IN VIVO THERAPY OF ONCOGENIC VIRUSES[1,2]

By M. A. Chirigos
Viral Leukemia and Lymphoma Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Introduction

The objective of this review is to fulfill a need for summarizing the diverse approaches employed for the prevention or control of viruses or both. It is impossible to review comprehensively the literature pertaining to all major groups of viruses; consequently, the present review is limited to the murine leukemia and sarcoma viruses and their induced diseases. Emphasis was placed on publications which represent new or unique experimental approaches to *in vivo* therapy. *In vitro* studies are included when they dealt with these viruses or were pertinent to the results achieved *in vivo*. In general, the publications reviewed are those of greatest interest to the reviewer and include publications that appeared up to June, 1968.

The author apologizes for any inadvertent omission of worthwhile publications pertaining to the subject. However, it is hoped that both the scope of the review and the items included will represent the type of research being conducted on oncogenic virus therapy throughout the world.

Virus-Induced Malignancies in Animals

The concept for a viral causation of malignancy in man is presently hypothetical and rests only on speculative foundations. There are five basic phenomena which strengthen the belief in a viral etiology of human malignancy:

(*a*) The virus-induced malignancies or benign tumors which have been demonstrated in a wide variety of warm-blooded animals and birds. Included among these are: horses, cattle, dogs, chickens, cats, ducks, guinea fowls, turkeys, rabbits, foxes, deer, gray squirrels, wood chucks, and pheasants. Virus-induced malignancies have also been reported to occur in cold-blooded animals such as frogs and salamanders. The most intensively studied virus-induced malignancies have been in laboratory animals: mice, rats, hamsters, guinea pigs, rabbits, mastomys, and monkeys. Since man is prone

[1] The survey of literature pertaining to this review was concluded in May 1968.

[2] The following abbreviations are used in this review: ALS (antilymphocyte serum), FLV (Friend leukemia virus), GLV (Gross leukemia virus), MLV (Moloney leukemia virus), MSV (Murine sarcoma virus), MSV-PV (Murine rine sarcoma virus—plasma variant), MTV (Mammary tumor virus), RLV (Rauscher leukemia virus), RSV (Rous sarcoma virus).

to viral infections, it would be difficult to consider him to be so unique as to escape virus-induced malignancy, especially in view of the many viral-induced malignancies found in animals. Several excellent reviews on virus-induced malignancies have been published (1–3).

(*b*) A viral etiology based on epidemiology grounds, such as the Burkitt's African lymphoma (4). Burkitt lymphoma is a neoplastic disease, the distribution of which is restricted to certain regions of equatorial Africa. It is seen most commonly in children between the ages of 5 and 12 years and very infrequently in persons older than 20 years. The disease occurs in children of all races and tribes and therefore does not appear to be connected with a genetic disorder. The distribution of the disease in areas where the climate favors the breeding of mosquitos has led to the suggestion that the disease may be caused by an arthropod-born virus. The outbreak of O'Nyong-Nyong fever seems to support this suggestion, since it is caused by an arbovirus, and was limited to the same region in which the Burkitt lymphoma is indigenous (5). In North America a clustering of cases of human leukemia have been reported in Niles, Illinois (6), Orange, Texas (7), and Los Angeles County (8).

(*c*) Electron microscopy has brought the hope of visualizing the responsible virus directly in the tissues of leukemia or cancer patients. Electron microscope studies of lymph-node biopsy specimens, bone-marrow aspirates, and blood plasma concentrates obtained from patients with different types of leukemia and lymphoma, discloses structures resembling virus particles, so-called "type C" murine leukemia particles, and mycoplasma, in a number of specimens. An imposing list of studies has appeared as individual reports or reviews concerning the presence of virus or virus-like particles in human cancer tissue, a few of which are included (9–11).

(*d*) Immunological factors resulting from viral-induced tumors have been elicited in the host. Even though viral genetic material as such, may or may not be directly demonstrable in virus-free transformed cells, it is possible that some virus-specific marker might reflect the continuing expression of an integrated viral genome. The recent development in the field of immunology of animal virus tumors has established that cells infected with tumor-inducing viruses develop foreign cellular antigens. These antigens fall into two major categories which are based on the methods used to demonstrate them: a transplantation type of antigen (12, 13), or a complement-fixing antigen (14). Using antisera produced by tumor-bearing animals the presence of reacting antigens has been demonstrated in Gross, Moloney, Rauscher, Graffi, and Friend mouse leukemia cells by a cytotoxic test (15). Antigens have also been demonstrated by fluorescence staining of Simian virus 40 and adenovirus tumor (16) and Rauscher, Graffi, and Moloney leukemia cells (17). The use of immunofluorescence techniques, cell-free extracts prepared from plasma of human leukemics, found by electron microscopy to contain virus-like particles, resulted in a positive immunofluorescence with the cells of a high percentage of leukemics (18, 19).

(*e*) A fifth basis is the observed sequential development of malignancy following a viral infection in the same tissue site (20). Such evidence, although circumstantial, is suggestive. Examples of viral co-carcinogenesis are the development of chorio-epithelioma following testicular swelling in rubella infection, malignant testicular tumors following mumps orchitis infection, squamous carcinomas following herpes simplex lesions, and a variety of malignancies in skin following herpes zoster infection.

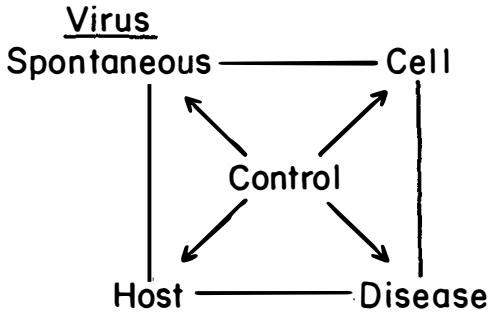
Biological Systems for Studying Control Measures for Virus Induced Malignancies

Many different biological systems employing nononcogenic viruses have been effectively used in searching for antiviral agents. Some excellent reviews discuss the best examples of both *in vitro* and *in vivo* virus test systems, citing examples of readily reproducible antiviral activity that have been demonstrated with these experimental procedures (21–26). The number of different viruses capable of inducing tumors or other malignant responses is large and continues to grow. These include the viruses of the avian leukosis complex, avian sarcoma, mouse leukemias, various papillomas and fibromas, polyoma, the frog renal carcinoma, and the mouse mammary carcinoma. These viruses have been reviewed (27, 28). Recently, viruses inducing murine sarcomas have also been added to the growing list of murine oncogenic viruses (29–31).

In the past, most of the therapy studies with viral tumors have been concerned with the effect of drugs on the tumor, with little attention or emphasis being placed on the viral aspects. More recently, with increasing evidence of the importance of viruses in maintaining experimental tumors, emphasis has been orientated toward the virus-tumor complex, rather than examining the virus or tumor as separate entities.

Chart I shows a simplified scheme by which one can study the effect of a control mechanism (i.e., drug, interferon, interference, antibody, radiation, etc.) on the virus or cell or both. Advances in tissue-culture techniques and the adaptation of viruses to growth in tissue cultures have provided useful tools for studying virus-host cell interactions at the cellular level, for the screening of potential antiviral agents, and the elucidation of their modes of action (32–35). A recent symposium was devoted exclusively to antiviral substances (36). When *in vivo* oncogenic virus systems are employed for evaluating therapeutic responses, three parameters may be employed for determining the effect of the therapy: oncogenic virus reduction; inhibition of viral-induced neoplastic cells (disease); and effect on the survival time of the host. The test systems to be described herein consist of those systems employing oncogenic viruses which produce disease by inducing normal cells to proliferate and become malignant, resulting in death of the host from cancer. These systems were especially selected for review because of the greater amount of information available concerning *in vivo* antiviral therapeutic testing.

BIOLOGICAL SYSTEMS FOR STUDYING CONTROL MEASURES FOR VIRUS INDUCED NEOPLASMS



① Virus Cell: In Vitro cell culture studies; Drug, Interference, Interferon, Irradiation

② Virus-Cell-Disease-Host: In Vivo studies; Drug, Irradiation, Immunotherapy, Surgery, Interference, Interferon.

Virus-induced leukemias.—

(*a*) Friend leukemia virus (FLV).—Friend (37) reported isolation of a leukemia virus from the spleen of an adult Swiss mouse which had received a cell-free extract of Ehrlich carcinoma when newborn. The virus was effective in adult Swiss and DBA/2 mice. Friend (38) and Furth (39) have interpreted the virus-induced disease as a reticulum cell neoplasm associated with a pronounced erythroblastosis. On gross examination, the infected mice exhibited marked hepatosplenomegaly with little or no lymph node involvement. The time from virus inoculation to manifestation of the Friend disease is extremely short. The spleens of infected animals became infiltrated and palpable as early as 2 weeks after virus injection. In the terminal phase of the disease a marked increase occurs in the number of white blood cells, approaching or exceeding 300,000 per mm^3. Anemia is also present. In differential examination of the peripheral blood smears, a considerable number of normoblasts and smudge cells are observed with many characteristic mononuclear cells with lobular, or horseshoe-shaped, nuclei and other abnormal white cells. Rowe (40) described a graded response assay for the

Friend virus in which mean log spleen weight at 2 weeks post-inoculation was a function of initiating virus dose. Since the mean log spleen weight in mice inoculated with this virus was a function of log virus dose, Chirigos (41) employed spleen weight as a measure to quantitate virus titer in an antiviral assay with FLV. Soon after FLV infection, extracellular virus can be readily recovered. The intensity of the viremia and splenomegaly was found to increase with time.

(*b*) Moloney leukemia virus (MLV).—Sarcoma 37, a transplantable connective tissue neoplasm of mice, served as the original source of the Moloney leukemia virus (42). The virus extracted from this tumor when inoculated into mice of various ages and strains, and into rats, produced a generalized lymphocytic neoplasm in 100 per cent of the recipients within a relatively short period. The virus has a latent period of 2 to 3 months and produces a generalized lymphatic leukemia with greatly enlarged spleens and livers, and massive involvement of the thymus and of the peripheral and mesenteric lymph nodes. The blood cell counts show an anemia and an increase in the number of white cells of 29,000 per mm^3. The virus-induced leukemia is freely transplantable within the mouse strain where induced and in compatible F_1 hybrid mice. Soon after infection, virus is detactable in extracellular fluid and in different organs. The viremia becomes more intense with time and prior to death.

(*c*) Rauscher leukemia virus (RLV).—Rauscher (43) reported the isolation of a virus from an ascites cell line of a leukemia which he obtained from Dr. H. M. Schoolman. The virus produces an acute fatal disease in mice of various ages and many strains. The response occurs in 100 per cent of the animals and is characterized by a very rapid and marked proliferation of erythrocytic and leukocytic elements, accompanied by gross enlargement of the spleen and an intense viremia, as early as 7 days after inoculation. Death with markedly enlarged spleens occurs within 3 to 6 weeks in 50 per cent of the animals. The early erythropoiesis is followed by the development of lymphocytic leukemia in the surviving mice 5 to 7 weeks after infection and is characterized by splenomegaly, hepatomegaly, enlarged lymph nodes, and leukemic cells in the peripheral blood. Chirigos (44) established that a dose-response relationship occurred between the degree of splenomegaly and the dose of virus used in inoculation; he employed spleen weight as the parameter for quantitating virus titer. The Rauscher and Friend leukemia virus systems, because of their short latent period, and early appearance of detectable virus in blood and organs, lend themselves to the evaluation of therapeutic agents. When used in the proper experimental designs, therapeutic agents can be evaluated in their ability to (i) decrease or increase the latent period in the appearance of splenomegaly; (ii) retard or enhance splenomegaly; (iii) decrease the amount of virus in blood and organs; (iv) suppress leukemic cells in the spleen and blood (41, 44); and (v) protect or extend survival time of the host.

(*d*) Gross leukemia virus (GLV).—Gross (45) reported that cell-free

extracts of the hematopoietic organs of AK mice with spontaneous lymphoid leukemia were capable of inducing the same disease when inoculated into newborn susceptible hosts. After continued serial cell-free passage in highly susceptible newborn C_3H/Gs mice, the tumor-producing activity of the leukemic tissue filtrate was increased (46). This more active virus is leukemogenic in rats of the Sprague-Dawley strain. This virus strain, designated "Passage A," produces generalized lymphocytic leukemia, following a short period of latency, in compatible mice inoculated as late as 14 days of age.

Virus-induced sarcomas.—

(*a*) Rous sarcoma virus (RSV).—In 1911, Rous first reported the induction of sarcomas in chickens by a cell-free extract prepared from a tumor found in a barred Plymouth Rock hen (47, 48). The virus-induced tumors microscopically proved to be a spindle-cell sarcoma, with a widespread necrotic center. Various techniques for the assay of RSV have been reported which employ single- or multiple-site inoculations and utilize tumor incidence, latent period to tumor induction, and relative sizes of tumors at a definite time following infection, as criteria for interpreting results. The use of these techniques, together with the source of experimental variations encountered and methods for their control, have been reviewed and discussed in detail by Bryan (49). Loomis (50) demonstrated that typical sarcoma cells could be observed histologically as early as 48 hr after injection of virus into the wing-web of susceptible chickens. Since a quantitative response could be expected to occur within 48 hr, i.e., macroscopic tumors in the translucent wing-web of injected chickens, virus-induced Rous sarcoma could be used as a test system for the detection and evaluation of potential antitumor or antiviral agents.

(*b*) Murine sarcoma virus (Moloney)—MSV (M).—Moloney (29) recently described the isolation of a virus that induces sarcomas in murine animals. This agent was derived from solid tumors that appeared in BALB/c mice following inoculation with high concentrations of Moloney leukemia virus. Perk (51) described the pathogenesis of the virus-induced rhabdomyosarcoma in mice. Inoculation of MSV(M) intramuscularly in the leg muscle results in the development of a tumor within a relatively short latent period (6 to 16 days). The induced tumor arises and progresses rapidly in muscle tissue. Histologically the tumor is composed of elongated cells resembling young muscle cells, often with centrally located and frequently multiple large nuclei possessing several distinct nucleoli. Death occurs within 2 to 4 weeks in mice when inoculated as newborns, and, when inoculated as adults, within 6 to 8 weeks. Since typical sarcoma cells can be observed histologically as early as 6 days after injection of virus into the leg muscle, tumor incidence could be effectively utilized to detect therapeutic activity.

(*c*) Plasma variant of murine sarcoma virus (MSV-PV).—With con-

tinuous passage of tumor, from a BALB/c mouse, originally induced by MSV(M), a variant of the MSV(M) has been isolated (31). When this variant is inoculated intraperitoneally into adult BALB/c mice, it induces a progressive splenomegaly with diffuse tumor cell infiltration, and multiple tumor formation in or adjacent to lymph nodes, muscle tissue, thymus, kidney, and lungs. Of particular interest is the development of an intense viremia detectable in plasma as early as 7 days after inoculation. This tumor virus system lends itself to studies of therapeutic agents possessing either antiviral or antitumor activity. These virus systems have been employed more extensively in evaluating antiviral and antitumor therapeutic agents. Several other murine and chicken virus-induced tumors have been reported and are discussed in detail in several excellent reviews (28, 52, 53).

CHEMOTHERAPY

Of the several approaches considered for treatment of viral infection, chemotherapy has been investigated most extensively. However, the bulk of available information on antiviral chemotherapy deals with the treatment of nononcogenic viruses (21–26, 32–36). The Cancer Chemotherapy National Service Center, through a viral chemotherapy contract program, tested hundreds of compounds for potential antiviral activity against four RNA and four DNA oncogenic virus systems (Table I). Against DNA viruses, more active compounds were of a pyrimidine structure, indicating that the antiviral effect was a result of interference with the synthesis of infective viral DNA. However, against oncogenic RNA viruses, of eight classes of compounds tested, the purines, alkylating agents, antibiotics, and a miscellaneous category comprised of synthesized chemicals, possessed antiviral activity. Several of these drugs possess *in vivo* activity against some of the murine leukemogenic viruses by both decreasing virus replication and retarding the primary virus-induced disease.

Moloney leukemia virus.—Chirigos (54) investigated the effect of several antitumor drugs on leukemia produced in mice by the MLV. When treatment was started at the time that the disease was diagnosed, methotrexate, triethylene melamine, and cyclophosphamide (Cytoxan) were effective in retarding lymphadenopathy, thymus, spleen, and liver enlargement, resulting in moderate increases in survival time. Cyclophosphamide and triethylene melamine were also found effective against the transplantable MLV-induced leukemia with cyclophosphamide, triethylene melamine, melphalan, and X-radiation resulting in an appreciable number of long-term survivors. However, most of the animals subsequently succumbed with widely disseminated lymphocytic neoplasia. This relapse was shown to be caused by virus associated with the initial cellular implant, which re-induced the disease. It is reasonable to assume that the therapy was effective on the tumor cells which arose from MLV infection, but did not materially effect the MLV (55).

Gross leukemia virus.—The thymus plays a central role in lymphocytic

TABLE I

CHEMOTHERAPY OF ONCOGENIC VIRUSES

	RNA Virus Systems[a]		DNA Virus Systems[b]	
	in-vitro	*in-vivo*	*in-vitro*	*in-vivo*
Number of compounds tested	114	679	268	15
Percentage active	4	13	5	27
Class of active compounds	Compounds active against RNA viruses		Compounds active against DNA viruses	
Purines	13		—	
Pyrimidines	5		12	
Folic Acid Antagonists	2		—	
Alkylating Agents	19		—	
Steroids	2		—	
Miscellaneous	17		5	
Natural Products:				
Alkaloids	1		—	
Antibiotics	11		—	

[a] Rous Sarcoma, Rauscher, Friend, Moloney.

[b] Polyoma, Myxoma, Adenovirus 12, Adenovirus 18.

viral leukemia induced by the GLV. This virus proliferates in a number of tissues, but the thymic lymphocyte appears to be the target cell for transformation. The incidence of viral-induced leukemia is markedly reduced in thymectomized mice (56). Upton & Furth (57) demonstrated that depletion of lymphocytes from the thymus, as a result of food restriction, irradiation, or treatment with cortisone, delayed or reduced the incidence of spontaneous leukemia of AK mice, the high leukemic strain of mice from which the Gross Passage A virus was derived. Striking results of controlling spontaneous leukemia in AK mice (58) and of leukemia induced by GLV (59) were accomplished by continuous treatment with cortisone. In the latter study, treatment initiated during the latent period of the disease produced a more marked lengthening of life-span than when treatment was delayed until the overt disease appeared. The results indicate that viral replication was retarded because of thymic atrophy and reduction of target-cell thymic lymphocytes. Utilizing AKR mice with overt spontaneously induced lymphatic leukemia, Salgamik (60) reported excellent therapeutic response in these mice by treatment with deoxyribonuclease. Treatment of leukemic mice with 5 mg of DNAse every second day over a 4-month period significantly extended their life-span. The action of the DNAse also resulted in marked decreases in weights of the thymus, spleen, and liver.

Rauscher leukemia virus.—Chirigos (61–63) reported the antiviral and antitumor chemotherapeutic response in mice infected with RLV. Of eight drugs tested against the Rauscher virus-induced leukemia, netropsin, melphalan, 6-mercaptopurine, and 5-fluorouracil resulted in a two-fold increase in survival time. A relationship was shown to exist with the inhibition of viral-induced splenomegaly in animals subjected to effective therapy. In an *in vivo* assay system, designed to assess antiviral activity, 12 drugs were tested. Dactinomycin (actinomycin D), 6-mercaptopurine, methotrexate, cyclophosphamide, 5-fluorouracil, 6-thioguanine, vincristine, and 1, 3-bis(2-chloroethyl)-1-nitrosourea were effective in reducing the viremia in RLV-infected mice by 1 to 3 logs. The antiviral effect of a few of these drugs, however, was considered to be cell-mediated. Suppression of splenomegaly was related to a reduction of extracellular virus, indicating that a reduction in the number of spleen cells resulted in a decrease of virus replication. *In vitro* exposure of RLV or RLV-containing cells, to two alkylating agents, melphalan and triethylene melamine, resulted in a more marked cytocidal effect than a virucidal effect. From *in vitro* studies, Bases (64) reported that RLV replication could be inhibited by actinomycin D, which also inhibits cellular RNA synthesis, but not by cellular DNA inhibitors such as cytosabine (cytosine arabinoside) and 5-fluorodeoxyuridine.

Friend leukemia virus.—Of the several murine leukemia viruses, the FLV system has been most extensively employed for testing potential antiviral and antitumor agents. Sugiura (65) and Sugiura & Stock (66) tested over 110 compounds in mice against the FLV. Eight compounds were found capable of inhibiting the leukemia, namely: Triethylene melamine, busulfan (Myleran) 1, 9-di (methanesulfonoxy)-nonane, mitomycin C, netropsin, thioguanine, and two steroids (estradiol and 9-α-fluoro-2-α-methyl-hydrocortisone acetate). From their bioassay studies, the first four compounds appeared to exert an antiviral effect. From these and subsequent studies (67, 68), 12 compounds were shown to possess marked inhibiting effect on FLV. It is interesting to note that most of the effective compounds were nitrogen mustard analogues or purine antagonists. The same compounds or analogues of these compounds were also reported to be effective against the MLV, RLV, and RSV.

Mirand (69, 70) demonstrated a correlation of splenomegaly and erythropoiesis in FLV-infected mice attributable to an increase in splenic uptake of Fe^{59}. Employing these parameters, effective chemotherapy resulting in increased life-span was related to a decrease of splenic uptake of Fe^{59} and splenomegaly. In this study and in a separate study reported by Regelson (71), two alkylating agents were shown to exert anti-FLV activity: benzyl-N-[bis-(ethylenimido)-phosphoro] carbamate (AB-103) and ethyl N-[bis-(2,2-dimethylethylenimido) phosphoro] carbamate (AB-132). Dawson (72) employed a transplantable reticulum cell sarcoma, originally induced by FLV, to test the effect of drugs on the neoplastic cell and the cell-contained FLV. Mitomycin C, triethylene melamine, 6-mercaptopurine,

and urethane were effective in inhibiting growth of the cellular implant; however, no significant effect on the virus was noted. This response is similar to that reported for the MLV (55), where the therapy was effective in the tumor cells but did not materially affect the MLV contained in the tumor cells. Chirigos (41), employing a bioassay technique developed for assessing potential antiviral agents, reported the activity of 11 agents. Treatment initiated when mice were viremic, with 6-mercaptopurine, 6-thioguanine, dactinomycin, streptonigrin, vincristine, and cytarabine, resulted in a marked reduction of extracellular FLV. Sidwell (73), employing a similar system, evaluated the antiviral activity of 132 compounds. Seventeen compounds inhibited FLV-induced splenomegaly and significantly reduced virus titers in spleen and plasma of treated animals. The seventeen compounds included a carbamate; urethane; five alkylating agents, busulfan, thio-TEPA, mannitol mustard, nitrogen mustard N-oxide, and 1,3-bis(2-chlorethyl)-1-nitrosourea); two antibiotics (also considered to be alkylating agents); mitomycin C and porfiromycin; and nine purines: 6-thioguanine, 6-mercaptopurine, 6-mercaptopurine ribonucleoside, 9-cyclopentyl-6-mercaptopurine, 9-n-butyl-6-mercaptopurine. 6-(2,2-dimethylhydrazino) purine, 6-benzylthiopurine ribonucleoside, 6-thioguanosine, and 6-[(-methyl-4-nitroimidazol-5-yl) thio] purine hydrate). More recently, DeLong (74) reported excellent antiviral activity with the compound, 1-(4-fluorophenyl)-1-phenyl-2-propynyl-N-cyclohexylcarbamate (FPPC). These results showed that FPPC was capable of inhibiting both FLV-induced splenomegaly as well as FLV replication. Of particular interest was the fact that the compound did not produce any host body weight loss and that the compound was almost equally effective when administered orally or by injection.

From the reported effective antiviral activity achieved by chemotherapy in the murine leukemia virus systems, suppression of the reticuloendothelial system was most often observed. It is reasonable to assume that this suppression may be responsible for the observed reduction of viremia. For the GLV, thymectomy alone markedly delayed development of overt disease, indicating that a depletion of the target thymic lymphocyte cells leads to a delay in virus replication and subsequent development of disease. Suppression of splenomegaly in RLV- and FLV-infected animals by effective drug therapy results in a concomitant decrease of viremia. Splenectomy alone was shown to delay the induction of FLV- and RLV-induced disease (75–77), and reduced extracellular FLV titer (78). Splenectomy, after viremia was established in FLV-infected mice, resulted in a decrease of virus in several tissues with a concomitant increase in survival time. Treatment with drugs or total body X-irradiation similarly resulted in marked virus reduction (78). The mechanism responsible for the virus reduction was considered to be the retardation of susceptible spleen cells. Lowered virus titers were observed in animals which were treated with total body irradiation,

drugs, and splenectomy—singly or in combination. This virus reduction was shown to be totally reversed when normal syngeneic spleen cells were administered along with the different treatments. The results imply that the spleen is the primary organ involved in FLV replication and that leukemic blood cells in FLV disease are released by this organ.

Rous sarcoma virus.—This virus-induced tumor has properties similar to human sarcoma; e.g., it arises from the host's own tissues, metastasizes, and is endowed with immunologically specific cell-bound antigens as well as viral antigens. It is understandable why this system has been employed extensively as a model system for chemotherapeutic testing. The prophylactic and antitumor activity of several agents employing this system have been reported (22). Tumor formation in chicks inoculated with RSV was suppressed by parenteral administration of xerosin or statolon. Xerosin appeared to exert its effect through an anti-inflammatory reaction. Statolon was considered to have exerted its effect by interfering with some stage of intracellular viral synthesis. In view of the recent reports that statolon stimulates host interferon, it is conceivable that interferon delayed tumor induction by retarding viral replication.

Pienta (79) evaluated the activity of 88 materials, active in other biological systems, as well as 227 filtrates and extracts prepared from soil isolates of actinomycetes and gram-negative bacteria in chicks infected with RSV. Of 88 selected materials, five compounds significantly delayed the latent period for tumor development. These were cylophosphamide, 6-thioguanine, 5-fluorodeoxyuridine, actidione, and 4, 6-diamino-1-(3-bromophenyl)-1,2-dihydro-2, 2-dimethyl-S-triazine. Cylophosphamide, an alkylating agent, was by far the most effective. Three compounds were found to possess good prophylactic activity when treatment was initiated 2 days prior to virus infection. All three possessed steroid structures: prednisolone, 9-fluoroprednisolone and 2 α-methylhydrocortisone-21-acetate.

Bather (80) demonstrated more beneficial antitumor activity with combined chemotherapy against RSV in chicks. Aminopterin combined with 6-mercaptopurine resulted in a significant reduction of tumor mass and increase in survival time; however, no antiviral effect was demonstrable. Bliznakov (81), employing a nontoxic lipid, restim, extracted from shark livers, demonstrated effective antitumor activity in young chicks infected with RSV. However, the effectiveness of this extract was considered to be a result of its capacity to enhance the immunological competence of the host, rather than a chemotherapeutic response.

Murine sarcoma virus (Moloney).—Rhabdomyosarcoma is generally considered a rare tumor in children. However, 16 children with this malignancy were studied and treated. Pinkel (82) reported that dactinomycin produced temporary regressions in some patients while cyclopliozdiamide was effective in others. The treatment was unsatisfactory in most instances. The recent isolation and characterization of viruses which induce rhabdomyosar-

comas in animal hosts (29–31), allow for the development of *in vivo* animal model systems for evaluating potential antiviral and antitumor agents. With such systems, different therapeutic approaches can be studied, and promising or successful treatment applied to human cases. Pratt (83), employing the murine sarcoma virus (Moloney), studied the effect of vincristine, dactinomycin, and cylophosphamide on the virus-induced rhabdomyosarcomas. Reduction in tumor size was achieved by the three drugs.

Murine sarcoma virus plasma variant (MSV-PV).—Employing a transplantable rhabdomyosarcoma tumor, originally induced by MSV-PV, significant reductions in tumor mass were achieved by treatment with melphalan and dactinomycin. Melphalan was most effective, resulting in a 40 per cent increase in life-span over untreated controls in two separate experiments (83a).

Host Factors

The ultimate goal in viral chemotherapy is to synthesize a drug which has a specific deleterious effect on the virus. Results of *in vivo* drug therapy, to date, indicate that most antiviral effects observed against oncogenic viruses are nonspecific. The investigator must thus address himself to the question of what body defenses of the host can be influenced to exert a resistance to oncogenic viruses or their induced diseases. A number of factors have been investigated, and in some cases represent unique antiviral therapeutic approaches.

Vaccines.—Live virus vaccines are generally best adapted to preventing diseases caused by viruses. However, the nature of disease induced by oncogenic viruses precludes use of live virus. Oncogenic viruses that have been formalin-treated and then used as vaccines include the Rous sarcoma virus (84) and the Friend (85), Moloney (53) and Rauscher (86) leukemia viruses. Friend showed that formalin-treated virus rendered a significant number of mice resistant to challenge with live virus and, to a lesser extent, with leukemic cells. Moloney (53) demonstrated a significant amount of neutralization antibody to the virus in sera from immunized mice. Fink (86), employing formalin-treated Rauscher leukemia virus plus complete Freund's adjuvant, immunized against a dose of virus sufficient to kill 100 per cent of the controls. The inoculation of heated virus plus adjuvant provided much less immunity.

Long-term monolayer cultures of mouse thymus and spleen cells, chronically infected with RLV, produced an attenuated virus with a low leukemia-inducing capacity when inoculated into mice. Use of this attenuated RLV to immunize isologous mice resulted in significant protection against challenge with viable RLV. Better protection was achieved when mice were immunized by repeated inoculations with frozen and thawed cells from these cultures (87). Wright (88) similarly demonstrated that immunization with a 100 × concentrate of attenuated RLV, grown in tissue culture, resulted in significant protection against challenge with a potent preparation

of infectious RLV. Mirand (89, 90) demonstrated that FLV was transmitted through successive generations from the infected mother to her offspring through milk. In a subsequent experiment, Mirand also showed that immunization of pregnant mice with FLV vaccine resulted in the transmission of passive immunity to the offspring during the suckling period. Passively acquired antibodies were transmitted postnatally only for 36 hr (90).

Passive immunization.—Relatively few experiments have been conducted on controlling oncogenic viruses by serum therapy. Where mice were treated with specific antiserum prior to viral infection, good protection has been attained. Fink (86) reported that a single injection of anti-RLV antiserum 19 hr prior to challenge with RLV resulted in protection of 50 per cent of the challenged animals and a delay of onset of disease in the remaining 50 per cent. Therapeutic studies, in which mice were infected with RLV and treated intermittently with homologous antiserum initiated as late as 3 days after infection, resulted in a significant delay in disease induction and a reduction of 2.6 logs in virus titer (91). In a similar study, homologous antibody was employed for treating FLV-infected mice (92). The complete protection achieved in animals receiving treatment 4 hr prior to FLV infection was attributable to a prophylactic effect. Delaying treatment until the fourth day after infection, when extracellular virus was present in infected mice, still afforded good protection. Thirty per cent of the treated animals were free of the disease.

Passive immunization is most effective when it is used prophylactically. The inability of sero-therapy to control an acute viremia after it is established, is attributable to several factors: (*a*) insufficient number and volume of treatments to neutralize extracellular virus completely; (*b*) inability of neutralizing antibody to penetrate virus-infected cells which are replicating virus; and (*c*) inability of neutralizing antibody to reach distal organs such as brain, which is known to harbor murine leukemia viruses and which therefore could serve as a reservoir for reinfection. Old (93) was successful in suppressing a transplanted Gross + leukemia by passive immunization with specific antiserum. Complete protection was achieved in mice inoculated with 10^5 leukemic cells and initially treated with immune serum 27 hr after cell inoculation. A forty per cent suppression of tumor growth was demonstrated when treatment was delayed as late as 3 days after inoculation. Animals that were successfully treated and free of tumor were subsequently rechallenged to test whether active immunization had occurred. The rechallenged mice died with leukemia with no significant delay in comparison to controls.

Law (94) employed antiserum prepared against MSV(M) for passive immunization studies in mice inoculated with a MSV(M)-induced transplantable tumor. By beginning treatment 1 day prior to tumor inoculation and treating every second day for a total of 12 treatments with antiserum, significant retardation of tumor growth was achieved. All inoculated

animals which received normal serum died with large local tumors. However, of the animals treated with MSV(M) antiserum, 50 per cent were free of tumor and 50 per cent bore tumors of strikingly decreased size. The effects of the antiserum were considered most likely directed toward the transplanted cells. Levi (95) employed a unique method in preparing antiserum to leukemic tissue from AKR/J mice, which have a high incidence of lymphatic leukemia when they attain an age of 8 to 12 months. Newborn rabbits were made tolerant to normal AKR/J mouse tissue by repeated inoculation of normal tissue brei, and at 8 weeks of age were immunized with tissues from AKR/J mice which developed lymphatic leukemia. This method produced antisera which were very unique in having minimal mouse toxicity combined with strong antileukemic activity. Treatment with antiserum of mice inoculated with leukemic cells, resulted in significant retardation of tumor growth and prolonged survival time. Of particular significance was the fact that death of treated mice, from serum sickness, was not a problem, as would normally occur if sera prepared in a heterologous host were used.

Adoptive immunotherapy.—This therapeutic approach is considered under host factors because immunological reactions can be manifested by the direct action of immuno-competent cells which are induced in a host or, particularly, when transferred to another host.

Mathé (96–97) has described the application of adoptive immunotherapy to leukemia employing autogeneic and allogeneic immuno-competent cells with some success. Similarly, Delorme & Alexander (98) obtained regressions and occasionally complete cures of primary sarcoma in the rat by intravenous injection of immune thoracic duct lymphocytes. Autologous spleen cells, obtained from the autochthonous host bearing the primary tumor, were also shown to prevent the growth of sarcoma cells on transplantation into syngeneic recipients (99). More recently, successful treatment was achieved in DBA/2 mice bearing a chemically induced leukemia, L5178Y, originating in this strain, with spleen cells and sera from allogeneic mice immunized with these leukemic cells (100). Adoptive immunotherapy to tumor cell systems *in vivo* was more significantly applied by Law (101) in a completely syngeneic system. Polyoma virus-induced neoplasms in C57B1 strain mice were totally prevented through adoptively acquired immunity. Sensitized lymphoid cells inoculated intravenously were effective if administered as late as 30 days after virus infection. This observation is included in this review because the experimental design employed fulfilled those requirements in demonstrating adoptive immunotherapy (102), in that the primary and secondary hosts employed were members of the same highly inbred strain. Mathé (103) successfully reduced the plasma concentration of FLV in mice by combining total body irradiation with intravenous injection of allogeneic bone-marrow cells from C57B1 mice previously vaccinated with FLV. The objective of irradiation prior to injection of bone-marrow cells was to immuno-suppress the mice to accept the alloge-

neic bone-marrow cells. However, total body irradiation alone was shown to be capable of suppressing viremia in FLV-infected mice (78). Thus, the therapeutic response achieved can be considered a result of the combined therapy, i.e., irradiation and adoptive immunotherapy.

Sinkovics (104), working within a syngeneic system, demonstrated significant antiviral effect against RLV with adoptively acquired immunity. Adult Swiss mice were vaccinated with formalin-killed Rauscher virus. Viable spleen cells from the immunized mice were inoculated intravenously into newborn Swiss mice and at 3 weeks of age, these mice were challenged with RLV. Eighty-seven per cent of the spleen-treated mice were asymptomatic in contrast to a 100 per cent leukemic incidence in control mice. The results indicate that the spleen cells transferred from actively immunized mice were capable of producing virus-neutralizing antibodies. Spencer (105) employed an allogeneic system for demonstrating adoptive immunotherapy of RLV. Transferring immunologically competent bone-marrow cells from an F_1 hybrid, previously vaccinated with live RLV, to the susceptible DBA parent, resulted in significant protection against an RLV challenge.

Interferon.—Natural host substances which have the capacity to increase cellular resistance to virus infection may play a role in recovery from viral disease. Interferons are viral inhibitory substances of protein composition that are commonly formed by cells in response to stimulation by viruses and by other microbes or their components (106–111). Interferons appear in blood and other tissue of animals as a result of viral infection. Fruitstone (112) reported the importance of the spleen in interferon production in mice. The elicited host factors appear to provide a mechanism for recovery from viral disease which is separate and distinct from specific immunological mechanisms. To date, the administration of exogenously prepared interferons, prophylactically or therapeutically, shows little promise in clinical medicine for preventing or treating viral disease. However, stimulation of endogenous interferon in the host by selected materials may significantly enhance host defense. Present evidence indicates that viruses, foreign nucleic acids, certain bacteria, coli polysaccharides, and polysaccharides such as statolon, may all stimulate production or release of interferons. Double-stranded polynucleotides of inosine and cytosine have also been reported to release preformed interferon when inoculated into mice (113). Recent findings indicate that the polyanionic polysaccharide, statolon, was not the responsible agent inducing interferon (114); this was a result, rather, of a polyhedral RNA virus found in cultures of *P. stolonif-erum* (115). Of particular interest is the recent report (116) of induction of circulating interferon by synthetic anionic polymers of known composition. Variations in the structure of the maleic acid copolymer resulted in a reduction of its interferon-inducing capacity. Regelson (117) earlier reported the ability of this synthetic compound to inhibit Friend leukemia virus-induced splenomegaly.

To date, the predominant emphasis on the role of interferon on virus infection is with nononcogenic viruses. Any consideration for control of cancer caused by viruses must necessarily take account of the importance of interferon in viral infection. Recent studies have demonstrated that exogenous and endogenous interferon exerts an effective antiviral action on several oncogenic viruses. Lampson (118) reported a marked suppression in the number and size of sarcomas in chicks, infected with Rous sarcoma virus (RSV), when purified and concentrated chick interferon was given prophylactically. Rous sarcoma virus alone has demonstrated capacity to induce interferon. Bader (119) showed that interferon production, as well as malignant transformation, can be induced in chick embryo cells exposed to RSV. *In vivo* confirmation for the formation of interferon has been reported by Force (120). Examination of wing-web tissue and serum of chicks infected with RSV, disclosed an interferon-like inhibitor in RSV-infected wing-web tissues of the chick prior to the appearance of infectious RSV, tumor formation, viremia, and circulating antibody. Gresser (121–124) and Wheelock (125–129) have reported the effect of interferon on the Friend leukemia virus in mice. Gresser (124) reported effective suppression of splenomegaly in FLV-infected mice by treatment with exogenously prepared interferon. When treatment was delayed as late as 7 days after FLV infection, a marked reduction of viral replication was achieved, as judged by the reduction in splenomegaly and the number of foci of Friend cells formed on the spleen. Several spleens from interferon-treated mice were shown to contain less FLV than untreated controls. Wheelock (125–129) investigated in detail the protective effect achieved in mice infected with FLV by inoculation with Sendai virus, treatment with Statolon, or mouse-prepared interferon. Inoculation with Sendai virus 6 days prior to FLV resulted in a marked reduction in splenomegaly and leukocytosis. Peripheral white blood cell counts were reduced from 78,500 in FLV-infected controls to 20,500 in mice pre-infected with Sendai virus. Spleens of the latter mice were found to contain 1.5 logs less of FLV than controls.

From studies on the effect of varying doses of Sendai virus on interferon production, and inhibition of FLV-induced splenomegaly, the maximum concentration of Sendai virus-induced interferon was found to occur in mice inoculated with $10^{8.2}$ EID_{50}. This same dose was shown to cause the most marked protection against FLV challenge. Possible *in vivo* neutralization of FLV by Sendai virus-induced antibody was ruled out by *in vitro* neutralization tests conducted with FLV and anti-Sendai virus-immune serum. Survival of mice, infected with Sendai virus 3 weeks prior to FLV challenge, was extended by 3 weeks, with 10 per cent of the dually-infected mice free of any FLV disease symptoms. Challenge with Sendai virus 20 or 30 days after FLV infection significantly prolonged the survival time over control FLV-infected animals. This observation is significant in that the inhibitory effects of Sendai virus inoculation occurred in mice with established Friend leukemia and during the period when mice were dying

with the disease. Protection was also shown to occur in FLV-infected mice when challenge with Sendai virus was delayed as late as 6 weeks, but not as late as 9 weeks, after RLV infection. In studies with Statolon, a known inducer of circulating interferon in mice (130), Wheelock (129) demonstrated a marked inhibition of the splenomegalic response to FLV in mice receiving Statolon 6 days prior to, or 3 days after, FLV inoculation. Treatment 3 days prior to FLV infection resulted in a significant number of survivors which were free of symptoms of Friend leukemia and detectable virus after a 4-month observation period. Of particular interest was the resistance of these survivors to a second challenge of FVL. Since both Sendai virus and Statolon were found effective in prolonging survival of leukemic mice, Wheelock (129) considered that these two agents may be effectively employed sequentially. Animals infected with FLV were inoculated with Sendai virus 29 days later, at a time when the animals were leukemic. Statolon treatment was withheld until 24 days after Sendai inoculation (i.e., 53 days after FLV infection). The results showed that this sequential treatment could extend the life of leukemic mice beyond the usual period of prolonged survival induced by Sendai virus alone. Although Sendai virus challenge 30 days after FLV inoculation was very effective in inducing interferon and prolonging survival, a second inoculation of Sendai virus was without effect.

Vandeputte (131) reported on the role of interferon in RLV-infected mice. Although high interferon levels were observed in mice inoculated with Newcastle disease virus, Sindbis virus, or endotoxin, no protection against RLV-induced splenomegaly could be detected. Further, no protection was noted in mice which were treated with high concentrations of interferon. Glasgow (132), however, reported that interferon injections could render suckling mice resistant to RLV infection. By treating mice with NSC-46015, a polycarboxylic pyran copolymer (pyran-2-succinic anhydride-4,5-dicarboxytetrahydro-6-methyl-anhydride), prior to FLV infection, Regelson (117) reported a marked inhibition of FLV-induced splenomegaly. A similar response was achieved when treatment was initiated after FLV infection. Merigan (116) confirmed these findings, and further characterized the protein induced by the drug as an interferon of 70,000 mol wt. Following intraperitoneal injection of this compound, at 125 mg/kg, peak interferon titer occurred at 18 hr. From dose-response studies, a broad optimum level of interferon induction was shown to occur between doses of 75 and 500 mg/kg.

Of the variety of agents reported to stimulate interferon production, very few are of a defined structure. One, the antibiotic cyclohexamide has been studied (133). However, the dose of this antibiotic required for interferon production *in vivo* results in irreversible inhibition of protein synthesis, leading to death of the animal. The demonstration of induction of interferon *in vivo* by chemicals of defined structure is of particular significance. Further studies with these compounds are required to determine their

ability to effect sufficient suppression of virus, permitting host mechanisms subsequently to control the infection, and to determine that treatment does not lead to any irreversible damage to host cells.

The prophylactic activity of interferon induced by the compound NSC-46015, on three murine oncogenic viruses was examined in our laboratory. Employing the spleen-focus assay for FLV (134) and RLV (135) and the spleen-weight assay for quantitating FLV (41, 136) and RLV (44), treatment with NSC-46015 prior to virus challenge was shown to be very effective in markedly reducing splenomegaly and viremia. Six daily treatments with a dose of 25 mg/kg prior to infection with FLV or RLV resulted in marked reduction of FLV- and RLV-induced spleen foci, splenomegaly, and more than a 3 log reduction in virus titer. Over 80 per cent of the treated animals survived for a significant period of time, apparently free of the disease. A single treatment with the compound, at doses of 200 to 400 mg/kg, either at 24 or 48 hr prior to infection, was not as effective as multiple treatment. Employing the same treatment regimen, effective antiviral therapy was attained with a sarcoma virus (31). A greater than 2 log reduction in detectable virus titer was observed in the treated animals at the time when untreated controls began to die with the disease. The treatment significantly prolonged the survival time of the animals, 40 per cent of them being free of any gross symptoms of the disease 9 weeks after the last untreated control animal died.

Since interferons appear in blood and other tissues of animals as the result of viral infection, it was pertinent to determine what role murine leukemia virus infection had on interferon induction. Gresser (122) employed two strains of mice (Swiss and IC), both susceptible to FLV, to ascertain whether FLV infection could induce circulating interferon. Tissues were examined at selected time intervals after FLV infection for the presence of interferon. Small quantities of an interferon-like substance were detected in serum and spleen 24 hr after infection but not at any other time. Peries (137) similarly reported the absence of interferon in spleens of susceptible BALB/c mice infected with RLV. Vandeputte (131), employing RLV, could not detect any interferon in liver or spleen at any time after RLV infection in MRI and Swiss mice. In contrast, Glasgow (132) noted that interferon induced by RLV was implicated in the resistance of adult CD-1 mice to RLV. The author reported the presence of relatively high levels (200 to 2,000 units per ml) of circulating interferon in the serum of 45 to 63 day old CD-1 mice infected with RLV. Only a 25 per cent mortality resulting from RLV infection was noted in this age group, in contrast to a 90 to 100 per cent mortality occurring in younger animals. This observation is of particular interest because histocompatibility factors have been implicated in the susceptibility of different strains of mice to murine leukemia viruses (138–140). Susceptibility to RLV in different strains of mice has also been reported to vary according to the age of the mouse at the time of virus infection (43).

Antilymphocyte serum.—A fifth, and very promising host factor, capable of exerting an immunological response to an oncogenic virus infection, is antilymphocyte serum (ALS). ALS is well known to have the capacity of prolonging the life of homografts on the animals into which it is injected (141–143). A high proportion of its immuno-suppressive power appears to reside in the 7S globulin fraction. Interest in the use of heterologous antilymphocyte serum as a means of suppressing the homograft reaction was stimulated by the work of Woodruff & Anderson (144). Xenografting of human tumor cell lines to mice has been possible to a limited extent, by the use of ALS (145).

Since the diseases induced by the majority of the murine leukemia viruses involve the lymphoid system, it would appear that the use of ALS for the prevention or control, or both, of leukemia virus infection would be worthwhile. To date, the results of two separate studies indicate that ALS potentiates viral-induced neoplasia. Allison (146) treated BALB/c mice intermittently with ALS from the day of birth and at 3 weeks of age they were inoculated with MLV. A significantly larger number of animals developed lymphoid neoplasms at an earlier time than did the control non-ALS-treated mice. Hirsch (147) described the effect of antilymphocyte serum treatment on the RLV-induced disease in mice. The virus-induced splenomegaly was greatly potentiated in mice that were treated with rabbit anti-mouse thymocyte serum either before or immediately after virus inoculation. Hook (148), employing a murine sarcoma-inducing virus [MSV(M)] demonstrated that the tumor regressions, often observed in adult mice inoculated with this virus (149), could be abrogated by treatment with rabbit antilymphocyte serum. In addition, the incidence and progressive growth of tumors induced in mice with low concentrations of virus were potentiated in mice treated with ALS. The results were considered to be the result of the immuno-supprcssive action of the administered ALS.

Theoretically, ALS should exert some virus-static control of viruses replicating in the lymphoid tissue. However, no such control has been reported. Further studies employing different doses and treatment schedules with ALS may yet result in a beneficial therapeutic response in viral-induced diseases of the lymphoid tissue.

Natural Products

Several studies dealing with the antiviral properties of biological materials have been reported. A few are included in this review to demonstrate the diversity of materials that possess demonstrable antiviral activity.

Okabe (150) successfully inactivated FLV by *in vitro* incubation with snake venom. It was concluded that phospholipase A was responsible for the inactivation and that FLV contained phospholipids.

Extracts of *Mercenaria mercenaria,* the common edible quahog or clam, and other shellfish have prevented growth of transplantable tumors (151)

and virus-induced hamster tumors (152). Judge (153), employing partially purified extracts of *Mercenaria mercenaria,* tested for antiviral activity against MLV and FLV. The extract prolonged the survival time in animals inoculated with an MLV-induced transplantable tumor, and inhibited the splenomegalic response characteristic for animals infected with FLV. However, no prolongation of survival time was attained in the FLV-infected mice. In a subsequent study, Judge (154) demonstrated that FLV-infected mice maintained on a thiamine-free diet experienced both a body weight loss and a decrease of FLV-induced splenomegaly. Sidwell (155) reported that body weight loss produced by food restriction leads to a decrease in FLV-induced splenomegaly. However, Judge attributed his results to factors other than body weight loss, since histologic sections of spleens from FLV-infected mice maintained on a thiamine-free diet were compatible to normal mouse spleens. When malignancy-free mice on thiamine-deficient diet were re-fed thiamine, leukemia became manifest. From these studies, Judge suggests that the antiviral effects attained with the shellfish extracts may have been due to destruction of thiamine by a heat-labile enzyme contained in the extracts.

Erythropoietin has been firmly established as the principal regulator of erythropoiesis. This hormone is of a particular significance when one considers the erythropoietic stimulation observed in mice inoculated with murine leukemogenic viruses. Mirand (156) demonstrated that when mice were induced into a hypertransfused-polycythemic condition and challenged with a polycythemic virus (a possible variant of FLV), marked erythropoiesis ensued. However, no erythropoietin could be detected in plasma or urine. Nakao (157), employing *in vitro* techniques, was able to show that when spleen cells from hypertransfused-polycythemic mice were incubated in the presence of erythropoietin, immature large erythroblasts appeared with marked incorporation of radioiron. Leaders (158) reported a 57 per cent increase in the incidence of leukemia occurring in mice injected with a single dose of erythropoietin. In contrast, Stansley (159) reported that in mice infected at 2 days of age with a virus that induces reticulum cell sarcoma and myeloerythroleukemia, and treated with erythropoietin-containing serum obtained from rabbits, a marked inhibition of tumor induction occurred. Delayed treatment with erythropoietin resulted in instances of regression of overt neoplasia. The possibility was considered that the virus disturbed normal erythropoiesis, resulting in leukemia. Injection of the erythropoietin modified the course of the virus-induced disease, resulting in a more prolonged survival time.

Dunn (160) reported similar results with the RLV. Mice inoculated with RLV were treated with different substances to determine whether the characteristic erythroblastic reaction and the survival time could be altered when compared with those of untreated virus-inoculated controls. Propylthiouracil added to the food of RLV-inoculated mice prolonged life and altered the morphology of the spleen. Repeated blood transfusions inhibited

the erythroblastic reaction and prolonged the survival time. Blood transfusions would be expected to lower erythropoietin levels and thereby reduce erythropoiesis. As expected, the erythroblastic reaction was inhibited, which could explain the prolonged survival time.

Virus-Virus Interactions

There is substantial evidence that *in vivo* and *in vitro* replication of one virus may be inhibited or enhanced by dual infection with another virus. Of particular significance, however, is whether infection with one virus will influence the course of a virus-induced neoplastic disease.

Yashikura (161) employed a mouse lung-cell line, persistently infected with FLV of low leukemogenicity but with high virus-particle count, to demonstrate interference between a low-leukemogenic and a high-leukemogenic virus. A mixed culture of normal mouse-lung cells and the lung-cell line from mice with the low leukemogenic virus was infected with high-leukemogenic FLV. The replication of FLV was markedly suppressed in the mixed cell line when compared to the virus-titer observed in the normal mouse lung-cell culture.

Latarjet (162) injected newborn AKR mice with irradiated cell-free extracts of leukemic tissue prepared from AKR mice with spontaneously occurring leukemia. Littermate controls were either nontreated, or treated similarly with an irradiated extract of isologous normal tissues. There was a 29 per cent reduction in the incidence of leukemia in animals treated with the X-irradiated extracts. Since the cell-free extract contained GLV, three possible mechanisms by which this difference was effected were considered: (*a*) an interferon-like mediated action against the Gross virus, hereditarily present in the AKR mouse strain; (*b*) steric interference of the inactivated virus with the active one; and (*c*) an immunological effect.

Squartini et al. (163) demonstrated a reciprocal interference between the Moloney leukemia virus (MLV) and the mammary tumor virus (MTV). Inoculation of mice with MLV 20 days prior to challenge with MTV resulted in a significant reduction of breast nodules. Conversely, a reduction in leukemia incidence of 29 per cent was noted in MTV-carrying mice inoculated with MLV at 28 days of age.

All the above studies indicate a prevention or delay of disease induction resulting from co-infection with an inactivated virus or a dissimilar virus. In contrast, Turner (164) reported that co-infection with Guaroa virus, an arbovirus, results in a markedly enhanced replication of Friend and Rauscher leukemia virus. The titers of leukemia virus were significantly higher in plasma and spleen of the dually infected mice.

Conclusions

The main objective of this review was to correlate, succinctly, the several effective and potentially effective methods for controlling oncogenic viruses. The several methods described fall into three categories: chemothera-

peutic, immunologic, and stimulation of natural host resistance mechanisms.

The chemotherapeutic approach suffers from the lack of a selective antiviral attack. Studies leading to a fuller understanding of the stages of viral adsorption, penetration, assembly, and release may lead to the synthesis of reagents selective for any of these stages.

Based on the many murine and avian leukemogenic viruses which have been isolated and which possess subtle antigenic differences, indications are that the vaccine approach may be limited and may be of no benefit in suppressing disease once infection is established. Passive immunization, although very effective when used prophylactically, suffers from the same disadvantage in that its effectiveness is lessened once infection has become well established in target cells.

Interferon has been employed successfully and provides an exciting approach to viral disease control. Of particular importance is the potential development of safe and effective means for inducing interferon in the disease host. The interferon-inducing substances must not however, be deleterious to host cells. In brief, the prospects for practical application of our knowledge of the interferon system seems excellent for prophylaxis, and hopeful for therapy of virus infection.

Another approach, worthy of mention, is combined therapy. Chemotherapy, although not selective, combined with interferon or passive immunization holds promise. Irradiation of diseased host, followed by transfer of immunologically competent cells from a syngeneic host capable of producing specific virus-neutralizing antibody, deserves further investigation.

LITERATURE CITED

1. Eddy, B. E., *Health Lab. Sci.*, **1,** 84–95 (1965)
2. Stanley, W. M., *Ann. N.Y. Acad. Sci.*, **71,** 1100–13 (1958)
3. Hilleman, M. R., *Health Lab. Sci.*, **1,** 70–78 (1965)
4. Burkitt, D., *Postgrad. Med.*, **38,** 71–79 (1962)
5. Burkitt, D., in *Viruses, Nucleic Acids and Cancer,* A Collection of Papers Presented at the 17th Symposium on Fundamental Cancer Research, 613 (Williams & Wilkins, Baltimore, 1963)
6. Schwartz, S. O., Greenspan, I., Brown, E. R., *J. Am. Med. Assoc.*, **186,** 106–08 (1963)
7. Heath, C. W., Manning, M. D., Zelkowitz, L., *Lancet,* **2,** 136 (1964)
8. Glass, A. G., Hill, J. A., Miller, R. W., *J. Pediat.*, **73,** 101–07 (1968)
9. Dalton, A. J., Moloney, J. B., Porter, G., Frei, E., Mitchell, E., *Trans. Assoc. Am. Physicians,* **77,** 52–64 (1964)
10. Dmochowski, T., Yumoto, T., Grey, C. E., Hales, R. L., Langford, P. L., Taylor, H. G., Freireich, E. J., Schullenberger, C. C., Shively, J. A., Howe, C. D., *Cancer,* **20,** 760–77 (1967)
11. Dmochowski, L., ***Subviral Carcinogenesis,*** Monograph on the 1st International Symposium on Tumor Viruses, 362–407, July (1967)
12. Sjogren, H. O., Hellström, I., Klein, G., *Cancer Res.*, **21,** 329–37 (1961)
13. Habel, K., *Proc. Soc. Exptl. Biol.*, **106,** 722–25 (1961)
14. Huebner, R. J., Armstrong, D., Okuyan, M., Sarma, P. S., Turner, H. C., *Proc. Nat. Acad. Sci. U.S.*, **51,** 742–50 (1964)
15. Slettenmark, B., Klein, E., *Cancer Res.*, **22,** 947–54 (1962)
16. Pope, J. H., Rowe, W. P., *J. Exptl. Med.*, **120,** 577–88 (1964)
17. Klein, E., Klein, G., *J. Nat. Cancer Inst.*, **32,** 547–68 (1964)
18. Fink, M. A., Malmgren, R. A., Rauscher, F. J., Orr, H. C., Karan, M., *J. Nat. Cancer Inst.*, **33,** 581–88 (1964)
19. Fink, M. A., Karan, M., Rauscher, F. J., Malmgren, R. A., Orr, H. C., *Cancer,* **18,** 1317–26 (1965)
20. Hilleman, M. R., in *Viruses, Nucleic Acids and Cancer,* 580–614 (Williams & Wilkins, Baltimore, 1963)
21. Sadler, P. W., *Pharmacol. Rev.*, **15,** 407–47 (1963)
22. Pienta, R. J., Group, V., in *Experimental Chemotherapy,* Vol. 3, 525–86 (Schnitzer, R. J., Hawking, F., Eds., Academic Press, Inc., New York, 1964)
23. Stuart-Harris, C. H., Dickenson, L., *The Background to Chemotherapy of Virus Diseases,* (Charles C Thomas, Springfield, 1964)
24. Thompson, R. L., in *Advan. Chemotherapy,* **1,** 85–131 (1964)
25. Appleyard, G., *Brit. Med. Bull.*, **23,** 113–18 (1967)
26. Prusoff, W. H., *Pharmacol. Rev.*, **19,** 209–50 (1967)
27. Beard, J. W., *Perspectives Virol. Symp. New York,* 197–230 (1959)
28. Gross, L., *Oncogenic Viruses,* (Pergamon Press, Inc., New York, 393 pp 1961)
29. Moloney, J. B., *Nat. Cancer Inst. Monograph,* **22,** 139–42 (1966)
30. Harvey, J. J., *Nature,* **204,** 1104–05 (1965)
31. Chirigos, M. A., Scott, D., Turner, W., Perk, K., *Intern. J. Cancer,* **3,** 223–37 (1968)
32. Eggers, H. J., Tamm, I., *Ann. Rev. Pharmacol.*, **6,** 231–56 (1966)
33. Kaufman, H. E., *Progr. Med. Virol.*, **7,** 116–59 (1965)
34. Levintow, L., *Ann. Rev. Biochem.*, **34,** 487–526 (1965)
35. Tamm, I., Eggers, H. J., *Am. J. Med.*, **38,** 678–98 (1965)
36. Herrman, E. C., Jr., Ed., *Ann. N.Y. Acad. Sci.*, **130,** Art. 1 (1965)
37. Friend, C., *J. Exptl. Med.*, **105,** 307–18 (1957)
38. Friend, C., *Ann. N.Y. Acad. Sci.*, **68,** 522–31 (1957)
39. Furth, J., Metcalf, D., *J. Chronic Diseases,* 8, 88–97 (1958)
40. Rowe, W. P., Brodsky, I., *J. Nat. Cancer Inst.*, **23,** 1239–48 (1959)
41. Chirigos, M. A., Luber, E., March, R. W., Pettigrew, H., *Cancer Chemotherapy Rep.*, **45,** 29–33 (1965)
42. Moloney, J. B., *J. Nat. Cancer Inst.*, **24,** 933–51 (1960)
43. Rauscher, F. J., *J. Nat. Cancer Inst.*, **29,** 514–43 (1962)
44. Chirigos, M. A., *Cancer Res.*, **24,** 1035–41 (1964)

45. Gross, L., *Proc. Soc. Exptl. Biol. Med.*, **76,** 22–27 (1951)
46. Gross, L., *Proc. Soc. Exptl. Biol. Med.*, **94,** 767–73 (1957)
47. Rous, P., *J. Am. Med. Assoc.*, **56,** 198 (1911)
48. Rous, P., *J. Exptl. Med.*, **13,** 397–411 (1911)
49. Bryan, W. R., *J. Nat. Cancer Inst.*, **16,** 285–315 (1955)
50. Loomis, L. M., Pratt, A. W., *J. Nat. Cancer Inst.*, **17,** 101–23 (1956)
51. Perk, K., Moloney, J. B., *J. Nat. Cancer Inst.*, **37,** 581–99 (1966)
52. Wolstenholme, G. E. W., O'Connor, M., *Ciba Foundation Symposium on Tumor Viruses of Murine Origin.* (Little, Brown & Co., Boston, 441 pp., 1962)
53. Moloney, J. B., *Federation Proc.*, **21,** 19–31 (1962)
54. Chirigos, M. A., Moloney, J. B., Humphreys, S. R., Mantel, N., Goldin, A., *Cancer Res.*, **21,** 803–11 (1961)
55. Glynn, J. P., Moloney, J. B., Chirigos, M. A., Humphreys, S. R., Goldin, A., *Cancer Res.*, **23,** 269–78 (1963)
56. McEndy, D. P., Boon, M. A., Furth, J., *Cancer Res.*, **4,** 377–83 (1944)
57. Upton, A. C., Furth, J., *Blood,* **9,** 686–95 (1954)
58. Wooley, G. W., Peters, B. A., *Proc. Soc. Exptl. Biol. Med.*, **82,** 286–87 (1955)
59. Leventhal, J. D., Eaton, M. D., *Cancer Res.*, **26,** 470–73 (1966)
60. Salganik, R. I., Martynova, R. P., Matienko, N. A., Ronichevskaya, G. M., *Nature,* **214,** 100–02 (1967)
61. Chirigos, M. A., Rauscher, F. J., Kamel, I. A., Fanning, G. R., Goldin, A., *Cancer Res.*, **23,** 762–69 (1963)
62. Chirigos, M. A., Rauscher, F. J., Kamel, I. A., Fanning, G. R., Goldin, A., *Cancer Res.*, **23,** 1646–50 (1963)
63. Chirigos, M. A., *Cancer Res.*, **24,** 1035–41 (1963)
64. Bases, R. E., King, A. S., *Virology,* **32,** 175–83 (1967)
65. Sugiura, K., *Gann,* **50,** 251–64 (1959)
66. Sugiura, K., Stock, E. C., *Acta Unio Intern. Contra Cancrum,* **16,** 780–88 (1960)
67. Sugiura, K., *Cancer Res.*, **22,** 93–135 (1962)
68. Sugiura, K., Schmid, F. A., Schmid, M., *Cancer Res.*, **21,** 1412–20 (1961)
69. Mirand, E. A., Prentice, T. C., Hoffman, J. G., Grace, J. T., Jr., *Proc. Soc. Exptl. Biol. Med.*, **106,** 423–26 (1961)
70. Mirand, E. A., Back, N., Prentice, T. C., Ambrus, J. L., Grace, J. T., Jr., *Proc. Soc. Exptl. Biol. Med.*, **108,** 360–63 (1961)
71. Regelson, W., Pierucci, O., *Radiation Res.*, **22,** 368–82 (1964)
72. Dawson, P. J., Fieldsteel, A. H., Bostick, W. L., *Proc. Soc. Exptl. Biol. Med.*, **119,** 206–08 (1965)
73. Sidwell, R. W., Dixon, G. J., Sellers, S. M., Schabel, F. M., *Cancer Chemotherapy Rep.*, **50,** 299–312 (1966)
74. DeLong, D. C., Baker, L. A., Easton, N. R., Dillard, R. D., *Proc. Soc. Exptl. Biol. Med.*, **127,** 845–49 (1968)
75. Metcalf, D., Furth, J., Buffett, R. F., *Cancer Res.*, **19,** 52–58 (1959)
76. Mirand, E. A., Hoffman, J. G., Grace, J. T., Jr., Trundel, P. J., *Proc. Soc. Exptl. Biol. Med.*, **107,** 824–929 (1961)
77. Yokoro, K., Thorell, B., *Cancer Res.*, **26,** 536–41 (1966)
78. Chirigos, M. A., March, R. W., *Antimicrobial Agents & Chemotherapy,* 489–96 (1966)
79. Pienta, R. J., Bernstein, E. H., Groupé, V., *Cancer Chemotherapy Rep.*, **31,** 25–39 (1963)
80. Bather, R., *Acta Unio Intern. Contra Cancrum,* **16,** 545–51 (1960)
81. Bliznakov, E. G., *J. Reticuloendothel. Soc.*, **1,** 360 (1964)
82. Pinkel, D., Pickren, J., *J. Am. Med. Assoc.*, **175,** 293–98 (1961)
83. Pratt, C. B., *Proc. Am. Assoc. Cancer Res.*, **8,** 54 (1967)
83a. Chirigos, M. A., Scott, D., Unpublished observations.
84. Fink, M. A., Rauscher, F. J., *J. Nat. Cancer Inst.*, **26,** 519–22 (1961)
85. Friend, C., *J. Exptl. Med.*, **109,** 217–28 (1959)
86. Fink, M. A., Rauscher, F. J., *J. Nat. Cancer Inst.*, **32,** 1075–82 (1964)
87. Barski, G., Youn, J. K., *Science,* **149,** 751–52 (1965)
88. Wright, B. S., Lasfargues, J. C., *Nat. Cancer Inst., Monograph,* **22,** 685–700 (1966)
89. Mirand, E. A., Buffett, R. F., Grace, J. R., Jr., *Proc. Soc. Exptl. Biol. Med.*, **121,** 970–76 (1966)

90. Mirand, E. A., Grace, J. R., Jr., Buffett, R. F., *Nature*, **209**, 696–98 (1966)
91. Chirigos, M. A., Luber, E. D., Vadlamudi, S., Fink, M. A., *Nat. Cancer Inst., Monograph*, **22**, 643–56 (1966)
92. Chirigos, M. A., March, R. W., Hook, W., Hoemann, R., *Proc. Soc. Exptl. Biol. Med.*, **125**, 35–37 (1967)
93. Old, L. J., Stockert, E., Boyse, E. A., Geering, A., *Proc. Soc. Exptl. Biol. Med.*, **124**, 63–68 (1967)
94. Law, L. W., Ting, R. C., Stanton, M. F., *J. Nat. Cancer Inst.*, **40**, 1101–12 (1968)
95. Levi, E., Golden, R., Zerubavel, R., Fisher, J., *Cancer Res.*, **26**, 659–667 (1966)
96. Mathé, G., *Presse Med.*, **75**, (19) 947–49 (1967)
97. Mathé, G., Schwarzenberg, L., Amiel, J. L., Cattan, A., Schneider, M., Schlumberger, J. L., *Semaine Hop. Paris.*, **43**, (7) 450–57 (1967)
98. Delorme, E. J., Alexander, P., *Lancet*, **II**, 117–20 (1964)
99. Mikulska, Z. B., Smith, C., Alexander, P., *J. Nat. Cancer Inst.*, **36**, 29–35 (1965)
100. Alexander, P., Connell, D. I., Mikulska, Z. B., *Cancer Res.*, **26**, 1508–15 (1966)
101. Law, L. W., Ting, R. C., Leckband, E., *Proc. Natl. Acad. Sci. U.S.*, **57**, 1068–75 (1967)
102. Billingham, R. E., Brent, L., Medawar, P. B., *Proc. Roy. Soc. London, Ser. B*, **143**, 58 (1954)
103. Mathé, G., Bernard, C., Amiel, J. L., *Compt. Rend.*, **259**, 4408–10 (1964)
104. Sinkovics, J. G., Schullenberger, C. C., Howe, C. D., *Texas Rep. Biol. Med.*, **23**, 94–109 (1965)
105. Spencer, H. J., *Proc. Am. Assoc. Cancer Res.*, **7**, 66 (1966)
106. Isaacs, A., Linderman, J., *Proc. Roy. Soc. London, Ser. B*, **147**, 258–67 (1957)
107. Isaacs, A., *Advan. Virus Res.*, **10**, 1–38 (1963)
108. Ho, M., *Bacteriol. Rev.*, **28**, 367–81 (1964)
109. Wagner, R. R., *Am. J. Med.*, **38**, 726–37 (1965)
110. Finter, N. B., in *Interferons* (Neuberger, A., Tatum, E. L., Eds., W. B. Saunders Company, Philadelphia, 1966)
111. Buckler, C. E., Wang, K. I., Baron, S., *Proc. Soc. Exptl. Biol. Med.*, **127**, 1258–62 (1968)
112. Fruitstone, M. J., Michaels, B. S., Rudloff, D. A. C., Sigel, M. M., *Proc. Soc. Exptl. Biol. Med.*, **122**, 1008–11 (1966)
113. Youngner, J. S., Hallum, J. V., *Virology*, **35**, 177–79 (1968)
114. Ellis, L. F., Kleinschmidt, S. J., *Nature*, **215**, 649–50 (1967)
115. Banks, G. T., Buck, K. W., Chain, E. B., Himmelweit, F., Marks, J. E., Tigler, J. M., Hollings, M., Last, F. T., Stone, O. M., *Nature*, **218**, 542–45 (1968)
116. Merigan, T. C., *Nature*, **214**, 416–17 (1967)
117. Regelson, W., Foltyn, O., *Proc. Am. Assoc. Cancer Res.*, **7**, 58 (1966)
118. Lampson, G. P., Tytell, A. A., Nemes, M., Hilleman, M. R., *Proc. Soc. Exptl. Biol. Med.*, **112**, 468–78 (1963)
119. Bader, J. P., *Virology*, **16**, 436–39 (1962)
120. Force, E. E., Stewart, R. C., *J. Immunol.*, **97**, 126–30 (1966)
121. Gresser, I., Coppey, J., Falcoff, E., Fontaine, D., *Proc. Soc. Exptl. Biol. Med.*, **124**, 84–91 (1966)
122. Gresser, L., Fontaine, J., Coppey, J., Falcoff, R., Falcoff, E., *Proc. Soc. Exptl. Biol. Med.*, **124**, 91–94 (1966)
123. Gresser, L., Coppey, J., Fontaine-Brouty-Boye, D., Falcoff, R., *Nature*, **215**, 174–75 (1967)
124. Gresser, I., Falcoff, R., Fontaine-Brouty-Boye, D., Zajdela, F., Coppey, J., Falcoff, E., *Proc. Soc. Exptl. Biol. Med.*, **126**, 791–97 (1967)
125. Wheelock, E. F., *Proc. Natl. Acad. Sci. U.S.*, **55**, 774–80 (1966)
126. Wheelock, E. F., *J. Nat. Cancer Inst.*, **38**, 771–78 (1967)
127. Wheelock, E. F., *Proc. Soc. Exptl. Biol. Med.*, **124**, 855–58 (1967)
128. Wheelock, E. F., Bryce-Larke, R. P., *Science*, **158**, 537–38 (1967)
129. Wheelock, E. F., Bryce-Larke, R. P., *Proc. Soc. Exptl. Biol. Med.*, **127**, 230–38 (1968)
130. Kleinschmidt, W. J., *Virology*, **29**, 484–88 (1965)
131. Vandeputte, M., DeLafonteyne, J., Billiau, A., DeSomer, P., *Arch. Ges. Virusforsch.*, **20**, 235–54 (1967)
132. Glasgow, L. A., Friedman, S. B., *J. Pediat.*, **69**, 953–54 (1966)

133. Youngner, J. S., Stinebring, W. R., Taube, S. E., *Virology*, **27**, 541–47 (1965)
134. Axelrad, A. A., Steeves, R. A., *Virology*, **24**, 513–18 (1964)
135. Pluznik, D. H., Sachs, L., *J. Nat. Cancer Inst.*, **33**, 535–45 (1964)
136. Chirigos, M. A., Schwalb, E. D., Scott, D., *Cancer Res.*, **27**, 2249–54 (1967)
137. Peries, J., Boiron, M., Canivet, M., *Ann. Inst. Pasteur*, **109**, 595–613 (1965)
138. Tennant, J. R., Snell, G. D., *Nat. Cancer Inst. Monograph*, **22**, 61–72 (1966)
139. Axelrad, A., *Nat. Cancer Inst. Monograph*, **22**, 619–29 (1966)
140. Lilly, F., *Nat. Cancer Inst. Monograph*, **22**, 631–42 (1966)
141. Woodruff, M. F. A., Anderson, N. F., *Ann. N.Y. Acad. Sci.*, **120**, 119–28 (1964)
142. Monaco, A. P., Wood, M. L., Russell, P. S., *Science*, **149**, 432–35 (1965)
143. Levey, R. H., Medawar, P. B., *Ann. N.Y. Acad. Sci.*, **129**, 164–77 (1966)
144. Woodruff, M. F. A., Anderson, N. F., *Nature*, **200**, 702 (1963)
145. Phillips, B., Gayet, J. C., *Nature*, **215**, 548–49 (1967)
146. Allison, A. C., Law, L. W., *Proc. Soc. Exptl. Biol. Med.*, **127**, 207–17 (1968)
147. Hirsch, M. S., Murphy, F. A., *Nature*, **218**, 478–79 (1968)
148. Hook, W. A., Chirigos, M. A., Chan, S. P., *Cancer Res.*, (In press)
149. Fefer, A., McCoy, J. J., Glynn, J. P., *Cancer Res.*, **27**, 1626–31 (1967)
150. Okabe, K., Sugimura, T., Kasuga, T., *Gann*, **55**, 19–23 (1964)
151. Schmeer, M. R., *Science*, **144**, 413–14 (1964)
152. Li, C. P., Prescott, B., Eddy, B., Caldes, G., Green, W. R., Martino, E. C., Young, A. M., *Ann. N.Y. Acad. Sci.*, **130**, 374–83 (1965)
153. Judge, J. R., *Proc. Soc. Exptl. Biol. Med.*, **123**, 299–302 (1966)
154. Judge, J. R., *Proc. Soc. Exptl. Biol. Med.*, **128**, 98–103 (1967)
155. Sidwell, R. W., Dixon, G. J., Sellers, S. M., Maxwell, C. F., Schabel, F. M., *Proc. Soc. Exptl. Biol. Med.*, **119**, 1141–43 (1965)
156. Mirand, E. A., *Proc. Soc. Exptl. Biol. Med.*, **125**, 562–65 (1967)
157. Nakao, K., Miura, Y., Takaku, F., *Blood*, **27**, 646–52 (1966)
158. Leaders, F. E., Chapman, A. L., Kornhaus, B., Nielsen, A. H., Werder, A. A., *Intern. J. Cancer*, **2**, 365–69 (1967)
159. Stansley, P. G., Schiop, P. E., *Science*, **152**, 1082–83 (1966)
160. Dunn, T. B., Malmgren, R. A., Carney, P. G., Green, A. W., *J. Nat. Cancer Inst.*, **36**, 1003–24 (1966)
161. Yashikura, H., Hirokawa, Y., Yamada, M. A., Sugano, H., *J. Virol.*, **2**, 85–86 (1968)
162. Latarjet, R., *Ann. Inst. Pasteur*, **106**, 1–26 (1964)
163. Squartini, F., Olivi, M., Bolis, G., Ribacchi, R., Giraldo, G., *Nature*, **214**, 730–32 (1967)
164. Turner, W., Chirigos, M. A., Scott, D., *Cancer Res.*, **28**, 1064–73 (1968)