159 1:20	1:80
1st passage transplant of primary SV40 hamster tumor, #2007				
Unheated	16	≥64	8	
Heated 56° 30′	0	0	0	
Transformed hamster cells, #3775*				
Unheated	32	32	8	
Heated 56° 30′	0	0	0	
Transformed hamster cells, #3797*				
Unheated	32		≥16	
Heated 50° 30′	(1)†		0	
Heated 56° 30′	0		0	
SV40 Virus				
Unheated	0	4		8
Heated 50° 30′	0	≥ 2		8
Heated 56° 30′	0	≥ 2		8

* #3775 and #3797 are tissue culture cell lines of SV40 transformed hamster embryo cultures (Melnick). See Table 2.
† Partial reaction undiluted.

the tumor antigens were chiefly "soluble," remaining unsedimented after centrifugation, whereas the viral antigen was completely sedimented (Table 4).

Characteristics of antibody responses to viral and tumor antigens in tumor-bearing hamsters: In a previous report,[10] we described the patterns of neutralizing and viral CF antibody responses in hamsters developing tumor after inoculation with virus when 2 days old. Tumor development in these animals was accompanied by development of rising titers of neutralizing antibody, the titer tending to parallel the size of tumor. CF antibody to viral antigen also developed, but usually much later and in lower titer than the neutralizing antibody.

One hundred and seven sera from 34 hamsters with primary SV40-induced tumors were tested for CF antibody reactive with tumor extracts. These sera are serial samples from the animals described in reference 10. The upper portion of Table 5 summarizes the relationship of this CF antibody to size of tumor at the time of bleeding. No antibody was detected prior to tumor development, while antibody appeared in moderate titer in the majority of hamsters with early tumors, and tended to rise in titer as tumors enlarged. In all, 31 of the 34 animals developed antitumor CF antibody.

The great majority of hamsters with tumors produced by transplantation of the THK-1 line[16] of *in vitro* transformed hamster kidney cells, or of the clones derived therefrom,[17] also developed antibodies to tumor extracts (Table 5, bottom), and in generally higher titers than in the animals with primary tumors.

Many of these serial serum specimens were also tested for CF and neutralizing antibody to SV40; the relationships of titers of antiviral and antitumor antibodies are shown in Table 6. Hamsters with primary tumors showed a slight correlation between tumor CF antibody and neutralizing or viral CF antibody titers, in that

TABLE 4

ULTRACENTRIFUGATION OF CF ANTIGENS IN TUMOR EXTRACTS AND VIRAL HARVEST

Material tested[c]	Primary Tumor #2007[a]		Transformed Cells #3775[b]		SV40 Virus (BSC-1 TC)	
	Primary hamster tumor serum #5586 1:10 (16 antibody units)	SV40 immune monkey serum #2159 1:20	Primary hamster tumor serum #5586 1:10 (16 antibody units)	SV40 immune monkey serum #2159 1:20	Primary hamster tumor serum #5586 1:10	SV40 immune monkey serum #2159 1:80
Starting suspension	16[d]	8	16	4	0	2
Centrifugal fractions						
Upper supernatant	8	2	8	4	0	0
Lower supernatant	16	4	16	8	0	0
Pellet (10×)	16	(AC)[e]	1	0	0	8

[a] This tumor extract was not reclarified prior to ultracentrifugation. Precipitates formed as a result of storage in the frozen state may have been responsible for the appearance of antigen in the pellet fraction.
[b] For description see Table 2.
[c] For centrifugation procedure see *Materials and Methods* section.
[d] CF antigen titer.
[e] The low titer reaction observed could not be evaluated because of anticomplementary activity at a 1:2 dilution.

TABLE 5

RELATIONSHIP OF "ANTITUMOR" CF ANTIBODY TITER TO SIZE OF TUMOR

Type of tumor	Size of tumor*	CF Antibody Titer to Tumor Antigen <5	5–20	40–>160	No. sera pos†: no. tested	
Primary	Pretumor	35‡	0	0	0/35	
	Small	5	11	5	18/23	(78%)
	Medium	8	10	14	29/37	(78%)
	Large	2	3	6	10/12	(83%)
Transplanted THK-1 cells§	Pretumor	11	0	0	0/11	
	Small	6	2	2	4/10	(40%)
	Medium	3	4	27	31/34	(91%)
	Large	1	0	8	8/9	(89%)

* Small = 0.1–1.0 cm mean tumor diameter. Medium = 1.1–3.5 cm mean tumor diameter. Large = >3.5 cm mean tumor diameter.
† Includes 8 sera which were positive in screening test but on which titers were not determined.
‡ Number of sera with indicated titer.
§ For description of THK-1, see Table 2.

animals with high titer tumor CF antibody tended to have the highest antiviral titers. In view of the correlation of all three titers with tumor size, the lack of clear correlation here is all the more striking. Tumors induced with transformed cells rarely induced antiviral antibodies; the viral CF and neutralizing antibodies which were detected were confined to sera of two animals with large tumors and high anti-tumor CF antibody. Neither of these two tumors contained demonstrable infectivity, as judged by tests of three serial biopsy specimens from one, and an autopsy specimen from the other. Although these data suggest some relationships between viral and tumor antigens, they also provide clear evidence that they are not identical.

Discussion.—The data presented here have established that cells which have become tumorous or which have undergone transformation subsequent to infection with SV40 consistently elaborate a specific CF antigen(s) not found in normal tissue or other tumors. The predominant antigen is clearly distinct from the SV40 antigen(s) detected in viral suspensions by the routine CF and neutralization procedures, both in serologic reactivity, as evidenced by the reactions with hamster antisera, and physically, as shown by the differences in thermal inactivation and sedimentation characteristics. However, present data do not exclude the possibility that this "tumor" antigen may be present in subdetectable amounts in viral harvests, and do not necessarily imply that its presence is restricted to transformed cells or cells with an integrated cell-virus relationship.

TABLE 6

COMPARISON OF ANTITUMOR AND ANTIVIRAL ANTIBODIES IN HAMSTER SERA

"Antitumor" CF antibody titer	Number of Sera with Titers in Range Indicated: Primary Tumors				Transformed Cell-Induced Tumors			
	Antiviral CF antibody titer							
	0	5–10	20–40	≥80	0	5–10	20–40	≥80
0	9	3	1	0	20	0	0	0
5–10	17	3	0	0	1	0	0	0
20–40	12	2	0	0	9	0	0	0
≥80	6	5	3	1	32	2*	1*	0
	Neutralizing antibody titer							
	0	5–10	20–40	≥80	0	5–10	20–40	≥80
0	1	3	4	0	12	2	0	0
5–10	5	6	0	0	0	0	0	0
20–40	2	7	1	0	9	0	0	0
≥80	0	0	4	4	23	1*	2*	3*

* In the tests of sera from hamsters with tumors induced with transformed cells, the 3 sera positive in CF and the 6 sera positive in neutralization tests were all from 2 animals.

Although the predominant antigen in tumor extracts is distinct from the presently known "viral" antigen(s), it is not clear whether the latter is also present in the neoplastic cells in immunologically significant amounts. It appears that SV40 tumors[18, 19] and transformed cells[16, 17] contain the viral genome in a highly integrated state, and may occasionally liberate small amounts of infectious virus. However, as judged by the relation between infectivity and CF antigen titers in AGMK harvests, the quantity of virus produced by tumor cells is far too small to be detected by the CF test, and is probably inadequate even to induce antibody response. Previous studies of antibody responses of hamsters with primary SV40-induced tumors suggested that significant amounts of viral antigens were indeed elaborated, since neutralizing antibody titers rose proportionally to size of tumor, even in animals with noninfectious tumors.[10] However, a few animals with large tumors failed to develop neutralizing or viral CF antibody. As reported here, tumors induced with transformed tissue culture cells rarely stimulated antiviral antibody response, suggesting that there may be a difference between primary and transformed cell-induced tumors with regard to content of viral antigen. This discrepancy may be due to differences in degree of expression of the viral genome, or to the booster effect of small amounts of viral antigen in primary tumors on the minimal antibody response produced by the initial virus inoculum. In this regard it is of interest that two hamsters with apparently noninfectious tumors induced by transplantation of transformed cells developed rising titers of neutralizing and viral CF antibody similar to the responses observed in primary tumors.

The consistent reactivity of hamster tumor and transformed cell suspensions with the SV40 immune monkey serum could also indicate presence of viral CF antigens. However, the reacting antigen showed the heat instability and low sedimentation rate which were characteristic of the "tumor-specific" antigen; also, the quantitative aspects of the reactivity differed markedly from the reaction of this serum with viral antigen. It seems more likely that the monkey serum contains two distinct types of antibody, one against virus and one against "tumor-specific" antigen formed during the course of a subclinical and presumably nononcogenic infection.

The relationship of the new cellular antigen described here to that responsible for rejection of SV40 tumor transplants by hamsters immunized with SV40[7–9] is

not known. The only available evidence suggests that they are distinct, since animals hyperimmunized with SV40 did not develop CF antibody to tumor antigens (Table 1) and these animals presumably would be resistant to tumor challenge.

The presence of the tumor CF antigen in all species transformed cells tested provides strong evidence that it is formed from information present in the viral genome. The alternative, that the information for formation of the antigen is of host cell origin, made functional by the viral infection, seems incompatible with the specificity of the antigen and with its presence in five genera of mammals. The presence of the antigen, coupled with the difficulty of detection of infectious virus in transformed rabbit and mouse cells,[11] suggests that the genetic information of SV40 is present in these cells in an integrated state similar to that in hamster cells. It is noteworthy that this apparently integrated virus-cell relationship has not led to overtly malignant cell behavior in the case of the C3H mouse cells, which do not transplant in isologous hosts;[11] also, in the case of the bovine cell cultures, production of the antigen was not accompanied by noticeable alteration in cellular morphology or growth pattern.

The findings reported herein are similar in many respects to the findings of Heubner *et al.*[6] in virus-free hamster tumors induced by adenovirus types 12 and 18; however, in the adenovirus tumors, noninfectious viral antigens are easily demonstrated. There is also evidence for tumor specific antigens not found in viral harvests, but their identification as wholly new antigens is not clearly resolved.

Summary.—Suspensions of hamster tumors and various species cells transformed by SV40 contain a specific antigen(s) demonstrable by CF tests with sera of hamsters bearing SV40 tumors. This antigen has several properties which are distinct from the SV40 viral antigen(s); it is soluble, heat labile, and has a different serologic reactivity. These results, taken together with other evidence, support the hypothesis that the new antigen is synthesized by information from SV40 viral genome integrated in the tumor and *in vitro* transformed cells.

Antibody to the "new" cellular antigen appears in the serum of hamsters soon after the development of tumor, and rises in titer as the tumors increase in size. Response to this antigen is largely independent of antiviral antibody response.

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VIRAL INHIBITION IN THE METAPHASE-ARREST CELL

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RNA-virus development proceeds normally in cells specifically inhibited for DNA or RNA synthesis.[1-7] Thus, in cells exposed to inhibitors of DNA or DNA-dependent RNA synthesis, Mengovirus,[2-4] poliovirus,[5, 6] and Newcastle disease virus[1, 7] show characteristic growth curves and essentially normal yields. During mitosis, cellular RNA is not synthesized.[8, 9] The natural suppression of RNA synthesis from late prophase to early telophase provides a system to examine viral replication in a cell devoid of DNA and RNA synthesis. It permits inquiry into the question of whether normal regulatory processes of the cell can exert control over an invading viral nucleic acid. To explore this problem we have used large populations of cells arrested in metaphase. Primarily this study describes the inhibition of viral replication following the interaction of metaphase-arrest HeLa cells with an RNA virus of the Myxovirus family, Newcastle disease virus (NDV). Experiments reported here also demonstrate inhibition of a small RNA virus (poliovirus) and a DNA virus (vaccinia), under similar conditions, and provide evidence for viral inhibition in the normal metaphase cell. Actinomycin D-treated and metaphase-arrest cells are compared as hosts for viral replication.

Materials and Methods.—Preparation of metaphase-arrest cells: Clonal line HeLa S3 cells were grown as monolayers in attachment solution[10] minus Ca^{++} or Eagle's spinner medium[11] in which Na_2HPO_4 was replaced with NaCl, and calf serum added to a final concentration of 6%. Cells were arrested in metaphase using the spindle inhibitor, vinblastine sulfate (Velban, Lilly) added at 0.05 to 0.1 μg/ml of medium. Cells in metaphase-arrest were harvested from the monolayer preferentially by employing a shaking technique developed by Terasima and Tolmach.[12] Omission of Ca^{++} from the growth medium greatly facilitated this procedure by producing a tenuously attached metaphase-arrest cell. The populations were concentrated by centrifugation and resuspended in appropriate medium. It was possible to obtain readily over 10^8 cells of which 98–99% were in metaphase-arrest. Vinblastine-exposed cells not recruited into metaphase-arrest, i.e., those remaining attached to the glass substrate following harvesting of the arrested cells were detached by trypsinization and used as control-interphase cells.

Virus-cell interaction: Monodisperse HeLa cells were exposed to NDV-strain Calif. by means of the dual temperature-dilution sequence already described.[13] Virus attachment was effected at 2°C while dilution, virus penetration, eclipse, and growth were carried out at 37°C. This procedure imparts a relatively high degree of synchrony to the initiation of infection. Assessment of virus multiplicity was by plaque assay on chick embryo cell monolayers.[13] Infected cells were scored