ONCOGENES OF RNA TUMOR VIRUSES AS DETERMINANTS OF CANCER

By Robert J. Huebner and George J. Todaro

VIRAL CARCINOGENESIS BRANCH, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

Communicated September 16, 1969

Abstract.—Evidence from sero-epidemiological studies and from cell culture studies supports the hypothesis that the cells of many, and perhaps all, vertebrates contain information for producing C-type RNA viruses. It is postulated that the viral information (the virogene), including that portion responsible for transforming a normal cell into a tumor cell (the oncogene), is most commonly transmitted from animal to progeny animal and from cell to progeny cell in a covert form. Carcinogens, irradiation, and the normal aging process all favor the partial or complete activation of these genes. An understanding of how normal cells and normal animals prevent expression of endogenous viral information would appear to offer one of the best hopes for the control of naturally occurring cancers.

Several new lines of evidence have led us to propose that there exists a unique class of viruses present in most, and perhaps all, vertebrates that plays an important etiologic role in the development of tumors in these animals.[1] The unique property of this virus group we suggest is that viral information can be transmitted from animal to progeny animal and from cell to progeny cell as a repressed viral genome. In this sense these agents behave more like cellular genes than like infectious virus; consequently, horizontal transmission (from animal to neighboring animal and from cell to neighboring cell) as a natural mode of spread is infrequent and a relatively unimportant factor in the *natural* occurrence of cancer.

The viruses belong to the group of C-type RNA viruses[2] first demonstrated to be oncogenic in chickens by Ellerman and Bang[3a] and by Rous [3b] about 60 years ago and in mice by Gross in 1951.[3c] Since that time, but especially in the last few years, morphologically and functionally similar C-type viruses have also been isolated from hamsters[3d] and cats[4a–c] and have been seen, though infrequently, by electron microscopy in several other species, including man. While the complete infectious form of the virus is rarely observed under natural conditions, in certain inbred mouse strains with high leukemia incidence, such as AKR and C58, and some lines of chickens, overt virus is commonly observed and is demonstrable even prior to birth.[3c, 5, 6a–c] In low or moderate leukemia-incidence mouses trains, overt expression is generally absent early in life but appears in certain tissues later at a time when there is also an increased incidence of tumors.[6a, b] Whether infectious virus and/or tumor become expressed is determined largely by host genetic factors,[7a–c] but expression can be influenced by environmental factors such as radiation[8a, b] and exposure to carcinogens.[9a–c] While full viral expression can occur, we postulate that more frequently there is

only partial expresssion, with perhaps the only gene of the virus expressed being that responsible for transforming the cell into a tumor cell (the oncogene).

The new hypothesis predicts that both spontaneous cancers and cancers induced by chemical and physical agents will be the result of expression of the oncogene(s) of covert C-type RNA virus.[9d] Numerous studies, some of them only recently completed, have established these viruses as significant causes of cancer in mice, chickens, cats, and probably also in hamsters.[1, 4b-c, 10] The C-type RNA-virus particles have also been observed by electron microscopy in tumors of guinea pigs,[11a] rats,[11b] swine,[11c] snakes,[11d] and humans;[11e, f] thus, three classes of vertebrates are now known to have at least some natural expression of viruses of this class. The central hypothesis implies, therefore, that the cells of many if not all vertebrates carry vertically transmitted (inherited) RNA tumor virus information (virogenes) which serves as an indigenous source of oncogenic information (oncogenes) which transforms normal cells into tumor cells; additional phenotypic expression of viral information may or may not also occur.[12]

The broad concept we are proposing derives from the following observations: (1) The C-type RNA virus particles have been found in almost all species of vertebrates so far examined for them. (2) They have a proven role as naturally occurring causative agents in spontaneous cancers of certain well studied animal species. In contrast, the "oncogenic" DNA viruses, polyoma, SV40, and adenoviruses of various species, do not appear to be significant factors in the development of cancers in their natural hosts. (3) The types of cancer and the stochastic occurrences of cancer observed in a species, the human, where C-type RNA viruses do not have a known etiologic role, are similar to those observed in mice and cats[5, 14] wherein the C-type RNA viruses are known to be etiologically involved.

With new virus assay techniques, field studies of the natural prevalence of C-type RNA viruses have revealed previously undetectable evidence of partial and/or complete expression of the virogene in chickens, mice, and cats.[1, 4, 6a, b, 15-19] Murine strains having a low or intermediate incidence of leukemia, such as the BALB/c, reveal little or no phenotypic expression of virus early in life but considerable amounts appear later in life when tumors are also most frequent[6a, b] (see Table 1). Inbred low tumor-incidence strains, such as C_3H/Bi or C57B1, reveal little or no C-type RNA virus expression until late in life;[1, 6a, 20] however, various different breeding programs have resulted in the segregation of substrains having early expression of the viral antigen.[1, 7b] Cross-breeding experiments using high (100%)-incidence AKR males with low (<1%)-incidence C_3H/Bi females leads to an approximately 50-per cent incidence of leukemia in progeny.[7b, 21] Meier *et al.* have recently demonstrated that clinical expression of leukemia shows linkage with an autosomal recessive gene for hairlessness in the HRS congenic mice.[7c] While "vertical transmission"[22] has generally been accepted as a frequent mode of transmission of viral information, it has previously been explained in terms of transmission of infectious particles.[7b] We suggest, however, that what is transmitted is susceptibility to the expression of the endogenous virogenes and oncogenes that are present with varying degrees of expression in all mice.

A highly significant recent observation was that wild (feral) mice, free of in-

TABLE 1. *Natural expression of C-type RNA virus genome in mouse strains differing in their tumor incidence.*

Inbred strains	CF Antigen (spleen)	Infectious virus	Tumor (excluding mammary)
High			
(AKR/NB) (to 6 mo.)	+++	+++	−
Mid-life (6–12 month)	+++	+++	+++
Late (12 month or >)	All dead; 90–95% with leukemia		
Intermediate			
(BALB/c)/NB	−	−	−
Mid-life	+	+	±
Late	+++	+++	++
Low			
C57B1/NB	±	−	−
Mid-life	−	−	−
Late	2–5%	2–5%	Rare (2–5% lymphoma)
Noninbred strains			
CF-1 NB	+	+	−
Mid-life	++	++	−
Late	+++	+++	Rare
NIH			
Swiss NB	±	−*	−
Mid-life	+	−	−
Late	+++†	−*	Rare

* Not yet isolated in METC.
† Found in spontaneous and radiation-induced tumor.

fectious virus, when treated with methylcholanthrene developed fibrosarcomas, about 10 per cent of which contained the murine C-type RNA virus group-specific antigen; also, C-type virus particles were visualized in the carcinogen-induced tumors by electron microscopy.[23] Thus, while viral expression and oncogene expression are rare under natural conditions in wild mice, carcinogenic agents that promote virus expression apparently can be used to demonstrate the presence of the genomic information.

The experiments by Gross,[8a] Lieberman and Kaplan,[8b] Ageenko,[9a] Irino *et al.*,[9b] and Toth and Shubik,[9c] which showed that infectious leukemia and sarcoma viruses appear in the radiation and chemically induced lymphomas of low-incidence mice, led to the suggestion that latent tumor viruses could be activated in a manner resembling the activation of temperate bacteriophages by similar radiological and chemical agents.[24, 25a] Huebner, Kelloff, and Lane[25b] recently demonstrated sarcoma-inducing C-type virus in sarcomas of CF-1 mice induced by 3-methyl-cholanthrene. Similarly, Freeman *et al.*[26] have shown that while neither the chemical carcinogen, diethylnitrosamine alone, or murine leukemia viruses alone were able to transform rat embyro cells, cultures exposed to both showed a marked morphological alteration.

The most direct evidence for the hypothesis comes from cell culture experiments involving BALB/c embryo cells. Aaronson *et al.*[22] have found that under one set of culture conditions (frequent transfer at high cell densities), the cells (BALB/3T12-4) began to spontaneously release C-type RNA viruses. This occurred only after several months in culture and after many transfers. The

cells had become aneuploid, had lost contact inhibition of cell division, and had become neoplastic.[27b] The reproducibility of the phenomenon would appear to rule out accidental contamination. These cell culture experiments led to the conclusion that the viral genome having the capacity to make the virus must be endogenously present in normal mouse embryo cells under conditions where the most sensitive methods are unable to detect any expression of the C-type virus. It appears, therefore, that there is finite but low probability that expression of the C-type RNA viral genome will occur with time both in the animal and in cell culture systems; the critical events leading to spontaneous derepression of the virus genome may well reflect stochastic somatic mutational events. However, to prove, that C-type virus information is present in each and every murine cell it is necessary to isolate single cell clones from virus-free embryo cultures and to demonstrate that the virus or viral specific functions can be induced in these clonal lines.

The virus that emerges from the long-term cultures of BALB/c embryo cells has as one of its properties a greatly reduced ability to grow in BALB/c embryo cells. Yet when this virus is isolated and propagated on cells of another mouse strain, NIH Swiss, it grows readily.[6a, 27] BALB/c cell lines that apparently remain virus free, such as BALB/3T3, remain highly resistant to the virus that emerges from the BALB/3T12 cells; NIH Swiss embryo cells and cell lines derived from them remain virus sensitive.[27c] The most reasonable explanation would be that the BALB/c cells have a powerful repressor for the endogenous BALB/c virus and NIH Swiss cells lack the repressor. An understanding of how the normal cell and the normal animal prevent the expression of indigenous viral information would appear to us to offer one of the best hopes for the control of naturally occurring cancers.

The fact that mice, cats, and chickens appear to be completely tolerant to the group-specific virion antigen expression of their homologous C-type leukemia and sarcoma viruses provides additional evidence of vertical transmission of the virus genome and also evidence for at least a low degree of phenotypic expression of this antigen prior to birth.[1] These animals, however, do respond with antibodies to the virus envelope and to the purified infectious virus particle indicating that they have not become tolerant to the fully infectious virus.[1, 7b, 20, 28a, b, 29]

The unexpressed viral genome that we envision as being transmitted from cell to progeny cell could be transmitted as either (1) a number of replicate ribonucleic acid molecules or (2) part of the host genetic material located in the chromosomes. Support for the former possibility is derived from experiments by Montagnier[30] and Colby and Duesberg[31] in which they have reported double-stranded RNA in apparently uninfected cells; cells infected with avian sarcoma virus, however, contain considerably more double-stranded RNA.[30] Whether the low level of double-stranded RNA in the uninfected cells might be C-type viral genetic material is not yet known. If the information is present as RNA, we would have to predict that uninfected cells from practically any vertebrate should have some RNA replicase activity.[32] The second possibility, that it is carried as a cellular gene in the form of a DNA "provirus," has been suggested by the experiments of Temin,[33] Bader,[34] and others, which demonstrate that

Rous sarcoma virus and murine sarcoma virus require cellular DNA synthesis for their replication and to produce transformation. Harel *et al.* have reported homology between the C-type virus RNA and uninfected cellular DNA in both the avian[35a] and the murine[35b] systems. Genetic and antigenic data by Payne and Chubb,[36a] Altaner and Temin,[36b] Weiss,[36c] and Kelloff *et al.*[10] are most consistent with the hypothesis of endogenous viral information present in apparently virus-free cells of the chick, the rat, and the hamster. Like many cellular genes, the genes coding for the unique C-type viral functions may not be expressed under normal conditions because of potent repressors for expression. Viewed in this light, the application of radiation, chemical carcinogens, and the natural aging process are believed to "switch on" the viral genome, perhaps by decreasing the level of repressor activity.

When we say that a given animal is virus free, it is of course apparent that this may reflect the insensitivity of the test systems being used. Nevertheless, it is clear that different degrees of viral function can be expressed independently in C-type RNA-containing cells (see Table 2). Thus, the internal nucleoid (group-specific) antigen is frequently present when the infectious virus is not demonstrable.[6, 36a, 37a, b] Virus production, detected by 3H-uridine incorporation into C-type particles banding 1.16–1.18 on equilibrium sedimentation in a sucrose density, gradient, was found by Robinson[38a] in "non-producing" Rous sarcoma cells.[38b] Further, some sarcoma cell lines can be negative by all other tests and yet can frequently be shown to have a sarcoma virus genome which can be readily rescued.[10, 37] A potentially useful marker for the presence of a functioning sarcoma virus genome is a drastically lower K_m for glucose uptake. In mouse and hamster cells infected and transformed by mouse sarcoma virus[39a] as well as

TABLE 2. *Demonstrable independent expressions of C-type RNA virus.*

V	gs	T	Examples found in
+	+	+	"High incidence" inbred mice; lymphomatous and sarcomatous chickens and cats. C58, AKR spontaneous lymphomas (95% at 6 to 10 months).
			Spontaneous and chemically induced tumors in "intermediate incidence" mice. Tumors induced by Rous and mouse sarcoma viruses with high dosage virus in natural hosts.
0	+	+	Tumors of mouse and Rous sarcoma viruses in heterologous hosts or in homologous hosts given low dosage. Certain "low incidence" Swiss mice with spontaneous and induced tumors.
0	0	+	Hamster tumors induced by mouse sarcoma virus, demonstrable by genome rescue.
			Certain "low incidence" mouse strains with chemically induced tumors.
+	+	0	Spleens of CF-1 at weaning age (50%) and normal BALB/c mice at 8 to 30 months (percentage increasing with age).
0	+	0	Spleens and thymuses of "virus-free" Swissand feral mice. Livers of many "RIF-free" chick embryos.
0	0	0	Newborn mice of low incidence strains.
			C57 strains up to 30 months of age; activated to +++ in chemically and radiation-induced lymphomas.

V = infectious (virogene) expression including viral envelope subunits.
gs = group-specific antigen (virogene) expression (noninfectious internal nucleoid subunits).
T = tumor (oncogene) expressions (leukemias, sarcomas in mice, hamsters, cats, and chickens; carcinomas in chickens and probably also in other animals).

both "spontaneous" and mouse sarcoma virus-induced tumor cells, there is an alteration in glucose uptake not seen with normal cells or with DNA-tumor virus-transformed cells.[39b] It is noteworthy that cell lines derived from "spontaneous" sarcomas of man in five of five cases show glucose uptake values in the range found for tumors of hamsters and mice induced by mouse sarcoma virus.[39c] The hypothesis would predict that human sarcomas and leukemias are due to similar agents but that a transmissible C-type RNA virus of humans will only rarely be found in "spontaneous" tumors.

Lifetime studies of C-type viral expression in most established colonies of mice, including wild mice, have revealed no murine colonies that are wholly free of C-type RNA virus involvement.[6a, b, 17, 40] This implies that the arrangement between this virus and its natural host is a long-standing one. Its demonstration in nine different species and in three classes of vertebrates, together with the evidence of vertical rather than horizontal transmission as the chief mode of spread, suggests that this virus genome is an essential part of the natural evolutionary inheritance of vertebrate cells.

It is interesting that certain clones of 3T3 transformed by SV40 reveal low but reproducible levels of murine leukemia virus group-specific antigen; however, infectious virus has not been isolated.[27a, 41] This suggests that the "oncogenic" DNA viruses may function, in part, by activating previously repressed oncogenes in the cells they infect; thus, as part of the over-all hypothesis presented it is possible that the oncogenic DNA viruses may also serve as carcinogens, derepressing C-type RNA virus information indigenous to the cells. That the viruses can interact with one another is supported by the recent demonstration of Freeman *et al.*[42] of greatly accelerated *in vitro* transformation of rat cells by various adenoviruses when the cells are previously infected with C-type RNA viruses.

To summarize: Our hypothesis suggests that the cells of most or all vertebrate species have C-type RNA virus genomes that are vertically transmitted from parent to offspring. Depending on the host genotype and various modifying environmental factors, either virus production or tumor formation or both may develop at some time in these animals and/or in their cells grown in culture. This hypothesis implies that the occurrence of most cancer is a natural biological event determined by spontaneous and/or induced derepression of an endogenous specific viral oncogene(s). Viewed in this way, ultimate control of cancer will therefore very likely depend on delineation of the factors responsible for derepression of virus expression and of the nature of the repressors involved. We believe that the hypothesis provides a rational basis for a unifying theory and is consistent with the phenomena of radiation and chemically induced cancer as well as the stochastic occurrence of spontaneous cancer. The availability of *in vitro* test systems to study the derepressed virus in cells in culture should make it possible to analyze this phenomenon at the cellular and molecular level.

[1] Huebner, R. J., *et al.*, "Switched off vertically transmitted C-type RNA tumor viruses as determinants of spontaneous and induced cancer: A new theory of viral carcinogenesis," in preparation.

[2] Bernhard, W., *Cancer Res.*, **20**, 712 (1960).

[3] (*a*) Ellerman, V., and Bang, O., *Zentralbl. Bakteriol. Parasiterik. Abt. 1 Orig.*, **46**, 595 (1908). (*b*) Rous, P., *J. Exptl. Med.*, **13**, 397 (1911). (*c*) Gross, L., *Proc. Soc. Exptl. Biol.*

Med., **76**, 27 (1951). (*d*) Graffi, A., T. Schramm, E. Bender, I. Graffi, K. Horn, and D. Bierwolf, *Brit. J. Cancer*, **22**, 577 (1968).

[4] (*a*) Jarrett, O., H. M. Laird, D. Hay, and G. W. Crighton, *Nature*, **219**, 521 (1968). (*b*) Rickard, C. G., J. E. Post, F. Noronha, and L. M. Barr, *J. Natl. Cancer Inst.*, **42**, 987 (1969). (*c*) Snyder, S. P., and G. H. Theilen, *Nature*, **221**, 1074 (1969).

[5] Gross, L., "Mouse leukemia," in *Oncogenic Viruses* (New York: Pergamon Press, 1961) p. 153.

[6] (*a*) Hartley, J. W., W. P. Rowe, W. I. Capps, and R. J. Huebner, *J. Virology*, **3**, 126 (1969). (*b*) Peters, R., G. Spahn, L. Rabstein, and R. J. Huebner, unpublished data. (*c*) Dougherty, R. M., H. S. Di Stefano, and F. K. Roth, these PROCEEDINGS, **58**, 808 (1967).

[7] (*a*) Tennant, J. R., and G. D. Snell, *J. Natl. Cancer Inst.*, **41**, 597 (1968). (*b*) Aoki, T., E. A. Boyse, and L. J. Old, *J. Natl. Cancer Inst.*, **41**, 97 (1968). (*c*) Meier, H., D. D. Myers, and R. J. Huebner, "Genetic control by the hr-locus of susceptibility and resistance to leukemia," these PROCEEDINGS, **63**, 759 (1969).

[8] (*a*) Gross, L., *Acta Haematol.*, **19**, 353 (1958). (*b*) Lieberman, M., and H. S. Kaplan, *Science*, **130**, 387 (1959).

[9] (*a*) Ageenko, A. I., *Folia Biologica*, **8**, 7 (1962). (*b*) Irino, S., Z. Ota, T. Sezaki, K. Suzaki, *Gann*, **54**, 225 (1963). (*c*) Toth, B., and P. Shubik, *Cancer Res.*, **27**, 43 (1967). (*d*) In the case of mammary tumors the similar but apparently less widespread B-type RNA virus must be considered.

[10] Kelloff, G., R. J. Huebner, Y. K. Lee, R. Toni, and R. V. Gilden, "Hamster-tropic sarcomagenic and non-sarcomagenic virus(es) derived from hamster tumors induced by the Gross pseudotype of Moloney sarcoma virus," these PROCEEDINGS, in press.

[11] (*a*) Opler, S. R., *Scientific Proc., Amer. Assoc. Path. Bact.*, **20a** (1967). (*b*) Weinstein, R. S., and W. C. Moloney, *Proc. Soc. Exptl. Biol. Med.*, **118**, 459 (1965). (*c*) Howard, E. B., W. J. Clarke, and P. L. Hackett, in "Proceedings of the 3rd International Symposium on Comparative Leukemia Research," Paris, 1967: *Bibl. Haemat.*, **31**, 255 (1968). (*d*) Zeigel, R. F., and H. F. Clark, "Morphological aspects of a new "C" type viral agent of reptilian origin," *Proc. Electron Microscopy Soc. Amer.*, in press. (*e*) Dmochowski, L., "Viral studies in human leukemia and lymphoma," in *Proceedings of International Conference on Leukemia and Lymphoma* (Philadelphia: Lea and Febiger, 1968), p. 97. (*f*) Morton, D. L., W. T. Hall, and R. A. Malmgren, *Science*, **165**, 813 (1969).

[12] The latter consists of the internal nucleoid group-specific antigen[12a, c] and/or the type-specific Gross (G+) or Friend/Moloney/Rauscher antigens.[12b] These antigens are generally not detectable early in life but appear later in life and particularly in lymphomas and sarcomas. (*a*) Huebner, R. J., D. Armstrong, M. Okuyan, P. S. Sarma, and H. C. Turner, these PROCEEDINGS, **51**, 742 (1964). (*b*) Old, L. J., and E. A Boyse, *Fed. Proc.*, **24**, 1009 (1965). (*c*) Geering, G., L. J. Old, and E. A. Boyse, *J. Exptl. Med.*, **124**, 753 (1966).

[13] Rabstein, L., R. Madison, R. Peters, and G. Spahn, "Natural prevalence and pathologic types of cancer observed in a lifetime study of BALB/c mice," in preparation.

[14] Dorn, C. R., D. O. N. Taylor, R. Schneider, H. H. Hibbard, and M. R. Klauber, *J. Natl. Cancer Inst.*, **40**, 307 (1968).

[15] Rubin, H., these PROCEEDINGS, **46**, 1105 (1960).

[16] Sarma, P. S., H. C. Turner, and R. J. Huebner, *Virology*, **23**, 313 (1964).

[17] Myers, D. D., H. Meier, J. S. Rhim, and R. J. Huebner, "Excretion of murine leukemia virus and the failure of horizontal viral transmission," in preparation.

[18] Sarma, P. S., J. Baskar, and R. J. Huebner, "Complement-fixation test for the detection and assay of feline leukemia viruses," in preparation.

[19] Gardner, M. B., P. Arnstein, R. Rongey, P. S. Sarma, and R. J. Huebner, unpublished observations.

[20] Igel, H. J., R. J. Huebner, H. C. Turner, P. Kotin, and H. L. Falk, "Mouse leukemia virus activation by chemical carcinogens," *Science*, in press.

[21] Gross, L., and Y. Dreyfuss, "How is the mouse leukemia virus transmitted from host to host under natural life conditions," in *Carcinogenesis: A Broad Critique* (Baltimore: Williams & Wilkins Co., 1967), p. 9.

[22] Gross, L., *Proc. Soc. Exptl. Biol. Med.*, **78**, 342 (1951).

[23] Gardner, M. B., J. D. Estes, H. C. Turner, R. Rongey, and R. J. Huebner, unpublished observations.

[24] Kaplan, H. S., *Cancer Res.*, **27**, 1325 (1967).

[25] (*a*) Whitmire, C. E., R. L. Peters, L. S. Rabstein, R. J. Huebner, H. C. Turner, and J. W. Hartley, "Induced expression ("switch-on") of previously "switched-off" C-type RNA tumor

virus group-specific antigen during induction of neoplasia by 3-methylcholanthrene, I. Antigen expression in fibrosarcomas of BALB/c mice given 3-methylcholanthrene subcutaneously at different ages," in preparation. (*b*) Huebner, R. J., G. Kelloff, and W. T. Lane, unpublished observations.

[26] Freeman, A. E., P. J. Price, H. J. Igel, J. C. Young, J. M. Maryak, and R. J. Huebner, "Morphological transformation of rat embryo cells induced by diethylnitrosamine and murine leukemia viruses," *J. Natl. Cancer Inst.*, in press.

[27] (*a*) Aaronson, S. A., J. W. Hartley, and G. J. Todaro, "Mouse leukemia virus: "Spontaneous" release by mouse embryo cells after long term *in vitro* cultivation," these PROCEEDINGS, **64**, 87 (1969). (*b*) Aaronson, S. A. and G. J. Todaro, *Science*, **162**, 1024 (1968). (*c*) Jainchill, J. L., S. A. Aaronson, and G. J. Todaro, "Murine sarcoma and leukemia viruses: Assay using clonal lines of contact inhibited mouse cells," *J. Virology*, in press.

[28] (*a*) Sarma, P. S., R. J. Huebner, and D. Armstrong, *Proc. Soc. Exptl. Biol. Med.*, **115**, 481 (1964). (*b*) Kelloff, G., and P. K. Vogt, *Virology*, **29**, 377 (1966).

[29] Aoki, T., E. A. Boyse, and L. J. Old, *J. Natl. Cancer Inst.*, **41**, 89 (1968).

[30] Montagnier, M. L., *C. R. Acad. Sci.*, **267**, 1417 (1968).

[31] Colby, C., and P. H. Duesberg, *Nature*, **222**, 940 (1969).

[32] Spiegelman, S., N. R. Pace, D. R. Mills, R. Levisohn, T. S. Eikhom, M. M. Taylor, R. L. Peterson, and D. H. L. Bishop, *Cold Spring Harbor Symp. Quant. Biol.*, **33**, 101 (1968).

[33] (*a*) Temin, H. M., *Natl. Cancer Inst. Monograph*, **17**, 557 (1964). (*b*) Temin, H. M., "Formation and activation of the provirus of Rous sarcoma viruses" in *Biology of Large RNA Viruses*, ed. R. Barry and B. Mahy (New York: Academic Press, in press).

[34] Bader, J. P., *Virology*, **26**, 253 (1965).

[35] (*a*) Harel, J., L. Harel, A. Goldé, and P. Vigier, *C. R. Acad. Sci.*, **263**, 745 (1966). (*b*) Harel, L., J. Harel, and J. Huppert, *Biochem. Biophys. Res. Comm.*, **28**, 44 (1967).

[36] (*a*) Payne, L. N., and R. Chubb, *J. Gen. Virology*, **3**, 379 (1968). (*b*) Altaner, C., and H. M. Temin, "Carcinogenesis by RNA sarcoma viruses, XII. A quantitative study of infection of rat cells *in vitro* by avian sarcoma virus," in preparation. (*c*) Weiss, R., *J. Gen. Virology*, in press.

[37] (*a*) Huebner, R. J., these PROCEEDINGS, **58**, 835 (1967). (*b*) Huebner, R. J., J. W. Hartley, W. P. Rowe, W. T. Lane, and W. I. Capps, these PROCEEDINGS, **56**, 1164 (1966).

[38] (*a*) Robinson, H. L., these PROCEEDINGS, **57**, 1655 (1967). (*b*) Vogt, P. K., these PROCEEDINGS, **58**, 801 (1967).

[39] (*a*) Hatanaka, M., R. J. Huebner, and R. V. Gilden, "Alterations in the characteristics of sugar uptake by mouse cells infected or transformed by murine sarcoma viruses," *J. Natl. Cancer Inst.*, in press. (*b*) Hatanaka, M., G. J. Todaro, and R. V. Gilden, "Altered glucose transport kinetics in murine sarcoma virus transformed BALB/3T3 clones," *Int. J. Cancer*, manuscript submitted. (*c*) Hatanaka, M., and G. J. Todaro, unpublished observations.

[40] Nowinski, R. C., L. J. Old, E. A. Boyse, E. de Harven, and G. Geering, *Virology*, **34**, 617 (1968).

[41] Todaro, G. J., S. A. Aaronson, and J. W. Hartley, unpublished observations.

[42] Freeman, A. E., J. M. Maryak, and R. J. Huebner, unpublished observations.