Latent Viruses and Mutated Oncogenes: No Evidence for Pathogenicity

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"Circumstantial evidence is a very tricky thing," answered Holmes, thoughtfully. "It may seem to point very straight to one thing, but if you shift your point of view a little, you may find it pointing in an equally uncompromising manner to something entirely different. . . . There is nothing more deceptive than an obvious fact . . ."
—Sir Arthur Conan Doyle, in "The Boscombe Valley Mystery," 1928

The scientific community has been virtually unanimous in admiring its recent triumphs in biotechnology—above all, the detection and amplification of minute amounts of materials into workable and marketable products. However, in clinical diagnostic applications, the new detection methods have become a mixed blessing, which benefits medical scientists but not necessarily their clients. Since rare signals have become just as detectable as abundant ones, many latent viruses have been detected and have been assumed to be just as pathogenic as active prototypes (*1*–*3*). Likewise, cellular mutations have become detectable that do not, or just barely, affect the function and activity of genes. Yet when the affected genes are structurally related to retroviral oncogenes, they are assumed to be just as oncogenic as highly active retroviral oncogenes (*1*, *4*–*8*). However, the evidence for these hypotheses is only circumstantial—based on structural similarities to classical pathogenic viruses and viral oncogenes. Thus, without direct proof, these hypotheses may open the doors to psychologically harmful prognoses and clinically harmful prevention programs, termed "molecular genetics at the bedside" by Bishop (9).

I. New Technology and Old Theories in the Search for the Causes of Disease

A. A New Generation of Virologists Presents Latent Viruses as Pathogens

Although viral epidemics have all but disappeared in the Western world since polio was eliminated with vaccines in the 1950s, the number of viruses currently discovered and studied by virologists has reached epidemic proportions. For example, zealous virus hunters have been able to detect by ultrasensitive biological and biotechnical methods latent viruses that are neutralized by antiviral immunity in diseases such as AIDS, leukemias, lymphomas, hepatomas, hepatitis, cervical cancers, encephalitis, and many others (*1*, 3). Their proposals that latent viruses cause these diseases are widely accepted, because from the days when only the most pathogenic and abundant viruses were detectable, all viruses still have the reputation of being pathogens.

However, the diseases with which these newly discovered latent viruses are associated are not contagious—unless one makes bizarre assumptions. One assumption postulates that these viruses are "slow viruses" or "lentiviruses" causing diseases only up to 55 years after infection and only after they are neutralized by antibodies (see Sections II and III). Yet all of these viruses replicate and are immunogenic within weeks, not years, after infection just like conventional viruses. Another assumption is that these viruses can shift from a nonpathogenic dormant state to a pathogenic state without increasing their biochemical activity or abundance.

A case in point is the assumption that AIDS is caused by a virus. There were over 160,000 AIDS patients in the U.S. in the last 10 years, and there is no antiviral vaccine or drug. Yet at the time of this writing there is not even one confirmed case of a health care worker who contracted AIDS from a patient, nor of a scientist who contracted AIDS from the "AIDS virus" that is propagated in hundreds of research laboratories! The AIDS virus is just as inactive in patients as it is in asymptomatic virus carriers (see Section II).

Such assumptions are not compatible with classical criteria of viral pathogenicity. Conventional viruses are very active, abundant and replicating in many cells that are killed or transformed when they cause diseases such as polio, flu, measles, mumps, hepatitis, herpes, Rous sarcoma, and many others (3, *10–12*). Likewise, SV40 and adenoviruses inundate many cells with viral T-antigens when they cause

tumors, even though the respective host animals are not permissive for viral replication (*13*). Pathogenicity by these classical viruses results from high biochemical activity in large numbers of cells. These viruses are not pathogenic when they are latent or infect only small numbers of cells. Indeed, even the most pathogenic viruses depend for their survival on asymptomatic infections in which they are highly active in small numbers of cells before they are stopped by antiviral immunity, the reason that such infections are asymptomatic (*3*).

Furthermore, all conventional viruses are maximally pathogenic within weeks or months after infection before they are neutralized by antiviral immunity, causing disease as soon as they reach pathogenic thresholds in the host (*10–12*). In rare cases, they may be reactivated to resume replication, and hence pathogenicity, long after they are neutralized by antiviral immunity (e.g., the herpes simplex virus). Reactivation typically follows a transient immunodeficiency acquired by another primary disease or other immunosuppressive conditions (*12*). Except for these instances of viral reactivation, there are no known examples of viruses that cause diseases only after a long latent period and only after they have been neutralized by antibodies.

Thus, the evidence that latent viruses can be pathogenic is only circumstantial, based on structural similarities between latent viruses and active, pathogenic viral prototypes. Further, these hypotheses are based on the epidemiological evidence that latent viruses occur, or appear to occur, in diseases at a higher rate than would be expected from random infection (*3, 14, 15*) (see Section V).

B. From Retroviral to Cellular Oncogenes—The Oncogene Hypothesis

New technology detecting point-mutations, deletions, and truncations of cellular genes and latent or defective viruses put new life in the somatic mutation hypothesis of cancer (*16*). It was postulated in 1969 by Huebner and Todaro that latent viruses and covert cancer genes preexist in normal cells and are "activated" to cancer genes and cancer viruses by mutation (*17*). The proposal became known as the oncogene hypothesis. The discoveries in 1970 of retroviral oncogenes (*18, 19*) and in 1973 of cellular genes from which the coding regions of retroviral oncogenes are derived (*20–22*) put the oncogene hypothesis to its first test. It was proposed that mutation turns those genes from which the coding regions of retroviral oncogenes are derived into equivalents of viral oncogenes (*6*). These genes are now called either proto-*onc* genes or cellular oncogenes (*1, 5–8, 23, 24*)

or even "enemies within" the cell (*25*). And mutated cellular oncogenes are euphemistically termed "activated" cellular oncogenes (*1, 5–8*).

Examples of "activated" oncogenes are point-mutated proto-*ras* genes that are thought to be bladder or colon cancer genes (*23, 26–28*), truncated proto-*myc* genes that are thought to be Burkitt's lymphoma genes (*29, 30*), proto-*myc* genes with retroviruses integrated upstream (*31*) and downstream (*32*) that are thought to be avian lymphoma genes, and rearranged proto-*abl* genes that are thought to be myelogenous leukemia genes (*7, 8, 33*). By analogy to proto-*onc* genes, even genes that are not related to retroviral *onc* genes are now thought to be "activated" oncogenes if mutated by provirus integration, like the *int* genes of mouse mammary tumors with retroviruses integrated within or nearby (*5, 8, 34*).

However, mutated proto-*onc* genes and *int* genes with integrated retroviruses are either just as active or only slightly more active than their normal counterparts (see Section IV). Moreover, the mutant genes from tumors do not transform cells upon transfection. By contrast, proviral DNA copies of retroviral oncogenes transform susceptible cells and are about 100 times more active than normal proto-*onc* genes (*24, 35–38*). During the last 5 years, the transforming function of retroviral oncogenes, including those of Rous sarcoma, Harvey sarcoma, and MC29 and MH2 carcinoma viruses, has been shown to depend absolutely on transcriptional activity, rather than on mutations in the coding region (*39–44*). This high transcriptional activity of retroviral oncogenes results from retroviral promoters.

The latest modification of the oncogene hypothesis, the anti-oncogene hypothesis, proposes that constitutively active, but as yet unnamed, oncogenes are "activated" by mutational inactivation of tumor suppressors or anti-oncogenes (*8, 9, 45*). Examples are the retinoblastoma and p53 anti-oncogenes that are thought to cause retinoblastoma (*45*) and colon cancer (*46*) if they are inactivated by point-mutation, truncation, or deletion. However, unmutated anti-oncogenes do not revert tumor cells to normal (see Section IV).

Thus, the evidence for these hypotheses is only circumstantial, based on structural similarities between mutated "cellular oncogenes" displaying a normal level of activity and about 100 times more active viral oncogenes. Further, these hypotheses are based on the epidemiological evidence that mutated genes occur, or appear to occur, in diseases at a much higher rate than would be expected from spontaneous mutation (*4, 5, 7, 28, 47*) (see Section V).

C. From Autonomous Pathogens to Multifactorial Causes of Disease

In view of the apparent non-equivalence between the postulated pathogens and their prototypes, the original hypotheses have been supplemented by *ad hoc* hypotheses. Typically, these *ad hoc* hypotheses postulate second- or even higher-order mechanisms of pathogenesis that include cofactors and helper genes, in contrast to the classical prototypes, which all follow first-order mechanisms of pathogenesis. Moreover, the putative helper genes, like the putative primary pathogens, are not disease-specific, because they are also found in asymptomatic subjects. Indeed, "cofactors" are euphemisms for new hypotheses, which grant face-saving roles to failing incumbents with large constituencies.

D. The Search for Alternative Hypotheses

In the following, we have reinvestigated the evidence for the claims that latent viruses and mutated genes are pathogenic. Since the available evidence for pathogenicity is insufficient, we conclude that the latent viruses and mutated genes must be considered innocent until proven guilty.

Since falsification creates a vacuum, we have attempted to present brief alternatives, drawing in most cases from published work. However, in the case of AIDS, we have documented an alternative to the virus–AIDS hypothesis more extensively, because there is hardly any mention of alternatives in the over 60,000 papers published on the AIDS virus and AIDS since 1983 (*48*). By challenging currently unproductive hypotheses and by providing falsifiable alternatives, we hope to contribute to the search for what really causes these diseases.

II. Inactive Viruses and Diseases Resulting from the Loss of Cells

A. Human Immunodeficiency Virus (HIV) and AIDS

AIDS is a new syndrome of 25 previously known diseases (*49–52*). In America, 61% are microbial diseases such as pneumonia, candidiasis, tuberculosis, cytomegalovirus, and herpes virus disease (*50, 52*) that result from immunodeficiency due to a severe depletion of T-cells (*49, 51*). The remaining 39% of AIDS diseases are dementia, wasting disease, Kaposi sarcoma, and lymphoma, which are not consistently associated with immunodeficiency and microbes (*52–54*). In the U.S.,

32% of AIDS patients are intravenous drug users (*52, 55*), about 60% are male homosexuals (*52*) who frequently used drugs as aphrodisiacs (*54, 56–64, 103*), and most of the remainder have severe clinical or congenital deficiencies, including hemophilia (*52, 54, 61*). Over 80% of the American AIDS patients are 20- to 44-year olds, of which about 90% are males (*52*). Different AIDS-risk groups have different AIDS diseases. For example, homosexuals have 20 times more Kaposi sarcoma than other AIDS patients (*65*), intravenous drug users have a proclivity for tuberculosis (*66, 67*), "crack" (cocaine) smokers for pneumonia (*68*), and users of the cytotoxic DNA-chain-terminator AZT, prescribed to inhibit HIV, for anemia, nausea, and lymphoma (*69–71*).

About 50% of all American AIDS patients are currently confirmed to have antibodies to a retrovirus, termed **human immunodeficiency virus** (HIV) (*51, 54, 72*). However, all AIDS diseases occur in all risk groups in the absence of HIV (see Section II,A,3) (*54*). In the U.S., HIV is fixed to an extremely constant reservoir of about 1 million carriers, ever since 1985, when it became possible to detect antibody against HIV (the "AIDS test") (*54, 73*). HIV is naturally transmitted from mother to child, like other retroviruses, at an efficiency of about 50% (*54*). This efficiency might be higher than serological tests indicate, because some proviruses of other perinatally transmitted human retroviruses only become immunogenic with advanced age (*54*) (see Section III). Sex is another natural mode of transmission. However, it is highly inefficient, depending on an average of about 1000 sexual contacts (*54, 74*), because there is no HIV provirus detectable, even with the polymerase chain reaction (PCR), in semen in 24 out of 25 HIV-positive men (*75*). Since 1987, when AIDS was given its current definition (*50*), about 30,000, or 3% of the 1 million Americans infected by HIV (*53, 54, 73*), develop AIDS annually (*52*).

1. The Virus–AIDS Hypothesis

Currently, most medical scientists believe that AIDS is caused by HIV (*51*). The hypothesis assumes: (i) that AIDS is new because HIV is thought to be new in all countries with AIDS (*14, 51*); (ii) that AIDS is acquired by sexual and parenteral transmission of HIV; (iii) that HIV causes immunodeficiency by killing infected T-cells; (iv) that 50–100% of HIV infections lead to fatal AIDS diseases; (v) that AIDS occurs on average only 10 years after antibodies to HIV appear (a positive "AIDS test"), to reconcile the low (3%) morbidity with the large number of asymptomatic HIV carriers; (vi) that antibodies to HIV do not neutralize the virus (*53, 76, 77*), to reconcile AIDS with antibodies to HIV; and

(vii) that all unrelated AIDS diseases are caused by the same HIV (*49, 51, 54, 78*).

In view of this hypothesis, AIDS has been defined exclusively by the association of the 25 indicator diseases with antibody to HIV (*50, 51, 54*). Further, "safe sex" (*49, 51*) and "clean injection equipment" for recreational drugs (*55*) are recommended as AIDS prophylaxis for uninfected persons, and the cytotoxic DNA-chain-terminator, 3'-azidothymidine (AZT) is prescribed to infected healthy, as well as sick, persons to inhibit HIV (*51, 71, 80a, 79, 80*). The presence of antibody to HIV in a healthy person is interpreted as a prognosis for AIDS. Testing and counseling are provided routinely to applicants of the U.S. Job Corps (*81*). Several countries, including the U.S. and China, bar entry to HIV-positive persons. And a negative "AIDS test" for antibodies to HIV has become mandatory in the U.S. since 1985 for the approximately 12 million blood donations that are collected annually (*82*) by the American blood banks and the Red Cross (Irwin Memorial Blood Bank, San Francisco, personal communication, 1990) and for admission to the U.S. Army (*73, 83*).

Each of the seven assumptions of the virus–AIDS hypothesis can be challenged on epidemiological and virological grounds:

1. Since all new microbes spread exponentially in a population (*11*), the complete failure of HIV to spread from its 1985 level, when it became first detectable, indicates that the American "HIV epidemic" is old. This is particularly compelling if one considers that there is no antiviral vaccine and no antiviral drug. Thus, HIV is not new in the U.S.
2. Given that procreative sex is about 10% efficient (3 days per month) and sexual transmission of HIV only 0.1%, it follows that HIV depends on perinatal transmission for its survival (*54*). If HIV survives naturally via perinatal transmission, it cannot be pathogenic by itself, just like all other perinatally transmitted parasites (*12*)—except if one assumes latent periods that exceed the normal generation time of humans. Indeed, chimpanzees experimentally inoculated and health care workers accidentally inoculated with HIV do not develop AIDS (*51, 54*). Thus, sexual transmission of HIV cannot be a sufficient cause for AIDS.
3. Since no more than 1 in 500 T-cells of AIDS patients ever contains a DNA provirus of HIV and over 99% of infected T-cells survive infection (*84*), and since about 1 in 25 T-cells is regenerated during the 2 days it takes a retrovirus to infect a cell, HIV infection cannot be responsible for the loss of T-cells in AIDS (*53*). Thus, HIV, like all other retroviruses, does not kill cells (*53,*

85, 86). Indeed, HIV is propagated commercially for the "AIDS test" in cultured lines of the same human T-cells that it is said to kill *in vivo* (87).

4. The assumption that HIV is 50–100% fatal within 10 years cannot be correct, because about 1 million Americans carry HIV since 1985 but only about 30,000 develop AIDS annually since 1987, when AIDS received its current definition (50). Instead, it would take 33 years for all U.S. HIV carriers to develop AIDS diseases based on the current data (3% per year). An average latent period of 10 years would predict that 100,000 Americans would develop AIDS in 1 year.
5. Since viruses, as self-replicating toxins, are all fast immunogens and thus potentially fast pathogens, but AIDS diseases are estimated to occur on average only 10 years after HIV is neutralized by antiviral antibodies, the assumption that HIV needs 10 years to cause AIDS is arbitrary. The long intervals between infection and AIDS probably indicate that HIV is not even necessary for AIDS, because there is no "late" HIV activity, and because antibodies continue to neutralize the virus during AIDS (53, 54).
6. The complete absence of free HIV in nearly all AIDS patients (53, 54, 88)—the reason that the isolation of HIV had escalated into an international scandal (89, 90)—invalidates the assumption that antibodies to HIV do not neutralize HIV. Indeed, antiviral immunity effectively restricts HIV in AIDS patients (91, 92) to 1 provirus in about 500 T-cells, and viral activity to less than 1 in 10,000 T-cells (53, 54, 84).
7. Since all AIDS diseases occur in the absence of HIV in intravenous drug users, homosexuals, and hemophiliacs, HIV is not even necessary for AIDS diseases—except for their classification as AIDS (53, 54).

Because of the many virological and epidemiological inconsistencies of the virus–AIDS hypothesis, some, notably Montagnier (93) and recently Maddox (94–96), have proposed that HIV is not sufficient for AIDS. Accordingly, a number of "cofactors" such as mycoplasmas (85, 93) and other viruses (15, 76) have been postulated as helping HIV to cause AIDS. However, there is no consensus at this time about a specific cofactor that would be sufficient to cause AIDS in combination with HIV (76, 93). Moreover, there is not even one plausible hypothesis as to how a latent retrovirus such as HIV, which is present in no more than 1 in 500 T-cells, could possibly help another microbe to cause AIDS that, by itself, is not able to do so.

Indeed, there are at least six inconsistencies between AIDS and infectious disease:

1. Paradoxically, there is not even one case reported in the scientific literature of a health care worker who contracted AIDS from a patient, although there were over 200,000 AIDS patients in the U.S. in the last 10 years (52). Likewise, not even one scientist contracted AIDS from the "AIDS virus" or from other microbes from AIDS patients, which are propagated in hundreds of research laboratories and companies (53, 54, 87).
2. All new infectious diseases spread exponentially in susceptible populations (11). However, despite widespread alarm, AIDS claims since 1987 only about 30,000 or 0.03% per year from a reservoir of over 100 million susceptible, sexually active Americans. This is particularly paradoxical for a presumably infectious syndrome, because conventional venereal diseases are increasing in the U.S. (97) and because there is no anti-HIV vaccine and no anti-HIV drug.
3. The distribution of all infectious venereal diseases is almost even between the sexes (98). By contrast, 90% of American AIDS is restricted to males since 1981 (52). This is incompatible with infectious venereal disease.
4. Almost all (94%) of the Americans who develop AIDS have been subject to abnormal health risks (52). These risks include either long-term consumption of recreational, psychoactive, and aphrodisiac drugs and anti-HIV drugs such as the cytocidal DNA-chain-terminator AZT (see below) or congenital or acquired deficiencies such as hemophilia (52, 54). This indicates that specific health risks are necessary for AIDS.
5. The observations that distinct AIDS-risk groups have distinct AIDS diseases—e.g., homosexuals having 20 times more Kaposi sarcoma than HIV carriers from other risk groups (65), intravenous drug users having a proclivity for tuberculosis (66, 67), "crack" (cocaine) smokers for pneumonia (68), and AZT users for anemia, nausea, and lymphoma (69–71)—are also difficult to reconcile with a single infectious cause.
6. All AIDS diseases occur in all AIDS-risk groups in the absence of HIV (54).

In view of these inconsistencies between AIDS and infectious disease and the total lack of a common active microbe in AIDS, several investigators, including us, have concluded that AIDS may not be infectious (54, 56–62, 99–102).

2. The Drug–AIDS Hypothesis

An alternative hypothesis proposes that American AIDS diseases, above their normal background, are the result of the long-term consumption of (*a*) intravenous and (*b*) oral recreational drugs, and (*c*) anti-HIV drugs (*54, 60, 103*). The following epidemiological and drug-toxicity data support this hypothesis.

a. Intravenous Recreational Drugs. Currently, 32% of the American AIDS patients come from groups that use intravenous drugs such as heroin, cocaine, and others (*52, 55*). This group includes about 75% of the heterosexual AIDS cases, 71% of the females with AIDS, and over 10% of the male homosexuals and hemophiliacs with AIDS (*52, 55*). In addition, about 50% of American children with AIDS were born to mothers who are confirmed intravenous drug users and another 20% to mothers who had "sex with intravenous drug users" and are thus likely users themselves (*52, 55*). Likewise, 33% of European AIDS patients are intravenous drug users (*104*).

b. Oral Recreational Drugs. Approximately 60% of the American AIDS patients are 20- to 44-year-old male homosexuals (52). The following evidence indicates that they come from groups who use oral psychoactive and aphrodisiac drugs. A survey of 3916 self-identified American homosexual men, the largest of its kind, reported in 1990 that 83% had used one, and about 60% two or more, drugs with sex during the previous 6 months (*105*). These drugs include nitrite and ethylchloride inhalants, cocaine, amphetamines, methaqualone, lysergic acid, phenylcyclidine, and more (*59, 61–63, 101, 105–112*). A study of 359 homosexual men from San Francisco reported in 1987 that 84% had used cocaine, 82% alkylnitrites, 64% amphetamines, 51% quaaludes, 41% barbiturates, and 20% injected drugs, and 13% shared needles (*107*). This group had been randomly selected from a list of homosexuals who had volunteered to be investigated for hepatitis B virus infection and to donate antisera to hepatitis B virus between 1978 and 1980.

Nitrite inhalants and possibly other drugs are preferred by male homosexuals as aphrodisiacs because they facilitate anal intercourse (*105, 111, 113, 114*). For example, an early CDC study that included 420 homosexual men found nitrite use far more frequent among homosexuals than among heterosexuals and correlating directly with the number of different homosexual partners (*57*). Surveys studying the use of nitrite inhalants in San Francisco found that among homosexual men 58% were users in 1984 and 27% in 1991 compared to less than 1% among heterosexuals and lesbians of the same age group (*115*).

The nitrites are directly toxic as oxidants of biological molecules

such as hemoglobin, and are effective mutagens (*101, 103*). The National Institute on Drug Abuse reports correlations from 69% (*116*) to virtually 100% (*101, 113*) between nitrite inhalants and Kaposi sarcoma and pneumonia, which are diagnosed as AIDS in the presence of antibody to HIV (*50, 51, 54*). In view of this, a causal link between nitrite inhalants and Kaposi sarcoma and pneumocystis pneumonia in homosexuals was first suggested in 1982 by the CDC (*57*) and other investigators (*56, 58*). As a consequence, the sale of nitrite inhalants was banned by the U.S. Congress in 1988 (Public Law 100-690) (*117, 118*). The direct and indirect toxicity associated with the long-term use of other recreational drugs has been described elsewhere (*103*).

c. Anti-HIV Drugs. About 80,000 Americans and 120,000 persons worldwide with and without AIDS currently take the cytocidal DNA-chain-terminator AZT (*54*) and an unknown number take other DNA chain-terminators such as ddI and ddC (*71*). AZT has been prescribed since 1987 to symptomatic (*51, 70, 79, 119*), and since 1990 to asymptomatic, carriers of HIV, including babies and hemophiliacs (*80, 120*), in an effort to inhibit HIV DNA synthesis (*121*). Thus, an unknown, but possibly high, percentage of the 30,000 Americans that currently develop AIDS per year (*52*) have used AZT prior to or after the onset of AIDS. For instance, 249 out of 462 HIV-positive, AIDS-free homosexual men from Los Angeles, included in the above survey (*105*), are on AZT or ddI (*122*).

Although AZT is an inhibitor of HIV DNA synthesis, it is not a rational medication for persons with antibodies to HIV for the following reasons: (i) There is no proof that HIV causes AIDS. (ii) Since no detectable RNA-dependent viral-DNA synthesis occurs, and since the number of infected cells remains stable once the virus is neutralized by antibodies (*53, 54*) only cell DNA with and without proviruses of HIV is terminated by AZT treatment. Further, since AZT cannot distinguish infected from uninfected cells, and only 1 in 500 T-cells is infected in AIDS patients and asymptomatic carriers (*54, 84*), it kills 500 uninfected cells for every infected cell. Thus, AZT is inevitably toxic, killing 500 times more uninfected than infected cells. (iii) In view of the hypothesis that HIV causes AIDS by killing T-cells (*49, 51*), it is irrational to overkill infected cells with AZT.

As expected from an inhibitor of DNA synthesis, many studies report AZT-mediated toxicity. Anemia, neutropenia, and leukopenia occur in 20–50%, with about 30–50% requiring transfusions within several weeks (*70, 71, 123–125*). Severe nausea from intestinal intoxication is observed in up to 45% (*70, 71, 80*) and severe muscle atrophy in 6–8% (*70, 126–128*). Acute hepatitis, insomnia, headaches, demen-

tia seizures, and vomiting are also reported effects of AZT (*71*). Lymphomas appear in about 9% within 1 year on AZT (*69*). AZT is also mutagenic and carcinogenic in mice (*129, 130*) and transforms cells *in vitro* as effectively as methylcholanthrene (*131*). AZT toxicity varies a great deal with the patient treated, due to differences in kinases involved in its uptake and in AZT metabolism (*71, 121, 131, 132*). All of these results explain Temin's profound observation that ". . . the drug generally becomes less effective after six months to a year . . ." (*134*).

Nevertheless, AZT is thought to have serendipitous therapeutic benefits based on the only placebo-controlled study of its effects on AIDS patients (*70, 119*). The study was sponsored by Burroughs-Wellcome, the manufacturer of AZT (*70, 119*). In this study, T-cell counts were observed to increase from 4 to 8 weeks and then to decline to pretreatment levels. Above all, AZT was claimed to "decrease mortality" because only 1 out of 143 in the AZT-treated group died compared to 19 out of 135 in the placebo group.

However, 30 out of the 143 in the AZT group depended on multiple transfusions for survival from anemia, compared to only 5 out of the 135 in the placebo group. Since the number of subjects in the AZT group who would have died from anemia if untreated (30) was larger than the AIDS deaths and anemias of the control group combined (19 + 5), the claim of decreased mortality is not realistic (*70, 119*). Moreover, 66 in the AZT group suffered from severe nausea and 11 from muscle atrophy, compared to only 25 and 3 in the control group. The lymphocyte count decreased over 50% in 34% of the AZT group and in only 6% of the control. The study is further compromised by "concomitant medication" (*70*), the failure to consider the effects of recreational drug use and of patient-initiated randomizations of blinded AZT and placebo controls (*135*). The brief AZT-induced gain of T-cells may reflect compensatory hemopoiesis and random killing of pathogenic parasites (*132*) and the influence of concomitant medication (*70*).

In view of the inevitable toxicity of AZT, its popularity as an anti-HIV drug can only be explained by the widespread acceptance of the virus–AIDS hypothesis and the failure to consider the enormous difference between the viral and cellular DNA targets. This may also be the reason that long-term studies of AZT in animals compatible with human applications have not been published (*71*).

3. The Drug- versus the Virus–AIDS Hypothesis

To distinguish between HIV and drugs as causes of AIDS, it is necessary to determine whether HIV carriers develop AIDS only

when they use drugs, and whether HIV-free drug users develop AIDS indicator diseases.

a. Drug Use Necessary for AIDS in Presumed or Confirmed Carriers of HIV.

(i) Epidemiological correlations suggest that nitrites are necessary for Kaposi sarcoma. (i) A 27- to 58-fold higher consumption of nitrites (*111, 115*) correlates with a 20-fold higher incidence of Kaposi sarcoma in male homosexuals compared to all other AIDS patients of the same age group (65). (ii) Among male homosexuals, those with Kaposi sarcoma have used nitrite inhalants twice as often as those with other AIDS diseases (*101*). (iii) During the last 6–8 years, the use of nitrite inhalants among male homosexuals decreased (e.g., from 58% in 1984 to 27% in 1991 in San Francisco) (*115*). In parallel, the incidence of Kaposi sarcoma among American AIDS patients decreased from a high of 37% in 1983 (*136*) to a low of 10% in 1990 (*52*). In fact, nitrites may be sufficient causes for these diseases, because there is no evidence that HIV was even present in any of these studies.

(ii) Specific correlations indicate that nitrites are necessary for AIDS. The first five cases diagnosed as AIDS in 1981, before HIV was known, were male homosexuals who had all consumed nitrite inhalants and presented with pneumocystis pneumonia and cytomegalovirus infection (*137*). Early CDC data indicate that, in 1981 and 1982, 86% of male homosexuals with AIDS had used oral drugs at least once a week and 97% occasionally (*57, 138*), and that every one of 20 Kaposi sarcoma patients had used nitrites (*56*). The National Institute on Drug Abuse reports correlations from 69% (*116*) to virtually 100% (*101, 113*) between nitrite inhalants and Kaposi sarcoma and pneumonia. Again, drugs may have sufficed to cause these diseases, because HIV was not diagnosed (*50, 51, 54*).

(iii) The incidence of AIDS diseases among 297 HIV-positive, asymptomatic intravenous drug users over 16 months was three times higher in those who persisted than in those who stopped injecting drugs (*139*).

(iv) The T-cell count of 65 HIV-infected drug users from New York dropped over 9 months in proportion with drug injection—on average, 35%—compared to controls who had stopped (*140*).

(v) A placebo-controlled study, investigating AZT as AIDS prophylaxis in HIV-positive, AIDS-free 25- to 45-year-old male homosexuals and intravenous drug users, indicates that AZT induces diseases from within and without the AIDS definition (*80*). During 1 year of taking 500 mg of AZT per day, a group of 453 developed 11 AIDS cases, and a group of 457, taking 1500 mg of AZT per day, developed 14 cases. The placebo group of 428 developed 33 AIDS cases.

However, the price for the presumed savings of 22 and 19 AIDS cases with AZT was high, because 19 more cases of anemia, neutropenia, and severe nausea appeared in the 500-mg AZT group, and 72 more such cases appeared in the 1500-mg AZT group, than in the placebo group. This indicates cytocidal effects of AZT on hemopoiesis and on the intestines. Although these AZT-specific diseases were not diagnosed as AIDS, neutropenia generates immunodeficiency. Surprisingly, in view of its toxicity on leukocytes and red cells, a consistent loss of T-cells was not observed in this study. A recent study investigating AZT as AIDS prophylaxis observed leukopenia, e.g., T cell depletion, in 82% within 1 to 1.5 years of AZT treatment (*140a*). The study is further compromised by the failure to report and to consider the recreational drug-use histories and the many AZT-treatment adjustments of the subjects analyzed.

(vi) Within 48 weeks on AZT, 172 (56%) out of 308 AIDS patients developed additional AIDS diseases, including pneumonia and candidiasis (*125*). This indicates that AZT induces AIDS diseases within less than 1 year, and thus much faster than the 10 years HIV is said to need to cause AIDS (*54*). Likewise, no therapeutic benefits were observed for 365 French (*123*) and 4 Norwegian AIDS (*133*) patients after 6 months on AZT.

(vii) The annual lymphoma incidence of AZT-treated AIDS patients was reported to be 9% by the National Cancer Institute and was calculated to be 50% over 3 years (*69*). The lymphoma incidence of untreated HIV-positive AIDS-risk groups is 0.3% per year and 0.9% per 3 years, derived from the putative average progression rate of 10 years from HIV to AIDS (*54*, *141*, *142*) and the 3% incidence of lymphoma in AIDS patients (*52*). Thus, the lymphoma incidence is 30–50 times higher in AZT-treated than in untreated HIV-positive counterparts. In addition, "during the past three years [of AZT therapy] a progressive increase in the number of [AIDS] patients dying from lymphoma . . . ," to a current level of 16%, was noted in 1991 in a group of 346 AIDS patients in London, most of whom were on AZT (*143*).

It is likely that the chronic levels of the mutagenic AZT, at 10–30 μM (500–1500 mg/person/day), were responsible for the lymphomas. The alternative proposal that HIV-induced immunodeficiency was responsible for the lymphomas (*69*) is unlikely, since cancers do not reflect a defective immune system (*53*, *144*).

(viii) Ten out of 11 HIV-positive, AZT-treated AIDS patients recovered cellular immunity after discontinuing AZT in favor of an experimental HIV vaccine (*145*), indicating that AZT sufficed for immunodeficiency.

(9) Four out of five AZT-treated patients recovered from myopathy 2 weeks after discontinuing AZT; two redeveloped myopathy on renewed AZT treatment (*126*).

(10) Four patients with pneumonia developed severe pancytopenia and bone marrow aplasia 12 weeks after the initation of AZT therapy. Three out of four recovered within 4–5 weeks after AZT was discontinued (*124*), indicating that AZT was sufficient for pancytopenia.

b. Drug Use Sufficient for AIDS Indicator Diseases in the Absence of HIV.

(1) Among intravenous drug users in New York, representing a "spectrum of HIV-related diseases," HIV was observed in only 22 out of 50 pneumonia deaths, 7 out of 22 endocarditis deaths, and 11 out of 16 tuberculosis deaths (*66*).

(2) Pneumonia was diagnosed in 6 out of 289 HIV-free and 14 out of 144 HIV-positive intravenous drug users from New York (*146*).

(3) Among 54 prisoners with tuberculosis in New York State, 47 were street-drug users, but only 24 were infected with HIV (*67*).

(4) In a group of 21 heroin addicts, the ratio of helper to suppressor T-cells declined within 13 years from a normal of 2 to less than 1, which is typical of AIDS (*50, 51*), but only 2 were infected by HIV (*147*).

(5) Thrombocytopenia and immunodeficiency were diagnosed in 15 intravenous drug users on average 10 years after they became addicted, but 2 were not infected with HIV (*148*).

(6) Lymphocyte reactivity and abundance was depressed by long-term injection of drugs not only in 111 HIV-positive but also in 210 HIV-free intravenous drug users from Holland (*149*).

(7) The same lymphadenopathy, weight loss, fever, night sweats, diarrhea, and mouth infections were observed in 49 out of 82 HIV-free and 89 out of 136 HIV-positive, long-term intravenous drug users from New York (*150*), and in about 40% of 113 intravenous drug users from France, of which 69 were HIV-positive and 44 were negative (*151*). The French group had used drugs for an average of 5 years.

(8) Among six HIV-free male homosexuals with Kaposi sarcoma, five reported the use of nitrite inhalants (*152*).

(9) Similar neurological deficiencies were observed among 12 HIV-infected and 16 uninfected infants from drug-addicted mothers (*153*).

Thus, the long-term use of recreational and anti-HIV drugs appears necessary in HIV-positives and sufficient in HIV-negatives to induce AIDS indicator and other diseases.

It follows that the drug–AIDS hypothesis is epidemiologically and pathologically better grounded than the virus–AIDS hypothesis. About 32% of American AIDS patients are confirmed intravenous drug

users, probably 60% use aphrodisiac drugs orally, and an unknown but large percentage of both behavioral and clinical AIDS-risk groups use AZT. Moreover, the consumption of recreational drugs by AIDS patients is probably underreported, because the drugs are illicit, and because medical scientists and support for research are currently heavily biased in favor of viral AIDS (*68, 154, 155*). The pathogenicity of these drugs is empirically known for all, and mechanistically for some, drugs, notably for AZT and nitrites (*103*).

Nonetheless, evidence for the role of drugs in AIDS is rejected by proponents of the virus–AIDS hypothesis (*15, 77, 105*). This is certainly one reason why despite the current drug-use epidemic, there are no studies that investigate the long-term effects of psychoactive drugs and AZT in animals, compatible with the time periods and dosages used by AIDS patients (*155*).

By contrast to the near complete correlation between drugs and AIDS, antibodies to HIV are confirmed in only about 50% of AIDS patients (*51, 72*), and it is a complete mystery how HIV acts as a pathogen, despite enormous research efforts (*14, 15, 54, 156*).

The drug–AIDS hypothesis resolves all scientific paradoxes posed by the prevailing virus–AIDS hypothesis:

1. In America, HIV is a long-established, endemic virus, but AIDS is new—because the drug epidemic is new.
2. AIDS is restricted for over 10 years to 10,000 (52) or 0.01% of the over 100 million sexually active heterosexual Americans per year, and to 20,000 (52) or 0.25% of the 8 million homosexuals, estimated at 10% of the adult male population (*109, 111*). But conventional venereal diseases are on the rise in the U.S. (97), and there is no vaccine or drug against HIV. This is because AIDS is due to drug consumption rather than sexual activity.
3. Over 72% of American AIDS cases are 20- to 44-year-old males (52)—although no AIDS disease is male-specific (*50, 51*)—because males of this age group consume over 80% of all "hard" psychoactive and aphrodisiac drugs (*101, 103, 111, 115, 157, 158*).
4. Distinct AIDS diseases occur in distinct risk group—because they use distinct drugs (e.g., users of nitrites get Kaposi sarcoma, users of intravenous drugs get tuberculosis, and users of AZT get leukopenia and anemia).
5. Viral AIDS occurs, on average, 10 years after HIV infection (*51, 53, 54*), although infectious agents, being self-replicating toxins, typically strike within weeks or months after infection (*11, 12*).

Indeed, HIV is immunogenic, and may be mildly pathogenic in humans within weeks after infection and is then "effectively and rapidly limited" by antiviral immunity (*91, 92*). This is because HIV infection and AIDS are unrelated events. The duration and toxicity of drug consumption and individual thresholds for disease determine when AIDS occurs, irrespective of when and whether HIV infects.

6. HIV, as well as many other parenterally and venereally transmitted microbes and viruses, are mere markers for AIDS and AIDS risks (*54, 107, 159*)—because the higher the consumption of unsterile, injected drugs (*140, 151*) and sexual contacts mediated by aphrodisiac drugs, the more microbes are accumulated.
7. Some old diseases of hemophiliacs, other recipients of transfusions, and the general American population are called AIDS—if they coincide with perinatal or parenteral HIV infection (*54*).
8. Old African diseases such as slim disease, fever, diarrhea, and tuberculosis are called AIDS now, although they are clinically and epidemiologically very different from American AIDS. They occur in adolescents and adults of both sexes that are subject to protein malnutrition, parasitic infections, and poor sanitary conditions (53). Only because HIV is endemic in over 10% of Central Africans are over 10% of old African diseases now called AIDS (*51, 53, 54*).

The drug–AIDS hypothesis predicts that the AIDS diseases of the behavioral AIDS-risk groups in the U.S. and Europe can be prevented by controlling the consumption of recreational and anti-HIV drugs, but not by "safe sex" (*51*) and "clean injection equipment" (55) for unsterile(!) street drugs. According to the drug—AIDS hypothesis, AZT is AIDS by prescription. Screening of blood for antibodies to HIV is superfluous, if not harmful, in view of the anxiety that a positive test generates among the many believers in the virus–AIDS hypothesis and the toxic AZT prophylaxis, prescribed to many who test "positive." Eliminating the test would also reduce the cost of the approximately 12 million annual blood donations in the U.S. (82) by $11 each (Irwin Memorial Blood Bank, personal communication, 1990) and would lift travel restrictions for antibody-positives to many countries, including the U.S. and China. The drug–AIDS hypothesis is testable epidemiologically and experimentally by studying AIDS drugs in animals.

B. Hepatitis C Virus and Non-A Non-B Hepatitis

Non-A non-B hepatitis is observed primarily in recipients of transfusions and in intravenous drug users (*3, 12, 160*). It has been postulated to be a viral disease because inoculation of plasma or serum (3–75 ml) from hepatitis patients into chimpanzees induced some biochemical markers of hepatitis, such as alanine aminotransferase, in half of the animals (*160*). However, none of the animals developed hepatitis (*161, 162*). Trace amounts of presumably viral RNA have recently been detected in the liver of hepatitis patients. In addition, "non-neutralizing" antibodies to "nonstructural epitopes," from an apparently latent RNA virus, have been identified mostly in asymptomatic carriers (*160*). Cloning and sequencing indicated that the RNA is directly coding and measures about 10 kb. Therefore, the suspected virus has been tentatively classified as a togavirus (*160*). Viral RNA was only detectable after amplification with the PCR in 9 out of 15 non-A non-B hepatitis patients, and non-neutralizing antibodies were found in only 7 of the 9 RNA-positive and in 3 of the 6 RNA-negative patients (*163*). Likewise, liver tissues from chimpanzees inoculated with sera from hepatitis patients contain only one viral RNA molecule per ten cells (*160*).

In view of this evidence, the putative virus has been termed hepatitis C virus (HCV) to indicate that it is the cause of the hepatitis. Subsequently, the Food and Drug Administration has recommended, and the American Association of Blood Banks has mandated, as of 1990, the testing of the approximately 12 million annual blood donations in the U.S. (*82*) for antibodies to HCV at an approximate cost of $5 per test. The test was developed by Chiron Co., Emeryville, California (Irwin Memorial Blood Bank, personal communication, August 15, 1991).

However, several arguments cast doubt on the hypothesis that HCV causes hepatitis:

1. Virus-containing sera or plasma from hepatitis patients does not cause hepatitis if inoculated into chimpanzees, indicating that HCV is not sufficient to cause the disease. Moreover, since the virus has not been propagated in culture and isolated in a pure form, the possibility exists that the biochemical markers of hepatitis that are observed in chimpanzees inoculated with plasma were induced by another agent. Thus, HCV is not likely to be a sufficient cause of hepatitis in humans.
2. The presence of HCV in asymptomatic subjects at the same

concentration and activity as in hepatitis patients also indicates that the virus is not sufficient to cause hepatitis.

3. The absence of viral RNA in 6 out of 15 hepatitis C patients indicates that the virus is not necessary for the disease.

It appears that HCV either causes disease by unprecedented mechanisms with as little as one RNA molecule per 10 liver cells in some and even less in other carriers, or that the virus is not the cause of non-A non-B hepatitis. By contrast, the concentration of viral RNAs made by conventional pathogenic viruses, including togaviruses, ranges from 10^3 to over 10^4 per cell (*10*). Therefore, it seems plausible that a latent passenger virus was identified that survives by establishing chronic asymptomatic infections at very low, nonpathogenic titers (*164*).

C. Measles Virus, HIV, and Subacute Scleroting Panencephalitis

In 1967, a cytocidal measles virus was proposed to be the cause of a very rare, subacute scleroting panencephalitis of children (*165*), based on correlations with antibodies to the virus or trace amounts of virus (3, *10*, *12*). The encephalitis is observed only 1–10 years after an acute primary infection, in the face of antiviral immunity, and in only about 1 out of 1 million children infected by the virus (3, *10*, *12*). The virus can only be isolated from the brains of 2 out of 8 encephalitis patients after cocultivation of brain cells with susceptible human cells (*166*). Thus, only a few intact virus particles are present in the brains of some, but apparently not in all, children with encephalitis. Viral gene expression in brain autopsies is 10- to 200-fold lower than in virus-replicating control cells, amounting to as few as 10 mRNAs per cell (*167*). Moreover, mutations and deletions were observed in these viral RNAs compared to wild-type measles virus (*168*). Accordingly, some viral RNAs are not even translated (3). By contrast, the wild-type virus causes measles within weeks after infection, at very high virus titers, and prior to antiviral immunity (*10*, *12*).

The measles virus–encephalitis hypothesis has a number of epidemiological and virological shortcomings:

1. Since the disease does not occur concurrently with, or instead of, the conventional measles disease during a primary infection, and since antiviral immunity does not protect against the disease, measles virus cannot be sufficient to cause the subacute panencephalitis.
2. The virus cannot be a sufficient cause of the disease because only 1 in 10^6 infected persons develops panencephalitis, com-

pared to one in a few if not all who develop measles disease before antiviral immunity (*3, 10, 12*).

3. Since viruses are self-replicating toxins, all are potentially "fast" pathogens, but encephalitis is observed only 1–10 years after infection, measles virus cannot be sufficient for panencephalitis.
4. The absence of infectious virus in some panencephalitis cases, and the very low concentration of viral RNA in all cases, suggest that measles virus is either not causative, or is causative by a mechanism that is totally different from that causing measles disease. During conventional measles disease, the virus is abundant, making over 1000 RNA molecules per cell in large numbers of cells (*3, 10, 12, 167, 168*).

In view of these paradoxes, it was suggested that selection of viral mutants would account for the encephalitis-pathogenicity of the virus (*3, 167, 168*). However, this seems unlikely, because the virus does not replicate sufficiently in encephalitis patients to generate new pathogenic variants, and because natural variants with a neurotropic specificity would then be expected.

About 15 years after the measles virus–encephalitis hypothesis was advanced, others proposed that the encephalitis was caused by a latent retrovirus closely related to HIV (*169*). This hypothesis also suffers from the problem that the presumed viral pathogen is latent (*169*). In addition, an encephalopathy is hard to reconcile with the fact that retroviruses depend on mitosis for infection (*170*) and the fact that neurons stop dividing soon after birth (*1*).

D. Phantom Viruses and Neurological Disease

The strong belief in viruses as causes of diseases has in some instances even exceeded their very definition. For example, the Nobel Prize in 1976 was given for hypothetical, slow, and unconventional viruses that would cause neurological diseases such as kuru, Creutzfeld–Jacob's, and Alzheimer's diseases, after long latent periods of up to 30 years (*171*). Kuru is a now-extinct neurological disease of a small tribe of 35,000 in New Guinea that reportedly was transmitted by ritual cannibalism (*3, 12, 171*). "Slow and unconventional" viruses have been postulated because 4 out of 7 chimpanzees had developed neurological diseases about 1–2 years after they had been inoculated intracerebrally with brain suspensions from kuru patients (*172*). The presumed Creutzfeld–Jacob virus failed to induce neurological disease if presumably infected materials were inoculated into the brains of chimpanzees (*3*). A slow, unconventional virus has also

been claimed as the cause of scrapie, a neurological disease of sheep (*3, 12*).

Since the incubation periods from inoculation of brain suspensions from kuru patients to neurological disease in the animals (1–2 years) and from presumed infection of humans to kuru (up to 30 years) differ significantly, it is not clear whether the diseases were caused by the same agent. Considering the claim that the viruses are naturally transmitted by cannibalism, it seems inappropriate that the traumatic intracerebral inoculation was chosen to test the oral transmission hypothesis. Nevertheless, Gajdusek *et al.* pointed out, "To anyone who had the opportunity of observing the unique syndrome of kuru . . . the similarity of its clinical picture and course to the experimentally induced syndrome . . . is dramatically evident" (*172*).

The slow virus–neurological disease hypothesis suffers from several shortcomings:

1. None of these hypothetical viruses has ever been isolated and chemically analyzed. Their presumed properties all far exceed the known ranges of conventional viruses and even of known proteins and nucleic acids. For example, the kuru and Creutzfeld–Jacob viruses are said to resist boiling water, ionizing gamma radiation, ultraviolet radiation, and inactivation with formaldehyde (*3, 171*). Moreover, the viruses are not antigenic, and not visible under the electron microscope, although available preparations are reported to have titers of 10^7 lethal doses per milliliter (*3*). Paradoxically, the slow, unconventional viruses have since evolved into an infectious protein, termed prion, "derived from a normal cellular protein . . . through an unknown posttranslational process" (*173*).
2. The virus–kuru hypothesis fails to account for the long latent periods between presumed infection and disease and for the restriction of the disease to a very specific risk group.
3. A recent analysis of the original data on kuru transmission casts doubt on the virus–kuru hypothesis, because the evidence for cannibalism was fabricated (*174*).

In view of this, we agree with a review by Gibbs, a collaborator of Gajdusek, that "many paradoxes [were] thrust on us by the discovery of these unconventional viruses as the etiological agents of chronic, progressive, degenerative diseases of the central nervous system . . . " and that "toxic or genetic determinants and even trauma lead to the same pathogenesis . . . " (*3*). Indeed, it seems plausible that the toxic-

ity and trauma of intracerebral inoculations of human brain suspensions from kuru patients could cause neurological diseases without phantom viruses said to be the etiological agents. The restriction of the slow neurological diseases to specific ethnic groups or to sporadic cases could reflect genetic and acquired deficiencies rather than selective and slow viruses.

III. Viruses as Causes of Clonal Cancer

A. Human T-cell Leukemia Virus and Adult T-cell Leukemia

Human T-cell leukemia virus-I (HTLV-I) was originally discovered in a T-cell line from a leukemic patient (*175*). This line, termed HUT 102, only produced virus after it had been propagated *in vitro*, in the absence of the virus-suppressing immune system of the host, and after it had been treated with mitogens and mutagens such as iododeoxyuridine, an agent known to activate dormant retroviruses (*6*). Since the virus was isolated from a cell line that came from an adult patient with T-cell leukemia, the virus was proposed to be the cause of adult T-cell leukemia (ATL), and hence named human T-cell leukemia virus (*175, 176*). However, a parallel T-cell line, termed HUT 78, derived from another patient with T-cell leukemia, failed to yield a retrovirus (*87*).

Further support for the hypothesis was derived from epidemiological correlations between antibodies to HTLV-I and ATL in Japan and the U.S. (*3, 37, 176*). Based on 30,000 blood donations, the American Red Cross has reported that in 1986–1987 about 0.025% or 65,000 Americans were infected with HTLV-I (*3, 82*), but the American T-cell Leukemia/Lymphoma Registry had recorded in 1990 in the U.S. no more than 90 ATLs. Among these, 75 were non-Caucasians (*177*), a group in which HTLV-I is often endemic (*178*). However, the same Registry also reports, "although most cases of ATL are HTLV-I-associated . . . many are not" (*177*). As in the U.S., HTLV-I-free ATLs have been observed in Japan (*179*). A controlled study comparing the incidence of the leukemia in HTLV-I-positive and -negative control groups has never been published.

By definition, "The diagnosis of ATL is made from the characteristic clinical findings, the detection of serum antibodies to HTLV-I and, when necessary, the confirmation of monoclonal integration of HTLV-I proviral DNA in cellular DNA of ATL cells" (*180*). According to this tautology, ATL is defined and distinguished from virus-free T-cell leukemias solely by the presence of antibody to HTLV-I or viral DNA.

In addition, HTLV-I is also postulated to cause an HTLV-I-associated myelopathy (HAM), which is a neurological disease also defined only by the presence of HTLV-I (*3, 181*).

ATL is clonal, originating from a single cell, like virus-free T-cell leukemias. The clonality of the leukemia is defined by chromosome abnormalities, as well as by clonal proviral integration sites (*2, 176*). However, there are no specific integration sites of HTLV-I in different leukemias (*2*). In leukemic cells, the virus is always latent, suppressed by antiviral immunity, and sometimes even defective (*2*). It is for this reason that the virus was originally discovered only *in vitro,* after reactivation from latently infected leukemic cells grown in culture.

HTLV-I, like other non-oncogenic retroviruses (*6, 54*), is naturally transmitted from mother to child with an efficiency of 22% based on testing for antiviral antibodies (*176, 182, 183*). Indeed, latent proviruses appear to be transmitted perinatally at a higher efficiency than antibody tests indicate, because the antibody titers increase with age (*176*) at a much faster rate than could be accounted for even by thousands of sexual contacts (*183*). Thus, this virus, like all other retroviruses without oncogenes (*54*), survives from perinatal transmission. Sex is another, although highly inefficient, mode of transmission, depending on an average of over 1000 sexual contacts (*183*).

Based on epidemiological studies from Japan, HTLV-I is said to cause leukemia in only 1–5% of all virus carriers in a lifetime (*182*). The annual incidence of the leukemia per HTLV-I carrier in Japan is estimated to be only 1 in 1000 (*182, 184*). Since HTLV-I is a perinatally transmitted retrovirus, but leukemia typically appears, if at all, only in 50- to 60-year-olds, the latent period from infection to disease is estimated at 55 years (*176, 185*).

The following epidemiological and virological arguments cast doubt on the HTLV-I–leukemia hypothesis:

1. According to the American Red Cross, "ATL . . . as of September 1989, has not been reported in association with transfusion transmitted HTLV-I infection," although about 65,000 Americans were infected with HTLV-I and about 12 million blood donations are annually transfused to millions of recipients in the U.S. (*82*). Thus, HTLV-I cannot be sufficient to cause leukemia.
2. Since viruses, as self-replicating toxins, are all potentially fast pathogens, but leukemia is only observed about 55 years after infection, HTLV-I cannot be sufficient for leukemia.
3. Considering that 1% of HTLV-I carriers develop ATL per lifetime in Japan and about 0.1% (90 : 65,000) in the U.S., that the

leukemias are clonal deriving from single cells, and that each carrier must contain at least 10^7 latently infected T-cells (because the limit of provirus detection by hybridization is 1 in 1000 cells) and that humans contain 10^{10} to 10^{11} T-cells that go through at least 420 generations in a 70-year lifetime (see Section IV) (*37, 186*), then only 1 out of 10^2 (Japan) to 10^3 (U.S.) $\times$ $10^7 \times 420 = 10^{12}$ infected T-cells become leukemic. Thus, HTLV-I cannot be sufficient for leukemogenesis.

4. Antiviral antibodies that completely neutralize HTLV-I to virtually undetectable levels (2) do not protect against the leukemia. This also indicates that HTLV-I is not sufficient for leukemogenesis.
5. Retroviruses cause either polyclonal tumors via dominant, biochemically active oncogenes (*6, 37*), or possibly clonal tumors via site-specific integration that generates active virus-cell hybrid oncogenes (*31, 40, 42*). Yet HTLV-I neither expresses a leukemia-specific gene product that could function as an active oncogene, nor does it integrate at a specific site in different "viral leukemias" (*2, 187*). Thus, HTLV-I cannot be sufficient for leukemogenesis.
6. The statement of the American T-cell Leukemia/Lymphoma Registry that "although most cases of ATL are HTLV-I associated . . . many are not" (*177*) and the reports of virus-negative leukemias from Japan (*179*) and other countries (2) indicate that HTLV-I is not even necessary for the disease.
7. The HTLV-I–leukemia hypothesis fails to explain the clonal chromosome abnormalities that are consistently found in all ATLs (*2, 188*)—except if one makes the additional odd assumption that HTLV-I only transforms cells with a preexisting chromosome abnormality.

Thus, there are no virus-determined diagnostic criteria, besides the presence of antiviral antibodies, nor are there any controlled epidemiological and virological criteria to support the hypothesis that HTLV-I is the cause of ATL. Therefore, *ad hoc* hypotheses have been advanced proposing "a second oncogenic event, such as a chance translocation or a second oncogenic virus . . ." for viral leukemogenesis (*187*). Others estimate five steps in leukemogenesis, of which HTLV-I is postulated to be an "initiator" (*185*).

Since not even one transfusion-transmitted leukemia case has been recorded in the U.S., it seems surprising that a blood test for antibodies against HTLV-I has become mandatory for members of the American

Association of Blood Banks since February 1989. It raises the cost of each of the approximately 12 million annual blood donations in the U.S. (82) by $5–11 (*189;* Irwin Memorial Blood Bank, personal communication, 1990). Indeed, an HTLV-I epidemiologist pointed out, "Ironically, this route of [HTLV-I] transmission is numerically the least important," considering the 55-year average latent period from infection to leukemia, "the advanced age of most U.S. blood recipients, and the observation that as many as 60% of transfusion recipients may die within approximately 3 years of transfusion because of their underlying disease" (*183*). Nevertheless, in terms of blood testing expenses, HTLV-I has reached cost-parity with HIV, which adds another $11 test fee to each blood donation (Irwin Memorial Blood Bank, personal communication, 1990).

An alternative hypothesis suggests that spontaneous or perhaps radiation-induced chromosome abnormalities induce the clonal leukemias (see Section VI). Nuclear radiation from the Hiroshima and Nagasaki bombs is blamed for 147 leukemias (*190*). By proposing that one out of billions of normal HTLV-I-infected cells is transformed by a spontaneous chromosome abnormality, our hypothesis readily resolves the paradox of the clonal chromosome abnormalities in all "viral" leukemias.

B. Herpes Virus, Papilloma Viruses, and Cervical Cancer

Inspired by the SV40/adenovirus–cancer models, infection by herpes simplex virus (HSV) was postulated in the 1970s to be the cause of cervical cancer based on epidemiological correlations with HSV DNA (3). The virus is sexually transmitted and is latent in about 85% of the adult population of the U.S. (3). Infection by intact HSV typically kills the cell. However, defective and intact viruses that become latent do not kill cells (3).

The viral DNAs in cervical cancers are defective and integrated with cell DNA. Cervical cancers with defective HSV DNA are clonal, just like virus-free cancers (*191–194*). In agreement with the SV40/adenovirus models, HSV does not replicate in the tumors. But, unlike the SV40/adenovirus models, no set of viral genes is consistently present or expressed in human cervical cancers. Therefore, the "hit-and-run" mechanism of viral carcinogenesis was proposed (*195*). It holds that neither the complete HSV, nor even a part of it, needs to be present in the tumor. Obviously, this is an unfalsifiable, but also an unprovable, hypothesis.

Also inspired by the SV40/adenovirus models, and based on epidemiological correlations, infection by human papilloma virus (HPV)

was postulated in the 1980s by zur Hausen to be a causative factor in cervical and anogenital cancers (*3, 191, 196*).

Papilloma viruses are transmitted by sexual and other contacts, like the herpes viruses, and are widespread or "ubiquitous" in at least 50% of the adult population of the U.S. and Europe (*3, 191*). For example, using the PCR to amplify sequences of one particular strain of papilloma virus, 46% of 467 women in Berkeley, California, with a median age of 22 were found to carry HPV, but none of them had cervical cancer (*199*). Many other strains of HPV exist (*3, 191*) that could not be detected with the assay used in this study (*199*). Like the SV40/adenovirus models, HPV does not replicate in the tumors. But, unlike these models, HPV naturally replicates nonlytically (*13*), forming polyclonal warts with unintegrated viral DNA plasmids (*200*).

zur Hausen reports that cervical cancers occur in less than 3% of infected women in their lifetime, but the incidence in HPV-free controls was not reported (*191*). In the U.S., the incidence of cervical cancer in all women, with and without HPV, per 70-year lifetime is about 1% (*197*). In a controlled study of age-matched women, 67% of those with cervical cancer and 43% of those without were found to be HPV-positive (*198*). These cancers are observed on average only 20–50 years after infection (*191*).

Different sets and amounts of viral DNA are integrated into cell DNA of different carcinomas (*191*), and viral DNA is poorly expressed in some cancers and not expressed at all in others (*3, 191, 201*). Moreover, different HPV strains are found in different cancers (*3, 191, 196*). Viral antigens are found in only 1–5% of carcinomas (*3*). Accordingly, HPV does not replicate in the cancer cells and there are no reports of HPV-specific histological or physiological markers that set HPV DNA-positive apart from negative carcinomas (*191*). There is also no virus-specific integration site in HPV DNA-positive cancers (*191*), indicating that no specific cellular gene is activated, or that a tumor suppressor gene is inactivated by integration of viral DNA. HPV DNA-positive tumors are clonal and carry clonal chromosome abnormalities, just like virus-negative tumors (*191–194*).

The HPV–cancer hypothesis of zur Hausen proposes that HPV encodes a "transforming factor" that is suppressed in normal cells by a cellular interference factor (CIF). Inactivation of both CIF alleles by mutation is postulated to result in viral carcinogenesis (*191*). The low probability of developing mutations in both suppressor alleles is said to explain the long intervals between infection and cancer. This hypothesis correctly predicts that only a small fraction of infections lead to cancer. It further predicts clonal tumors with active HPV DNA and

mutations in both alleles of the suppressor genes, and it predicts no effects on the karyotypes of cells.

Howley *et al.* proposed that a viral protein neutralizes the proteins of the retinoblastoma and p53 tumor suppressor genes, and that neutralization of these suppressor proteins causes cancer (*202*). The proposal is modeled after the hypothesis that retinoblastoma is caused by a cellular cancer gene, provided that a complementary suppressor gene, termed the retinoblastoma gene, is inactivated (see Section IV). This hypothesis predicts polyclonal tumors.

The following epidemiological and biochemical arguments cast doubt on these HPV–cancer hypotheses:

1. Random allelic mutation of suppressor genes, as postulated by zur Hausen, predicts a few cancers soon, and more long after infection. Since cancers only appear 20–50 years after infection, cooperation between HPV and mutations cannot be sufficient for carcinogenesis.
2. Further, the proposal of zur Hausen that inactivation of host suppressor genes is necessary for viral transformation is not compatible with HPV survival. Since HPV, like all small DNA viruses, needs all of its 8-kb DNA for virus replication (*13*), suppression of one or more HPV proteins by normal cellular genes would effectively inhibit virus replication in all normal cells. Conversely, if viral transforming proteins were not suppressed by normal cells, virus-replicating wart cells should be tumorigenic because all viral genes are highly expressed in virus replication (*1*, *13*, *191*).
3. The clonality of cervical cancers rules out the Howley hypothesis.
4. The lack of a consistent HPV DNA sequence and of consistent HPV gene expression in HPV DNA-positive tumors is inconsistent with the zur Hausen and Howley hypotheses and indicates that HPV is not necessary to maintain cervical cancer.
5. The presence of HPV in no more than 67% of age-matched women with cervical cancer (*198*) also indicates that HPV is not necessary for cervical cancer.
6. The hypothesis also fails to explain the presence of clonal chromosome abnormalities consistently seen in cervical cancer (*16*, *192–194*)—except if one makes the additional odd assumption that only cells with preexisting chromosome abnormalities are transformed by HPV.

It follows that neither HPV nor HSV plays a direct role in cervical carcinomagenesis. Moreover, the HPV–cancer hypothesis offers no explanation for the absence of a reciprocal venereal male carcinoma.

Thus, detecting inactive and defective viral DNA from past infections in non-tumorigenic cells with a commercial hybridization test (Vira/Pap, Digene Diagnostics, Silver Spring, Maryland) or with the PCR (*199*) seems worthless as a predictor of rare carcinomas appearing decades later, in view of the "ubiquity" (*191*) of these viruses in women and the total lack of evidence that cervical cancer occurs in women with HPV more often than in those without. This test, at $30–150, is currently recommended for the 7 million Pap smears that appear "atypical" in the U.S. per year (Digene Diagnostics, personal communication, 1991). By contrast only 13,000 cervical cancers are observed annually in both HPV-positive and -negative women in the U.S. (*197*). Indeed, the test may be harmful, considering the anxiety a positive result induces in believers of the virus–cancer hypothesis.

An alternative cervical carcinoma hypothesis suggests that rare spontaneous or chemically induced chromosome abnormalities, which are consistently observed in both HPV and HSV DNA-negative and -positive cervical cancers (*192–194*), induce cervical cancer. For example, smoking has been identified as a cervical cancer risk (*204*). The controlled study of age-matched women described above suggests that 52% of the women with cervical cancer were smokers compared to only 27% of those without (*198*). Indeed, carcinogens may be primary inducers of abnormal cell proliferation rather than HPV or HSV. Since proliferating cells would be more susceptible to infection than resting cells, the viruses would be just indicators, rather than causes of abnormal proliferation. Activation of latent retroviruses like HTLV-I (Section III,A) (*2*), herpes viruses (*12*), and lambda phages (*205*) by chemical or radiation-induced cell damage and subsequent proliferation are classical examples of such indicators. Indeed, Rous first demonstrated that the virus indicates hydrocarbon-induced papillomas; it ". . . localized in these and urged them on . . . " and suggested that enhanced proliferation is a risk factor for carcinogenesis (*203*).

According to this hypothesis, HPV or HSV DNAs in tumor cells reflect defective and latent viral genomes accidentally integrated into normal or hyperplastic cells, from which the tumor is derived. This hypothesis readily reconciles the clonal chromosome abnormalities with the clonal viral DNA insertions of the "viral" carcinomas. The inactive and defective viral DNA in the carcinomas would be a fossil record of a prior infection that was irrelevant to carcinogenesis.

C. Hepatitis B Virus and Liver Carcinoma

Epidemiological evidence indicates that chronic hepatitis B virus (HBV) carriers in Asia have a 250-fold higher risk of developing hepatomas than do non-carriers (*3, 12, 206–208*). The virus is typically transmitted perinatally in Asia and Africa (*3, 207*). In over 95% of infections in Asia and 99.9% in the U.S. and Europe the virus is completely neutralized by antiviral immunity. In people with drug- or disease-induced immunodeficiencies the virus remains chronically active (*12*). Approximately 1 out of 70 chronic HBV carriers in Asia develop clonal hepatomas and 1 out of 300 develop liver cirrhosis in their lifetime (*3, 207*). However, the liver tumors appear only in 30- to 60-year-olds. Moreover, chronic HBV carriers in Asia are "more likely" to develop hepatomas than those in Europe and the U.S. (*12*). Inoculation of HBV into chimpanzees has failed to cause hepatomas (*3*).

The virus is thought not to kill infected cells and viral DNA is replicated as a plasmid and thus not typically integrated into the host DNA (*3, 12*). However, molecular studies have detected clonal inserts of HBV DNA randomly integrated into the cellular DNA of liver carcinoma tissues (*196, 209*). Viral DNA is defective and not replicated in HBV DNA-positive hepatomas (*209*), like SV40 and adenovirus DNAs in the corresponding viral tumors. By contrast to the SV40/adenovirus models, no subset of viral DNA is consistently found or expressed in HBV-positive tumors (*209, 210*). Only 11–19% of tumors in HBV-positive patients express some viral antigens, compared to 26–61% expressing them in surrounding non-tumorous tissues (*211*). In addition to clonal inserts of HBV DNA, the hepatomas carry clonal chromosome abnormalities (*16, 193, 196*).

On the basis of these data, it has been proposed that HBV causes liver carcinoma in a step-wise process that begins with antigenemia, followed by chronic hepatitis, cirrhosis, and cancer (*3, 207, 209*). However, cirrhosis is not a necessary precursor of a hypatoma (*3*).

The following epidemiological and biochemical arguments cast doubt on the HBV–hepatoma hypothesis:

1. The long intervals of 30 –60 years between infection and hepatomas indicate that HBV is not sufficient to initiate carcinogenesis.
2. The evidence that HBV is naturally transmitted perinatally also indicates that the virus is not sufficient to cause fatal diseases such as cirrhosis and hepatomas, because the viruses that depend on perinatal transmission for survival are not inherently pathogenic.

3. The evidence that the hepatoma risk among chronic HBV carriers in Asia is higher than in the U.S. and Europe also indicates that HBV is not sufficient for carcinogenesis.
4. The clonality of the HBV-positive hepatomas further indicates that HBV is not sufficient for carcinogenesis, because only one out of billions of chronically infected liver cells becomes tumorigenic.
5. The absence of an HBV-specific tumor marker, and of a specific HBV DNA sequence or integration site in viral hepatomas, both indicate that HBV is not necessary to maintain hepatomas.
6. The HBV–hepatoma hypothesis fails to explain the clonal chromosome abnormalities of hepatomas—except if one makes the additional odd assumption that HBV only transforms cells with preexisting chromosome abnormalities.

Thus, there is no convincing evidence that HBV DNA is functionally relevant for the initiation and maintenance of hepatomas. Its presence in the tumor could merely reflect that the tumor had originated from one of probably many liver cells of HBV carriers that contain defective, biochemically inactive viral DNA integrated randomly into their chromosomes (*196*). Therefore, molecular analysis of HBV DNA and of HBV DNA integration sites (*210*) is not likely to illuminate carcinogenesis.

However, chronically replicating HBV may function as an indirect carcinogen in the form of a long-term source of intoxication, inducing necrosis and tissue regeneration, a known risk factor for carcinogenesis (*1, 196, 203*). This view is consistent with the higher-than-normal incidence of hepatomas in persons with chronic HBV infection.

A competing hypothesis suggests that chronic HBV infection may only be an indicator of a chronic nonviral intoxication and immunodeficiency. Indeed, nonviral factors are involved in hepatomagenesis because the incidence of the hepatomas per HBV carrier varies with different countries (*12*). Intoxication could induce tissue regeneration and immune suppression, a classical precondition for opportunistic virus infections (see HPV in Section III,B). According to this view, the hepatoma would be caused by a rare virus-independent mechanism that generates chromosome abnormalities in one of many normal cells with HBV DNA inserts. This hypothesis would readily resolve the presence of the clonal chromosome abnormalities in all "viral" hepatomas. The defective and inactive viral DNAs in the hepatomas would be a fossil record of a prior infection that was irrelevant to carcinogenesis.

D. Epstein–Barr Virus and Burkitt's Lymphoma

In the early 1960s, Burkitt suggested that a B-cell tumor, now called Burkitt's lymphoma, which occurs in 1 out of 10,000 Central African children per year between 4 and 14 years of age, was caused by a virus (*3, 12*). Although not detectable in biopsies of lymphoma patients, a virus was found with the electron microscope in lymphoma cells grown in culture away from the suppressive immune system of the host (*212*). The Epstein–Barr virus (EBV) has since been postulated to be the cause of Burkitt's lymphoma (*3, 8, 12*).

In Central Africa, infection with the virus occurs perinatally in the first months of life in almost 100% of the population (*3, 207*). In the U.S. and Europe, infection occurs typically during or after puberty in about 50% of healthy adults (*3, 213*). However, the incidence of lymphomas with EBV in these countries is only less than 1 in 10^6 per year (*3*). Moreover, only 30% of otherwise indistinguishable lymphomas express EBV antigens (*3*). In America, Burkitt's lymphomas free of EBV DNA were described in 1973 (*214*). In China, EBV is also said to cause nasopharyngeal carcinoma in adults (*1, 3*).

During a primary infection, the virus may induce transient, polyclonal lymphoproliferative diseases, such as mononucleosis, if a large percentage of lymphocytes are infected prior to immunity. After antiviral immunity is established, the virus remains chronically associated with the host in a latent form (*3, 12*). During the chronic state of infection, viral DNA is detectable with the PCR in about 1 out of 10^5 lymphocytes (*213*) and viral antigens in only about 1 out of 10^7 lymphocytes (*12*).

In lymphomas, the virus is also suppressed, producing but a few viral antigens (*3*), as the history of its discovery had first indicated. Burkitt's lymphomas are clonal, deriving from single cells that carry characteristic chromosome translocations that often rearrange the proto-*myc* gene (see Section IV). Since EBV, like other herpes viruses, generally does not integrate into the host chromosome (*1, 3*), the time of infection of tumor cells (e.g., whether infection occured before, during, or after tumorigenesis) cannot be determined.

The EBV–lymphoma hypothesis suffers from numerous epidemiological and biochemical inconsistencies:

1. The clonality of the lymphomas that emerge from a single tumorigenic cell among billions of non-tumorigenic EBV-infected cells indicates that EBV is not sufficient for tumorigenesis.
2. The long intervals between infection and carcinogenesis, aver-

aging 10 years in Africa, and the incidence of only 1 lymphoma per 10,000 infected persons also indicate that EBV is not sufficient to initiate tumorigenesis.

3. The lymphoma incidence varies over 100-fold between African and European or American EBV carriers, also indicating that EBV cannot be sufficient to cause a lymphoma.
4. The lack of a lymphoma-specific EBV function in symptomatic carriers indicates that EBV is not necessary to maintain lymphomas.
5. The existence of EBV-free Burkitt's lymphomas in American and European patients indicates most directly that EBV is not even necessary for Burkitt's lymphoma.

Thus, EBV appears neither necessary nor sufficient for lymphomagenesis. Nevertheless, it has been argued that EBV plays at least an indirect role in lymphomagenesis, because only a minority of susceptible cells from EBV-positive patients are infected *in vivo*, but virtually all lymphoma cell lines in culture are infected by the virus (*215*, *216*). However, this could be an artifact of studying cells in culture, because the virus would spread unimpaired by immunity from a few infected, normal, or lymphoma cells to all lymphoma cells that survive in culture.

Since about 100% of the Central African and 30–50% of the American population carries latent EBV, and since EBV-negative Burkitt's lymphomas exist, it is likely that the correlations between EBV and tumors are accidental rather than causal. In view of this, an alternative hypothesis has been advanced, which holds that altered cellular proto-*myc* genes are the cause of Burkitt's lymphoma (see Section IV).

IV. Mutated Oncogenes, Anti-oncogenes, and Cancer

A. Mutated Proto-*myc* Genes and Burkitt's Lymphoma

The transforming gene of the directly oncogenic avian carcinoma virus MC29 contains a specific coding region, now termed *myc* (*217*), derived from a cellular gene termed proto-*myc* (*218*). Thus, the viral *myc* gene is a genetic hybrid that consists of a strong retroviral promoter linked to a coding region that is a hybrid of virus- and proto-*myc*-derived sequences (*219*). This viral *myc* gene, like synthetic hybrids in which the native proto-*myc* promoter is replaced with that of a retrovirus (*40*, *42*), is expressed to about 100-fold higher levels in all virus-

transformed cells *in vitro* and in viral tumors than the cellular proto-*myc* genes (*220–222*).

The cellular proto-*myc* gene, located on chromosome 8, is rearranged with immunoglobulin genes from chromosomes 2, 14 and 22 in all (*29*) or most (*30*) cell lines derived from Burkitt's lymphomas. However, direct cytogenetic studies show that chromosome 8 is rearranged in only about 50% of primary Burkitt's lymphomas (*223–226*). Analogous rearrangements have also been observed in the proto-*myc* genes of mouse plasmacytoma cell lines (*1, 8, 36*). The rearrangements do not alter the coding region of proto-*myc* genes. Most rearrangements link the proto-*myc* coding regions to genetic elements from cellular immunoglobulin genes in the opposite transcriptional orientation (*1, 8, 36*). Other rearrangements in Burkitt's lymphomas do not affect the location and structure of proto-*myc* on chromosome 8, but instead rearrange regions 3′ from proto-*myc* (*36, 227–232*). Because both retroviral *myc* genes and the rearranged proto-*myc* genes of most, but not all, Burkitt's lymphomas differ from normal proto-*myc* genes in truncations 5′ from the coding region, and because both were found in cancers, the viral and rearranged cellular *myc* genes were proposed to be equivalent oncogens (*6, 8, 29, 30*).

The transcriptional activity of the rearranged proto-*myc* genes in lymphomas is moderately enhanced, not altered, or even suppressed in Burkitt's lymphoma cells compared to normal proliferating cells (*5, 30, 36, 216, 227*). It is thus nearly 100-fold lower than that of viral *myc* genes or proto-*myc* genes artificially linked to retroviral promoters (*40, 42, 220–222, 233*).

Moreover, rearranged proto-*myc* genes from Burkitt's lymphomas do not transform any human or rodent cells upon transfection (*5, 36, 38*)—even if they are artificially linked to retroviral promoters (*234–236*). In efforts to develop a system that is more efficient than transfection for introducing mutated proto-*myc* genes into cells or animals, synthetic avian retroviruses with the coding region of the human proto-*myc* gene were constructed (*233, 237*). Since these viruses transform avian cells, it was concluded that "ungoverned expression of the gene can contribute to the genesis of human tumors" (*237*). However, transformation of human cells was not demonstrated. Moreover, three independent studies report that murine cells cannot be transformed by authentic avian (*238*) and synthetic murine retroviruses with *myc* genes (*239, 240*), signaling a restricted transforming host range of *myc* genes.

Several arguments cast doubt on the hypothesis that rearranged

proto-*myc* genes of Burkitt's lymphomas are functionally equivalent to retroviral *myc* genes and thus oncogenic:

1. Rearranged proto-*myc* genes from Burkitt's lymphomas or mouse plasmacytomas lack transforming function in transfection assays, while retroviral *myc* genes and proto-*myc* genes driven by retroviral promoters are sufficient to transform at least avian primary embryo cells (*40, 42, 237*). This indicates that the proto-*myc* genes from lymphomas and viral *myc* genes are functionally not equivalent.
2. Since expression of rearranged proto-*myc* genes from lymphomas is either the same as, or similar to, that of normal proto-*myc* genes, and their coding regions are identical, rearranged proto-*myc* cannot be sufficient for lymphomagenesis. By contrast, viral *myc* genes are oncogenic, owing to a 100-fold higher level of *myc* expression.
3. Primary Burkitt's lymphomas with normal chromosome 8, and with rearrangements of chromosome 8 that occur 3′ from proto-*myc* and thus do not affect the structure and regulation of the proto-*myc* gene, indicate that proto-*myc* translocation is not necessary for Burkitt's lymphomas.

It follows that rearranged proto-*myc* genes of human and animal tumors are transcriptionally and functionally not equivalent to viral *myc* genes, and that they are not necessary for lymphomagenesis.

In view of this, the demonstration (*237*) that human proto-*myc* transforms avian cells after it had been converted artificially to a retroviral *myc* gene is not relevant to its hypothetical role in human tumors. This claim is all the more questionable because even retrovirus-promoted *myc* genes appear unable to transform non-avian cells. Instead, such experiments model the genesis of a viral *myc* gene from a retrovirus and a cellular proto-*myc* gene by rare illegitimate recombination (*37*). The critical step in this process is the substitution of the weak cellular promoter by the strong retroviral counterpart (*40, 42*).

Thus, there is currently only circumstantial evidence for the hypothesis that rearranged proto-*myc* genes play a role in Burkitt's lymphomas. This evidence includes the structural, but not functional, similarity to viral *myc* genes, and the approximately 50% incidence of chromosome-8 rearrangements with breakpoints near proto-*myc* in primary lymphomas. In view of this, rearranged proto-*myc* genes either may be involved in a mechanism of leukemogenesis that is not

analogous to the viral model, or they may not be involved at all. Since the incidence of chromosome-8 rearrangements is higher in lymphoma cell lines than in primary lymphomas, it has been pointed out that the rearrangement may favor lymphoma cell growth in culture (225).

In efforts to link the proto-*myc* rearrangements with a role in tumorigenesis, despite these discrepancies with the one-gene model, it was postulated that rearranged proto-*myc* genes may cooperate with other genes for carcinogenesis (236, 238, 241). To test these *ad hoc* hypotheses, transgenic mice were constructed that carry rearranged proto-*myc* genes linked to artificial promoters and hypothetical helper genes in every cell of their bodies. However, only some of these mice developed clonal tumors late in their lives (236). This indicates that even these combinations are not sufficient for carcinogenesis. Consequently, further helper genes were postulated (236, 241).

An alternative hypothesis suggests that the appearance of certain chromosome abnormalities is sufficient for lymphomagenesis. It is consistent with this proposal that cytogenic studies have identified chromosome abnormalities in all Burkitt's lymphomas, even in those that lack rearranged proto-*myc* genes (224–226). The reason that a high percentage of these rearrangements include proto-*myc* and immunoglobulin genes may be a consequence of the natural functions of these genes in B cells, namely generating antibody diversity in which proto-*myc* genes may play an active or passive role.

B. Rearranged Proto-*abl* Genes and Myelogenous Leukemia

Human myelogenous or granulocytic leukemia develops in two stages. The first is a chronic phase that may last, on average, 3–4 years. During this phase, immature myeloblasts are overproduced in the bone marrow and appear in the blood, but may differentiate into functional cells. This hyperplastic stage is followed by a terminal blast crisis of several months, during which non-functional leukemic cells emerge (242, 243). The leukemic cells of both the chronic and terminal stages in 85–90% of patients are marked by a reciprocal translocation between chromosomes 9 and 22. The rearranged chromosome 22 is termed the Philadelphia chromosome (193). In the remaining 10–15% of cases, chromosome 22 is rearranged with other chromosomes (193, 242–245). The reciprocal translocation between chromosomes 9 and 22 substitutes the 5′ end of the coding sequence of the proto-*abl* gene on chromosome 9 with a 5′ regulatory and coding element of a gene of unknown function, termed *bcr* (for breakpoint cluster region), from chromosome 22 (33, 246–248). The breakpoints with regard to the proto-*abl* gene vary over 200 kb (249), but those within *bcr*

fall in a range of 5.8 kb (*8*, *247*, *248*). The transcriptional activity of the proto-*abl* gene is virtually unaffected by the translocation (*8*, *246*).

The proto-*abl* gene is the cellular precursor of the transforming gene of the murine Abelson leukemia virus (*6*). This virus is sufficient to cause terminal myelogenous leukemia in susceptible mice within 3–5 weeks after infection (*250*, *251*). In this virus, the promoter and 5′ coding sequence of proto-*abl* are replaced by retroviral counterparts. Since the 5′ proto-*abl* coding regions are substituted by heterologous genetic elements in both the virus and the leukemias, it has been postulated that the structurally altered proto-*abl* gene of the leukemia is a cellular oncogene that is functionally equivalent to the transforming gene of Abelson virus (*7*, *8*, *246*, *252*). However, the Abelson virus or provirus (*253*), but not the DNA of human leukemic cells, is capable of transforming the mouse NIH 3T3 cell line *in vitro* (*8*).

The failure of the *bcr*–proto-*abl* hybrid genes to function like the virus could be a technical problem, because the hybrid genes may not be transfectable due to their large size of over 200 kb (*8*, *249*). Therefore, the transforming function of a cDNA transcribed from the 8.5-kb mRNA of the *bcr*–proto-*abl* was tested in murine retrovirus vectors. In such vectors, as in wild-type Abelson virus (*251*), the transcriptional activity of the *abl* gene is about 100 times that of normal or rearranged cellular proto-*abl* genes (*252*, *254*, *255*). One such recombinant virus induced proliferation of lymphoid mouse cells *in vitro* (*254*). Another induced clonal lymphomas when introduced into the germline of transgenic mice (*255*). Finally, a myelogenous leukemia was obtained by infecting bone marrow *in vitro* with the synthetic virus and transplanting this marrow into irradiated syngeneic mice (*252*). The leukemias appeared after relatively short latent periods of 9 weeks (*252*), almost as fast as those caused by the wild-type Abelson virus (*250*). The karyotype of this leukemia was not described (*252*).

Yet several observations cast doubt on the hypothesis that the rearranged proto-*abl* gene from human chronic myelogenous leukemias is functionally equivalent to the transforming gene of Abelson virus and that it is leukemogenic:

1. The transcriptional activity of the rearranged proto-*abl* gene in the leukemias is about 1% of that of wild-type Abelson virus and those of the synthetic recombinant viruses. Thus, mutated cellular proto-*abl* genes and viral *abl* genes are functionally not equivalent.
2. Given estimates that chromosome translocations occur sponta-

neously in human cells in 1 out of 10^2 to 10^4 mitoses (*37, 256, 257*), it can be calculated that a *brc*–proto-*abl* rearrangement would be much more probable than chronic myelogenous leukemia. The probablity that a random reciprocal rearrangement falls within the 200-kb 5′ region of proto-*abl* and the 5.8-kb 5′ region of *bcr* of the 10^6-kb human genome is $(200 : 10^6) \times (5.8 : 10^6)$ or 10^{-9}. Thus, 1 in 10^9 translocations would generate a Philadelphia chromosome. Considering that humans carry about 10^{10} to 10^{11} lymphocytes (*186*), which are replaced at least six times per year (53), or 420 times in an average lifetime of 70 years, a human life represents at least 10^{13} mitoses of lymphocytes. Making the conservative assumption that a translocation occurs in 1 out of 10^4 human mitoses (*256, 257*), about 10^9 ($10^{13} : 10^4$) lymphocytes with rearranged chromosomes are generated in a lifetime. Accordingly, every human should, by the age of 70, develop 1, and possibly 100, lymphocytes with a Philadelphia chromosome ($10^9 : 10^9$) and thus leukemia. However, chronic myelogenous leukemia is observed in only 1 (*242*) to 2.4 (*197, 258*) out of 100,000 per year or about 0.1% of people in a 70-year lifetime. Therefore, a rearranged proto-*abl* gene appears not to be sufficient for leukemogenesis.

3. Since in 10–15% of the chronic myelogenous leukemia cases proto-*abl* is not rearranged (*193, 244*), proto-*abl* mutation is not necessary for leukemogenesis. According to Nowell, "These variants appear to have no significance with respect to the clinical characteristics of the disease, and so it appears that it is the displacement of the sequence of chromosome 22 that is of major importance, rather than the site to which it goes" (*193*).

Thus, a rearranged proto-*abl* is functionally not equivalent to the transforming gene of Abelson virus. The rearrangement appears to be more probable than a leukemia, and is not even necessary for the leukemia. It is consistent with the first point that the proto-*abl* translocation is observed in the rather benign, early stage of chronic myelogenous leukemia, in which cells can differentiate into functional myeloblasts (*242, 243*), whereas the Abelson virus causes a terminal leukemia within several weeks.

Since the transcriptional activity of retroviral *abl* genes is about 100 times that of normal and rearranged proto-*abl* genes, and since it is not known whether even a viral *abl* gene can transform a human cell, the claims that "retrovirus-mediated expression of the *bcr*–proto-*abl* protein provides a murine model system for further analysis of the

disease" (252) are not realistic. These claims fail to take into consideration the 100-fold transcriptional discrepancy between the retroviral and cellular *abl* genes and the question of whether the transforming host range of *abl* genes includes human cells. Therefore, synthetic proto-*abl* viruses are just experimental reproductions of the rare spontaneous genesis of retroviral transforming genes from normal cellular genes and retroviruses. The critical step in this process is the recombination of the coding region of a proto-*onc* gene with a retroviral promoter (37).

It follows that the 85–90% incidence of proto-*abl* rearrangements in chronic myelogenous leukemia and the structural similarity of the gene to that of Abelson virus are the only evidence to suggest that proto-*abl* plays a role in human leukemogenesis. In view of this, proto-*abl* either must be involved in human leukemogenesis by a mechanism that is not analogous to that of the viral counterpart, or it may not be involved at all.

An alternative hypothesis suggests that alterations of the normal balance of chromosomes cause the leukemia. According to this hypothesis, the Philadelphia translocation would only affect the growth control of the cell. This is consistent with the rather normal function of cells with the translocation during the 3–4 years prior to the blast crisis. In one case, a person with a Philadelphia chromosome did not develop a leukemia for at least 7 years (P. H. Fitzgerald, personal communication, 1985) (245). Indeed, the blast crisis of myelogenous leukemia is accompanied by further chromosomal abnormalities, which are observed in leukemia with and without rearranged proto-*abl* genes (193, 244).

C. Point-mutated Proto-*ras* Genes and Cancer

Two laboratories have reported that transfection of the DNA of a human bladder carcinoma cell line transforms morphologically the mouse NIH 3T3 cell line (259, 260). Subsequent cloning proved the transforming DNA to be the coding region of the proto-*ras* gene, the same gene from which the coding region of the *ras* gene of the murine Harvey sarcoma virus is derived. Sequencing indicated that the 3T3-cell-transforming proto-*ras* from the bladder carcinoma cells differs from normal proto-*ras* in a point-mutation in codon 12 that changes the native Gly to Val (23, 26, 261).

Further transfection analyses with the 3T3-cell-transformation assay detected point-mutated proto-*ras* genes in less than 1% to about 20% of most common human tumors (1, 6, 36, 262) and in up to 40% of colon cancers (28, 263, 264). The proto-*ras* genes from these tumors

were each from a closely related group that includes the Harvey, Kirsten, and N-*ras* genes. Like the Harvey gene, the Kirsten proto-*ras* gene is named after a sarcomagenic murine retrovirus with a coding region of that gene (*6*). Regardless of point-mutation, proto-*ras* expression is enhanced 2- to 10-fold in about 50% of tumors compared to normal control tissues (*44, 262, 265, 266*). Transcription of normal proto-*ras* is also enhanced in normal proliferating cells (*36*), as, for example, 8-fold in regenerating rat liver cells (*267*).

1. The Original *ras*—Cancer Hypothesis Postulates a First-Order Mechanism of Transformation

The observations that point-mutated proto-*ras* genes from human and some animal tumors transform mouse 3T3 cells became the basis for the hypothesis that point-mutations of proto-*ras* genes cause cancer (*23, 26, 27*). The hypothesis derived additional support from the observation that the *ras* genes of Harvey and Kirsten sarcoma viruses also differ from normal proto-*ras* in point-mutations in codon 12 (*5, 39, 268*). The hypothesis assumes that point-mutations confer to proto-*ras* genes dominant transforming function that is equivalent to that of sarcomagenic retroviral *ras* genes (*268*). Further, it assumes that the 3T3-cell transformation assay measures a preexisting function of mutated cellular proto-*ras* genes. Consequently, point-mutated proto-*ras* genes were termed "dominantly acting oncogens" (*4, 5, 9, 46, 259, 260, 269*). Subsequently, other proto-*onc* genes, such as proto-*myc* (*270, 271*) and proto-*src* and the *src* genes of Rous sarcoma virus (*275*), and even genes that are not structurally related to retroviral oncogenes, such as certain anti-oncogenes (see Section IV,E), were also proposed to derive transforming function from point-mutations (*1, 5, 6, 9, 46, 272–274*).

Numerous observations designed to test the *ras* point-mutation–cancer hypothesis indicate that point-mutation is not sufficient for carcinogenesis:

1. Point-mutated proto-*ras* genes from tumors do not transform diploid embryo cells from rodents or humans, as retroviral *ras* genes do (*238, 276*). However, upon simultaneous transfection with other viral oncogenes or cellular genes linked to viral promoters, proto-*ras* genes transform embyro cells (*234, 235, 238*). This indicates that point-mutation is not sufficient to convert proto-*ras* to a gene that can transform normal cells.
2. Numerical arguments based on relative probabilities of point-mutations versus cancer also indicate that point-mutated proto-

ras genes are not sufficient for carcinogenesis. The probability of point-mutations is 10^{-9} per nucleotide and per mitosis in eukaryotic cells (*37, 38, 47, 277*). Since eukaryotes carry about 10^9 nucleotides per cell (*278*) and consist of 10^{11} (mice) to over 10^{14} (humans) cells, mice carry 10^2 ($10^{11} : 10^9$) and humans carry 10^5 ($10^{14} : 10^9$) cells with the specific point-mutation that changes Gly to Val in codon 12 of proto-*ras* at any time (*37, 38*). Since the average cell is replaced about 100 times during a human lifetime of 70 years (*37, 277*), this number must be multiplied by 100. Moreover, since at least 50 different point-mutations in at least five different codons confer transforming function to proto-*ras* in the 3T3 assay (*39, 279*), mice would contain 5×10^3 and humans have 5×10^6 such cells.

3. Further, the existence of point-mutated proto-*ras* genes in non-tumorigenic, hyperplastic tissues (see Section VI) (*280–284*) and in transgenic mice (*236, 241*; R. Finney and J. M. Bishop, 7th Annual Meeting on Oncogenes, Frederick, Maryland, 1991, personal communication) indicates that these mutations are not sufficient for carcinogenesis.
4. Point-mutation is not necessary for the transforming function of Harvey and other murine sarcoma viruses, as mutants without point mutations in *ras* and synthetic retroviruses with normal proto-*ras* coding regions are almost as oncogenic as those with point-mutations (*41, 44, 285*). This indicates that viral *ras* genes derive transforming function from other virus-specific elements (*39, 41, 44*) and suggests that point-mutation may not be sufficient for proto-*ras* genes to transform.
5. In primary tumors, point-mutated proto-*ras* genes are expressed at nearly the same level as normal proto-*ras* genes (*36, 44, 262, 264, 280, 286*). By contrast, point-mutated proto-*ras* genes in cells transformed by transfection are expressed like viral *ras* genes, which is at a level at least 100-fold higher than native proto-*ras* genes (*44, 234, 235, 262, 280, 286, 287*). Thus, the 3T3-cell-transfection assay creates proto-*ras* expression artifacts that are transcriptionally about 100 times more active than native proto-*ras* genes from tumors. Their activity is similar to that of retroviral *ras* genes.

It appears then that a point-mutated but intact cellular proto-*ras* gene is not sufficient for carcinogenesis. Further, it follows that the transfection assay does not measure a genuine function of point-mutated proto-*ras* genes as they exist in tumors, but measures that of an

expression artifact created during the transfection assay. An analogous functional artifact has been observed upon transfection of an anti-oncogene (see Section IV,E) (*287a*).

Such artifacts could be generated during transfection by substituting by illegitimate recombination the native proto-*ras* regulatory elements by artificial promoters derived from carrier and helper gene DNA (*44*). Indeed, transformation of primary cells by cellular proto-*ras* genes depends on the presence of added viral helper genes or on other cellular genes linked to viral promoters (*234*, *235*, *288*, *289*), or on the presence of retroviral promoters alone (*44*). This recombination process is entirely analogous to the generation of retroviral *ras* genes, in which coding regions of normal proto-*ras* genes are recombined by transduction with heterologous retroviral promoters that enhance the transcription over 100-fold compared to proto-*ras* (*37*, *38*, *43*, *44*). In addition, transfection generates concatenated DNA multimers, an artificial gene amplification that would also enhance the dosage of *ras* transcripts (*290–293*).

The probable reason that proto-*ras* genes from tumors transform 3T3 cells, but not primary cells, is that mouse NIH 3T3 cells are much more readily transformed by exogenous genes, as well as spontaneously (*294*), than are embryo cells (*238*). Thus, the weak promoters acquired from random sources during transfection are sufficient to convert proto-*ras* genes with point-mutations to 3T3-cell transforming genes, but not to genes capable of transforming primary cells.

The reason that point-mutated, but rarely normal, proto-*ras* genes (*261*) are detected by transfection assays is that point-mutations enhance about 10- to 50-fold the transforming function imparted by heterologous promoters on proto-*ras* genes (*39*, *44*, *285*, *295*). Thus, proto-*ras* genes derive their transforming function from heterologous promoters, and certain point-mutations merely enhance this transforming function.

2. Ad Hoc *ras*–Cancer Hypotheses Postulating Second- and Higher-order Mechanisms of Transformation

In view of the evidence that native, point-mutated proto-*ras* genes detected in some tumors are not equivalent to viral *ras* genes and not sufficient for carcinogenesis, *ad hoc ras*–cancer hypotheses have been advanced proposing that cellular *ras* genes with point-mutations depend on helper genes for carcinogenesis (*6*, *28*, *46*, *236*, *238*). However, the hypothetical helper genes have not been identified in most tumors, except for colon cancers.

In the case of colon cancer, it has been postulated that point-

mutated Kirsten and N-*ras* genes depend on the mutation of at least three tumor suppressor genes for transforming function (*28, 46, 272*). Yet the incidence of these mutations in colon cancers is not convincing proof for their postulated function for the following reasons.

Among primary colon cancers, about 40% carry point-mutated Kirsten *ras* genes (*28, 263, 264*) and some others contain point-mutated N-*ras* genes (*28*). In addition 70% of all carcinomas carry deletions or mutations in the presumed tumor suppressor gene DCC (deleted in colon cancer) located on chromosome 18, 75% in the presumed suppressor gene p53 located on chromosome 17 and 30% in the presumed suppressor gene APC (adenomatous polyposis coli) on chromosome 5 (*28*). Thus, only about 6% ($0.4 \times 0.7 \times 0.75 \times 0.3$) of the colon cancers studied carry the genetic constellation postulated for colon cancer. About 87% carry various combinations of these mutations, and 7% carry none of the mutations (*28*). In addition, recent evidence indicates that mutations on chromosome 5 are scattered over several hypothetical suppressors or anti-oncogenes (*296*). Despite these radical mutational differences among colon carcinomas, the carcinomas do not differ from each other in any known histological or biological properties. In addition, all of these mutations alone, and even together, are also observed in benign colon adenomas (see Section VI) (*28*). Other tumors with point-mutated proto-*ras* genes are also histologically and morphologically indistinguishable from counterparts without these mutations (*262, 297*).

In view of such poor correlations and the absence of *ras*-specific tumor markers, a functional test is the only method to prove the hypothesis that point-mutated proto-*ras* genes have transforming function in conjunction with helper genes. However, the only functional test currently available is the 3T3-cell-transfection assay, which generates helper-independent proto-*ras* expression artifacts. Thus, the hypothesis that point-mutated proto-*ras* genes play a role in carcinogenesis is based only on circumstantial evidence, namely, structural, but not functional, similarity to viral *ras* genes. In addition, it is based on epidemiological evidence that mutated genes are more common, or are observed more commonly, in tumors than in normal cells (see Section VI). Moreover, the assumption that mutation of p53 is obligatory for carcinogenesis has not been confirmed in a recent study that generated developmentally normal mice without p53 genes (*319a*) (see Section IV,E).

It follows either that unrearranged, point-mutated proto-*ras* genes are oncogenic by a second- or higher-order mechanism of carcinogenesis that is not analogous to the first-order mechanism of viral *ras* genes

and of the transfection artifacts of proto-*ras* genes, or that they are not relevant to carcinogenesis. Since constellations of mutated proto-*ras* and helper genes that are tumor-specific have not been found, there is currently no evidence for a role in carcinogenesis.

Therefore, we propose that other events, such as chromosome abnormalities, which are consistently found in colon carcinomas with and without mutated oncogenes or anti-oncogenes (*28, 47, 192*), may cause colon cancers. The clonal mutations in proto-*ras* and hypothetical helper genes could reflect the origin of tumor cells from non-tumorigenic somatic cells with the same mutations (see Section VI).

D. *int* Genes with Integrated Mouse Retroviruses and Mouse Mammary Carcinomas

Mouse mammary tumor virus (MMTV) is one of the many endogenous retroviruses that are genetically and perinatally transmitted but hardly ever expressed by most strains of mice (*5, 6, 298*). However, inbred female mice of the C3H and GR strains express high titers of mammary tumor virus in their milk. Approximately 90% of the female offspring of C3H mice develop mammary tumors between the ages of 7–10 months (*299, 300*). Foster-nursing of C3H offspring by virus-free mothers of other strains reduces the risk of tumors to 20–40% and delays their appearance to 18 to 24 months (*299, 301*). However, wild mice foster-nursed by a C3H mother fail to develop mammary tumors (over a spontaneous background of 3% at 2 years of age), although they are infected by the virus (*302, 303*).

Virus replication at high titers enhances reversible, hormone-dependent mammary hyperplasias that are poly- or oligoclonal (*304*). Out of these hyperplasias, clonal tumors emerge that are hormone-independent (*304, 305*). Thus, infection by milk-borne virus initiates virus replication, hyperplasias, and frequently tumorigenesis at an earlier age compared to spontaneous virus activation and tumorigenesis—but only in certain inbred strains of mice. The virus is replicating in both early and late tumors (*305*). The tumors are clonal, defined by specific virus integration sites and chromosome abnormalities (*2*). Since only one out of millions of virus-producing mammary cells becomes tumorigenic, tumorigenesis may be virus-independent, or may be due to virus-mediated activation, or inactivation of a cellular gene, in which case, site-specific provirus integration must be postulated.

Site-specific integrations in mammary tumors were originally observed in three different mouse strain-specific loci, termed *int*-1 (*34*),

int-2 (*306*), and *int*-3 (*307*). In C3H mice, the provirus is primarily observed in *int*-1, in BR6 mice in *int*-2, and in some feral mice in *int*-3. Subsequently, "numerous" (*305*) *int* loci were observed in mouse mammary carcinomas (*308*). In light of the observation that proto-*myc* genes in certain chicken leukemias are transcriptionally acitvated by retrovirus insertion (*31*) these *int* loci have been postulated to be cellular proto-*onc* genes that are activated to cancer genes by the promoter of integrated MMT provirus (*1, 6, 8, 34, 306, 308*). This hypothesis is compatible with the clonality of the mammary tumors.

Integration sites within a given *int* locus are spread over 20 kb and occur in both transcriptional orientations (*1, 2, 8*). Viral integrations into *int* loci are also observed prior to tumorigenesis in hormone-dependent hyperplasias (*304, 309*). Only 1–10 copies of *int* RNAs are found in tumor cells that express *int* genes (*310*). By comparison, synthetic (*39, 40, 42, 44*) and natural (*31*) retrovirus-promoted proto-*onc* genes make about 10^3 to 10^4 copies of RNA per transformed cell. In many viral mammary tumors, the *int* loci are not expressed, and in some tumors the *int* loci are expressed but the MMT provirus is not integrated at or near *int*. For example, *int* loci are expressed in only 2 out of 9 clonal tumors of GR mice (*304*), and *int* loci are expressed in only 19 out of 46 clonal tumors of C3H mice (*305*). It has also been reported that *int* loci are expressed in tumors in which MMTV is integrated at non-*int* sites. Accordingly, there is no report of *int*-specific tumor markers.

In view of this, several arguments cast doubt on the *int*-activation hypothesis:

1. Since "numerous" *int* loci are observed in mammary carcinomas, and since integrations are scattered over 20 kb within a given locus and occur in both orientations, MMTV integration into *int* loci cannot be sufficient for carcinogenesis based on the following numerical arguments. Given random retrovirus integration (*6*) and 1×10^6-kb DNA per mouse genome (*278*), and assuming only five 20-kb *int* loci, about 1 out of every 10^4 (5×20 out of 10^6) infected mammary cells should become tumorigenic. Thus, tumors should appear very soon after infection. Since this is not the case, MMTV integration cannot be sufficient for carcinogenesis.
2. Since MMT proviruses integrate into *int* genes prior to tumorigenesis, provirus-mutated *int* genes cannot be sufficient for tumorigenesis.
3. Since wild mice are susceptible to the virus and produce the

same hormones as the inbred mice that develop mammary carcinomas, even the virus–hormone package is not sufficient for tumorigenesis.

4. Provirus integration into different *int* loci in different strains of mice indicates that integration is host-directed. Therefore, the virus is not sufficient for site-specific integration and thus for tumorigenesis, if site-specific integration proves to be relevant for tumorigenesis.
5. Since *int* loci are not expressed in many viral mammary tumors, transcriptional activation of *int* genes by any mechanism is not necessary for carcinogenesis. It is consistent with this view that proviruses are integrated into *int* genes in both directions and integration sites are spread over 20 kb, but retroviral promoters activate transcription in only one direction and only over limited distances (*42*).
6. Since the same tumors are observed with and without integration into *int* genes, site-specific integration is not necessary for carcinogenesis, because "clonal, hormone-independent tumors . . . seem to be the result of mutations that are unrelated to *int* activation" (*304*).
7. The retroviral *int*-activation hypothesis fails to account for the clonal chromosome abnormalities of all virus-positive tumors that have been characterized (2)—except if one makes the additional odd assumption that MMTV only transforms cells with preexisting chromosome abnormalities.

It thus appears that the MMTV plays only an indirect role in tumorigenesis as one of several factors that enhance mammary hyperplasias, a known risk factor for carcinogenesis (*1*, *203*). This role is similar to that of other highly expressed animal retroviruses in leukemogenesis. For example, inbred viremic mice and chickens have been described that develop virus-induced hyperplasias from which clonal lymphomas or leukemias emerge (2). Alternatively, high levels of retrovirus expression may just signal a heritable loss of intracellular suppressors which could themselves predispose to overgrowth and thus favor carcinogenesis (2). The incidence of 20–40% carcinomas in foster-nursed C3H mice compared to a background of 3% in other laboratory mice (*303*) supports this view. This would be analogous to the activation of other retroviruses in cells induced to proliferate by genetic damage from chemicals or radiation (see Section III).

In view of this, we propose that mammary carcinogenesis is a rare, spontaneous event initiated by chromosome abnormalities that occur

in one out of millions of virus-infected cells. This hypothesis would explain clonal viral integration sites as accidental consequences of the clonal chromosome abnormality that created a tumor cell from a normal virus-infected cell. It would also explain why the carcinomas are not distinguished by the type of *int* gene that is mutated. The *int*-loci would be strain-preferred provirus integration regions that are not relevant to tumorigenesis.

E. Constitutive Oncogenes, Mutated Anti-oncogenes, and Cancer

There are heritable and spontaneous retinoblastomas (*45*). Cytogenetic analyses of both have observed that chromosome 13 is either missing or deleted in 20 to 25% (*311, 312*). In addition, other chromosome abnormalities have been observed in all retinoblastomas (*311, 312*). On this basis, it was proposed that retinoblastoma arises from the loss of a tumor suppressor or an anti-oncogene, now termed *rb*, that is part of chromosome 13 (*45*). In the familial cases, the loss of one *rb* allele would be inherited and the second one would be lost due to spontaneous mutation. In the spontaneous cases, somatic mutations would have inactivated both loci. In the retinoblastomas with microscopically intact chromosomes 13, submicroscopic mutations were postulated.

This anti-oncogene hypothesis predicts that normal cells would constitutively express oncogenes that render the cell tumorigenic if both alleles of the corresponding suppressor are inactivated. The hypothesis further predicts that the suppressor genes must be active at all times in normal cells. In 1986, Weinberg *et al.* (*313*) cloned a human DNA sequence that was missing or altered in about a third of 40 retinoblastomas and in 8 osteosarcomas. Therefore, the gene encoded in this sequence was termed the *rb* gene. Reportedly, the *rb* gene was unexpressed in all retinoblastomas and osteosarcomas, even in those without *rb* deletions (*313*). The *rb* gene measures almost 200 kb, includes 27 exons and encodes, from an mRNA of 4.7 kb, a 110,000-dalton protein (*8, 278*).

An analysis of 34 primary retinoblastomas undertaken to test the hypothesis found deletions of the *rb* gene in only 4 of 34 tumors analyzed and transcripts of the *rb* gene were found in 12 out of 17 retinoblastomas and in 2 out of 2 osteosarcomas, casting doubt on the deletion hypothesis (*314*). The remaining tumors had apparently normal *rb* genes. However, subsequent studies of retinoblastomas have observed point-mutations and small submicroscopic deletions in *rb* genes that did not have macrolesions (*273, 274, 315, 316*). For example,

both Weinberg *et al.* (*273*) and Lee *et al.* (*274*) reported a point-mutation in a splice sequence of the *rb* gene. In view of this, it is now believed that point-mutations or other minor mutations of the *rb* genes are sufficient for tumorigenesis (*273, 315*). However, Gallie *et al.* reported point-mutations and deletions of *rb* genes in only 13 out of 21 tumors (*315*). In an effort to develop a functional assay, a DNA copy of the mRNA of the *rb* gene was cloned into a retrovirus; infection by this virus inhibited the growth of a retinoblastoma cell line *in vitro* (*274, 317*). However, two recent studies show that an intact, synthetic *rb* gene fails to inhibit tumorigenicity of human retinoblastoma and breast cancer cells in nude mice (*318, 318a*).

Clearly, the point-mutation hypothesis of the *rb* gene would never have emerged if the original chromosome deletion hypothesis had been confirmed. It advanced the anti-oncogene hypothesis into a virtually inexhaustible reservoir of hypothetical cancer genes: Any gene with any mutation in each of both alleles in a cancer cell could be a tumor suppressor or anti-oncogene. According to Weinberg, " . . . one can cast a broad net for tumor suppressor loci by using a large repertoire of polymorphic DNA markers to survey . . . for repeated instances of LOH (loss of heterozygosity). Indeed, this genetic strategy has revolutionized the research field" (*287a*). Over a dozen deleted or point-mutated anti-oncogenes are now considered to cause osteosarcomas, breast cancer, bladder cancer, lung cancer, colon cancer, Wilms' tumor, and neuroblastoma, in addition to retinoblastoma (*8, 9, 46, 287a, 317*). For example, a point-mutation in one of three genes of a colon cancer cell would signal an inactivated hypothetical colon cancer suppressor gene (*272, 296*). Further, the range of the *rb* suppressor gene has since been extended to other cancers, including small cell lung, bladder, prostate, and breast carcinomas, and osteosarcoma (*8, 317*).

The anti-oncogene hypothesis has been difficult to prove because (a) the oncogenes that are said to be suppressed have not been named or identified (*269*) and will be difficult to assay because all normal cells or animals should suppress them with the corresponding anti-oncogenes, and because (b) transfection of an intact *rb* gene (*274, 318*) has failed to revert transformed cells to normal and to suppress their tumorigenicity (*274, 318, 318a*). Likewise the hypothetical colon cancer suppressor gene p53 has failed to revert transformed cells to normal (*319*) and its complete absence has not affected the normal development of mice (*319a*). Nevertheless, 74% of these p53-free mice developed lymphomas and sarcomas at six months that probably derived from single cells, rather than through a systemic transformation as the anti-oncogene hypothesis would have predicted (*319a*).

At this time, the hypothesis suffers from the following shortcomings:

1. The probability of point-mutations and minor mutations in both alleles of the *rb* gene appears much higher than the cancers they are said to cause. Since the *rb* gene has 27 exons and each exon is flanked by at least four essential splice nucleotides, at least 108 (4×27) point-mutations could inactivate the *rb* gene. In addition, one can assume that point-mutations of at least 10% of the 928 amino acids of the 110,000-dalton *rb* protein would inactivate the gene (*8*). Thus, at least 200 point-mutations should be able to inactivate *rb*. Since 1 in 10^9 human cells contain any possible point-mutation of the human genome (see Section IV,C), about 1 in 5×10^6 would contain an inactive *rb* gene, and 1 in $(5 \times 10^6)^2$ or 2.5×10^{13} would contain two inactive *rb* genes in the same cell. This number would be even higher if other mutations such as minor deletions and chromosome nondysjunctions were included.

   Chromosome nondysjunctions are estimated to occur in 1 out of 10^4 human cells (*320, 321*). The probability of generating a retinoblastoma cell from a point-mutation in one *rb* gene and a missing chromosome 13 would be 1 in $5 \times 10^6 \times 10^4$ or 1 in 5×10^{10}. Thus, every adult human consisting of about 10^{14} cells would contain at least 1 and possibly 5×10^3 cells in which both *rb* genes are inactivated, and would develop over 100 to 100,000 such cells in a lifetime of 70 years, which represents about 10^{16} cells (*37, 277*). Since inactive *rb* genes are now said to cause retinoblastomas, osteosarcomas, small cell lung, breast, and bladder carcinomas, etc., and the corresponding tissues represent over 20% of the human body, one would expect at least 20% of humans to develop such a tumor per year.

   Moreover, 1 in 5×10^6 cells of every person with one inherited *rb* mutation should have defects in both *rb* alleles due to secondary mutation and 1 in 10^4 cells due to chromosome nondysjunction. A recent review on tumor suppressor genes reports exactly the same probabilities for *rb* mutations as we do (*287a*). Thus, all persons with an inherited *rb* deletion should develop retinoblastomas and other cancers. Since this is not the case (*45*), point-mutation or deletion of both *rb* alleles cannot be sufficient for carcinogenesis.
2. Since neither deletion nor minor mutation of *rb* genes is observed in all retinoblastomas or other specific tumors, *rb* deletion or mutation is not necessary for tumorigenesis.

3. The relevance of the growth inhibitory function of the artificial retrovirus with an *rb* coding region to the putative tumor suppressor function of *rb* is unclear for several reasons: (a) Expression from a retroviral promoter enhances the *rb* protein concentration at least 100-fold above physiological levels (*274*) and thus may not be relevant to its normal function. Similar reservations are expressed by Weinberg: ". . . many genes . . . will antagonize growth when they are forced on a cell by . . . gene transfer, but this provides no testimony as to whether these genes are normally used by the cell to down-regulate its own proliferation . . . " (*287a*). (b) Recently, elevated rather than reduced *rb* expression was observed in tumor cells (*322*). (c) Human retinoblastoma cell lines and breast cancer lines transfected with intact and artificially overexpressed *rb* genes are tumorigenic in nude mice, indicating that the *rb* gene does not suppress tumorigenesis by retinoblastoma and mammary carcinoma cells (*318, 318a*).

It follows that deletion or mutation affecting both alleles of the *rb* and p53 genes is not sufficient and probably not necessary for carcinogenesis since the same retinoblastomas and colon cancers occur in the presence and absence of these genes. An alternative hypothesis suggests that the many chromosome abnormalities associated with retinoblastomas (*311, 312*), other tumors with *rb* mutations (*194*) and colon cancers are to blame for carcinogenesis (see Section VI).

V. Conclusions

A. Evidence That Latent Viruses and Mutated Cellular Genes Are Pathogenic Is Circumstantial

Compared to classical prototypes, the presumably latent viruses and mutated cellular genes all suffer from activity, infectivity, and specificity gaps: (1) The viruses and genes that are postulated to be pathogenic or oncogenic are each orders of magnitude less active, and their products less abundant, than the transcriptionally active, pathogenic prototypes that have inspired these hypotheses; (2) infections with the viruses do not cause the postulated diseases, and transfections of appropriate cells with mutated cellular genes do not transform normal cells; (3) the hypothetical pathogens are not disease-specific, because (a) the same latent viruses and mutated cellular genes occur in

symptomatic and asymptomatic subjects (see Section VI) and because (b) histologically and clinically indistinguishable diseases occur without them.

Clearly, infection without disease involving limited numbers of cells also occurs with classical viral pathogens and is essential for their survival (*3, 12*). However, it becomes an important deficiency for hypotheses claiming pathogenicity for viruses that are equally inactive and restricted in diseased and healthy carriers.

Therefore, there is only circumstantial or epidemiological evidence for the role of mutated genes in cancer and latent viruses in disease. Indeed, Bishop writes that ". . . on the basis of circumstantial evidence of considerable variety, damage to diverse proto-*onc* genes has been implicated in the genesis of human tumors" (*7*). Varmus pointed out: "Although the dividends of conferring the status of proto-oncogenes upon these cellular genes have been considerable, it must be acknowledged that the basis for doing so, the genetic definition of v-*onc*'s [retroviral oncogenes], has not been uniformly rigorous" (*5*). And on the basis of epidemiological correlations, point-mutations in cancer cells are said to be "smoking guns" (*272*). Yet the same tumors occur without this circumstantial evidence (see Section VI).

Based on just two as yet unconfirmed studies from 1989, Baltimore and Feinberg pointed out that "HIV viremia cannot be said to be 'necessary' for AIDS on the basis of any available data, but the new results are a consistent feature of AIDS" (*77*). Further, Blattner, Gallo, and Temin (*14*) argued that ". . . the strongest evidence that HIV causes AIDS comes from prospective epidemiological studies . . . " and Weiss and Jaffe (*15*) concurred ("the evidence that HIV causes AIDS is epidemiological . . . "), although Gallo (*76*) conceded that epidemiology is just "one hell of a good beginning" for proving the virus–AIDS hypothesis. But even this beginning is flawed by the tautological definition of AIDS, which only diagnoses AIDS when antibodies to HIV are found (*54*) and ignores all AIDS indicator diseases that occur in the absence of HIV, even in AIDS-risk groups (*54*). For example, half of all American intravenous drug users (*55*) and 25% of all hemophiliacs (*54*) are HIV-free, so that their AIDS indicator diseases will not be reportable as AIDS.

The same is true for the epidemiological evidence that HTLV-I causes T-cell leukemia. The leukemia is solely defined by the presence of HTLV-I, although it has been observed in its absence (Section III). In an effort to link human myelopathy with latent HTLV-I, it is proposed that "similarities between HAM (HTLV-I associated myelopathy) and visna [are] the result of still deeper identities" (*323*).

Visna is a neurological disease that occurred in the 1930s in a now extinct strain of sheep. Its cause is believed to have been a latent retrovirus, termed visna virus, that is present in over 50% of healthy sheep in Europe and the U.S. (53). Likewise, there is no controlled study to show that the incidence of cervical cancer is higher in HPV-positive than in matched negative controls (see Section III).

Thus, latent viruses and mutated cellular genes are postulated to be pathogenic only because (i) they structurally resemble active pathogenic viruses or active viral oncogenes, and because (ii) they occur, or are assumed to occur, in the respective diseases more often than in normal tissues (*6, 49, 51, 296*).

B. Helper Genes and Cofactors to Close the Activity, Infectivity, and Specificity Gaps of Hypothetical Pathogens

Since the latent viruses and mutated cellular genes do not behave like the autonomous pathogens they were originally postulated to be, the original theories have been supplemented by *ad hoc* hypotheses.

For example, it has been postulated that the long latent periods, ranging up to 55 years, of the hypothetical viral pathogens are necessary for various, unproven cofactors to help the latent viruses to cause disease. Accordingly, the viruses have been termed "slow viruses" or "lentiviruses" (*3, 12*), although the viruses replicate within a few days and are immunogenic within a few weeks after infection (*13, 37, 91, 92*). Further, it has been postulated that antiviral antibodies consistently fail to neutralize viruses such as HIV (*76, 77*) or hepatitis C virus (*160*), although the respective viruses are almost undetectable for the duration of the diseases they are said to cause. Moreover, helper genes or cofactors are postulated for carcinogenesis by mutated genes that are not transcriptionally activated, including the point-mutated proto-*ras* genes, the *int* genes mutated by provirus insertion, or the rearranged proto-*myc* genes.

In reality, the cofactors are modern euphemisms for new hypotheses. The *ad hoc* hypotheses all assume second- or third-order mechanisms of pathogenesis relying on unproven cofactors for both latent viruses and mutated cellular genes to cause disease. Moreover, these *ad hoc* hypotheses all lack appropriate precedents because all available pathogenic viruses and viral oncogenes are helper-independent first-order pathogens. Yet the *ad hoc* hypotheses are popular because they leave arbitrary but face-saving roles for failing incumbents, which were all originally proposed to cause the diseases by themselves.

In the absence of functional proof, circumstantial and epidemiological evidence is only relevant for causation if the respective viruses and

cellular mutations and their hypothetical cofactors are at least disease-specific. This appears not to be the case.

VI. Alternative Hypotheses

A. Latent Viruses as Harmless Passengers

The inactive viruses associated with fatal diseases such as AIDS, hepatitis C, cervical cancer, T-cell leukemia, hepatoma, Burkitt's lymphoma, and encephalitis are all not disease-specific. They are common, like HSV, HPV, EBV, and HBV (*3, 12*), or rare, like HIV and HTLV-I (*54*), in healthy persons. The long "latent periods" and the low incidence of "viral" disease among virus carriers indicate that such infections are typically not pathogenic. Although the term "latent period" implies that the virus becomes active thereafter, even this is almost never true (see Section II and III). During the presumably virus-caused diseases, including AIDS, cervical cancer, T-cell leukemia, hepatoma, or panencephalitis, the virus remains typically inactive, leaving pathogenic functions to unnamed cofactors. And there is no cofactor that has been found only during the disease but not prior to it. It is hardly surprising that latent viruses or fragments of their DNAs are still there if their host develops a nonviral disease. Thus, the latent viruses are innocent bystanders or "passengers," rather than drivers, in nonviral disease processes (*159*).

B. Drugs as Alternatives to Hypothetical Viral Pathogens

The great triumphs in the pursuit of microbial and viral pathogens in the last 100 years have eclipsed, and even led to the ridicule of, alternative, less spectacular, explanations of disease, such as pathogenic drugs and toxins (*15, 324–326*). Although we are in the middle of a drug-use epidemic in America, the pathology and epidemiology of recreational drugs, and even of some medical drugs such as AZT, are virtually unstudied by the scientific community (*155*).

The drug–AIDS hypothesis described in Section II,A,2 is one example of how drug use could cause AIDS diseases (*54, 60, 103*). Psychoactive drugs and medical drugs could explain diseases caused by the depletion of many cells, such as the depletion of T-cells in AIDS or of hepatocytes in hepatitis C, much better than can dormant viruses. Indeed, both of these diseases are observed primarily in drug addicts (*54, 103, 160*). Drug toxicity is also much more compatible with the restriction of these diseases to risk groups, as, for example, AIDS, which is almost exclusively restricted to users of recreational drugs and anti-HIV drugs such as AZT (like lung cancer is to smokers).

Exogenous toxins could also explain the actions of putative viral tumors, such as nitrite inhalants causing Kaposi sarcomas and AZT causing lymphomas (*69, 103*), smoking possibly causing cervical cancer (*198, 204*), nutritional toxins causing hepatomas, and radiation possibly causing T-cell leukemia (*190*) (see Section III). Toxins would also provide a plausible explanation for the lack of contagiousness of these "viral" diseases. The cumulative effects of drug or nutrient toxicity over time are compatible with the appearance of these diseases relatively late in life and at unpredictable intervals after infection by presumed viral causes. By contrast, viruses as self-replicating toxins all cause diseases soon after infection. In light of this theory, hypothetical linkages between infection by a virus and a subsequent onset of disease via long and unpredictable latent periods of up to 55 years would dissipate, because infection and pathogenesis are independent events.

C. Mutated Genes and Latent Viruses as Trivial Genetic Scars of Cancer Cells

The spontaneous or virus-induced mutations in tumor cells are also not disease-specific. For example, point-mutated proto-*ras* genes have been observed in chemically induced skin hyperplasias of laboratory mice (*280*) and in spontaneous liver hyperplasias of B6C3F1 mice (*281*) that all spontaneously revert to normal. Further, they have been observed in reversible skin hyperplasias of humans (*282, 327*) and in human hemopoietic hyperplasias (*238, 284*). Moreover, a recent study of transgenic mice concluded that " . . . expression of the mutant [proto-Ha-*ras*] gene via its own promoter at the normal chromosomal locus is nontransforming" (R. Finney and J. M. Bishop, 7th Annual Oncogene Meeting, Frederick, Maryland, 1991, personal communication). In addition, point-mutations and all other mutations affecting hypothetical tumor suppressor genes are not tumor-specific. They are detected singly and in all combinations, including mutated proto-*ras*, in benign colon adenomas at about the same rates as in malignant carcinomas (*28*).

Proto-*abl* translocations are seen in functional granulocytes that are overproduced during the chronic, hyperplastic phase of myelogenous leukemia (*242, 243*) (see Section IV). Hormone-dependent mammary hyperplasias with *int* genes mutated by integrated MMT proviruses have been described (see Section IV). Also, the DNA of hepatitis B virus has been detected and is expressed in non-tumorigenic liver cells more consistently than in hepatomas (*196, 211*). Inactive and defective HPV DNA is routinely detected in non-tumorigenic tissues with the commercial Vira/Pap test or with the PCR (*199*). And HTLV-I is almost

only detected in normal rather than leukemic carriers (see Section III). Further, viable transgenic mice with mutated proto-*abl*, proto-*myc*, and proto-*ras*, and even with hypothetically cooperating combinations of proto-*myc* and proto-*ras*, have been constructed, and some are commercially bred ("OncoMouse-TM shortens the path to knowledge . . . ," Dupont Co., Wilmington, DE, 1990) (*236*). This argues either for even more cofactors or for other mechanisms altogether.

Thus, spontaneous and viral mutations of tumor cells are not disease-specific. These findings confirm the above calculations that the probability of these mutations is much higher than the incidence of cancer and that carcinogenesis even among hyperplasias is still a very rare event. In view of this, we agree with Bishop that "the nomenclature for the affected genes [oncogenes] is unfortunate, since it is based largely on occasional [presumed] pathogenic aberrations . . . " (*9*).

Nevertheless, since clonal tumors have been observed to emerge from hyperplasias and transgenic animals at a higher-than-normal rate, their mutated genes and latent viruses could play roles in carcinogenesis that are not analogous to those played by the biochemically active models that led to their discovery. For example, they could alter growth control genes and thus generate hyperplasias. However, even this is speculation because the mutations and latent viruses are not consistently found in hyperplastic cells, with the exception of HPV in papillomas (*13*) (see Section III). Therefore, they must be presumed innocent until proven guilty (*326*).

In view of this, we propose that the mutations and latent viruses that are found in tumor cells are trivial genetic scars that were picked up by non-tumorigenic somatic cells during many generations of growth in the presence of mutagens or viruses. Because of detection and reporting biases in favor of disease, the mutations and latent viruses would be reported more often in diseased than in healthy carriers. Further, the mutations and viruses would be more readily and more often observed in cancer cells than in non-tumorigenic somatic tissues, because cancers are clonal populations of cells (*192, 193, 328*) that provide multiple copies of identical mutations, biological equivalents of the PCR. In contrast, such mutations, including latent and fragmented viral DNAs, would not be detectable in mutationally "heterogenous" populations of normal cells, unless individual cells were cloned or their nucleic acids were amplified.

Since many of these somatic mutations could be incompatible with normal fetal development, they would not be seen in the germline (*329*) and thus not in an average normal cell. The many congenitally and genetically transmitted animal (*6*) and human retroviruses, including HTLV-I and HIV (*54*), would be notable exceptions. Apparently,

retroviruses are so harmless that they can be accepted as parasites even during development (2).

The hypothesis correctly predicts the same mutations and latent viruses in non-tumorigenic somatic cells and in tumors that emerged from these cells, as, for example, the proto-*ras* and other mutations or the many "tumor" viruses that are shared by tumorigenic and non-tumorigenic cells. Further, the hypothesis correctly accounts for the "too many mutations in human tumors" observed by Loeb (*47*), perhaps those that were considered irrelevant for carcinogenesis by Heidelberger ("I don't care if cells are 90% transformed, I am only interested in the last 10%.") (*330*). In view of this hypothesis, the latent viruses and nonactivating mutations of cellular genes in cancer cells would be genetic trivia.

D. Cancer by Somatic Gene Mutation Unconfirmed

The clonality, irreversibility, and predictable course of most cancers all indicate that cancer has a genetic basis. Yet an autonomous cellular cancer gene, or a complement of interdependent ones, that can be activated by the statistically cheap mutations observed in hypothetical oncogenes and anti-oncogenes is improbable on the following grounds.

(i) Nothing could be more terminal for a multicellular organism than a battery of latent cancer genes that are as easy to "activate" as the over 50 putative cellular oncogenes that have been named or the unnamed oncogenes that are said to be activated by inactivation of suppressor genes (*6*, *8*, *9*, *331*). The activation of just one dominant oncogene would be sufficient to initiate a clonal cancer and thus to kill the organism. By comparison, activation of a hypothetical death gene would kill only a single cell.

Indeed, since each of these oncogenes is thought to be activated via point-mutations, truncations, and virus insertions and since the probability of such mutational events is as high as 1 in 10^6 per mitosis and gene, and is as high as 1 in 10^9 per mitosis and nucleotide (see above estimates for proto-*ras*, proto-*abl*, and *rb*) (*37*, *38*, *47*, *277*), multicellular organisms such as humans, with about 10^{16} cells per average 70-year lifespan, would generate at least $50 \times 10^{16} : 10^9 = 5 \times 10^8$ cancer cells per lifetime. This number would be even higher if multiple mutational sites for the activation of specific oncogenes and for the inactivation of specific anti-oncogenes were considered (*6*, *8*). It would be further enhanced by the multiplicity of certain oncogenes that exist as large families, including proto-*myc* and proto-*ras* (*6*, *8*).

Nevertheless, the numerology of mutations could be reconciled with the real incidence of cancer by postulating adequate numbers of

cooperating mutations, as has been attempted in the case of colon cancer (see Section IV). However, this would be analogous to the invention of more and more Ptolomaic epicycles by geocentrists, in the face of Galileo's challenge that the earth was not the center of the solar system. Naturally, the relevance of these efforts to carcinogenesis would depend on functional proof.

(ii) Based on the only proven examples of "mutated cellular" oncogenes, the retroviral oncogenes, a cellular gene would have to become about 100-fold more active than normal to become a cancer gene. However, the odds of truly activating a gene about 100-fold over the level for which it has been optimized during 3 billion years of evolution by spontaneous mutation, must be much lower than the odds of the presumably "activating" point-mutations or truncations or virus insertions that are observed in the hypothetical proto- and anti-oncogenes of tumors. The rare, accidental recombinants with imported retroviral promoters, which in turn have been optimized during virus evolution to override cellular controls, are as yet the only known examples of oncogenic mutations (*37*).

The odds for activating a cellular gene 100-fold by spontaneous mutations would be particularly low for the many interdependent genes that must determine "how cells govern their replication . . . " (*7*), the presumed natural function of proto- and anti-oncogenes (*7, 287a*). According to Bishop, mutational "damage" to the "relays in regulatory circuitry" (proto-*onc* genes) and "governors of proliferation" (anti-oncogenes) is considered a "gain of function" sufficient to produce cancer (*9*). These oncogenic functions are postulated to be "dominant because . . . evil overrides good" (*9*). However, "damage" of the kind observed in putative oncogenes naturally inactivates genes causing diseases such as sickle cell anemia and hemophilia (*320, 332*). Such damage is a loss of function and thus recessive, because the remaining "good" gene overrides "evil." Ironically, the same kind of somatic mutations or damages to genes thought to "activate" oncogenes are said to perform conventional gene inactivations when they affect anti-oncogenes.

Indeed, it is one of the most common misconceptions that cancer is a consequence of unrestricted growth, because unrestricted growth produces benign hyperplasias, not cancer. According to Cairns, "It is a common mistake to assume that cancer cells multiply faster than the normal cells from which they were derived. . . . The fact is that the cells of most cancers divide at about the normal rate, and some even less frequently than their normal counterparts, but they are able to increase in number because a greater proportion of the cells' progeny remain in the dividing pool than is normally allowed" (*277*).

(iii) There is no functional proof for cellular oncogenes, because according to Stanbridge " . . . despite intensive efforts to transform normal human fibroblasts or epithelial cells with varying combinations of activated cellular oncogenes, the results have been uniformly negative" (269). Moreover, their presence, unlike that of related viral oncogenes, does not determine the character of a given type of tumor. Likewise, unmutated anti-oncogenes fail to revert tumor cells to normal, and mutated anti-oncogenes fail to distinguish tumors from those in which they are normal (see Section IV).

The somatic mutation hypothesis owes much of its popularity to the fact that, in the 1960s and 1970s, many carcinogens were found to be mutagens (335, 338), although substantial non-correlations between carcinogens and mutagens were also noted (335, 337). In the 1980s, the hypothesis derived further notoriety from the consensus that proto-*onc* genes and anti-oncogenes are the critical targets among the anonymous genes that are mutated by carcinogens (9, 287*a*). Says Weinberg: "Mutations that potentiate the activities of proto-oncogenes create the oncogenes that force the growth of tumor cells. Conversely, genetic lesions that inactivate suppressor genes liberate the cell . . . yielding the unconstrained growth of the cancer cell" (287*a*). However, not even one of the many somatic mutations observed to date in cancer cells has been shown to function as a cancer gene. According to Pitot: " . . . that carcinogens are mutagenic or may be converted to mutagens is important but not direct evidence for the genetic origin of neoplasia" (16).

In sum, the gene mutation hypothesis of cancer is numerically and evolutionarily implausible and is functionally unconfirmed. Similar conclusions were reached by Rous (203, 333) and Rubin (334) after studying oncogenic viruses and cancer for over 50 and 30 years, respectively. Rous concluded: "A favorite explanation has been that oncogens (Rous' term for carcinogens) cause alterations in the genes of the ordinary cells of the body . . . somatic mutations as these are termed. But numerous facts, when taken together, decisively exclude this supposition" (203). "A hypothesis is best known by its fruits. What have been those of the somatic mutation hypothesis? . . . It acts as a tranquilizer on those who believe in it, and this at a time when every worker should feel goaded now and again by his ignorance of what cancer is" (333). Likewise, Cairns " . . . suggests that most human cancers are not caused by conventional mutagens . . . " (335).

E. Chromosome Abnormalities as Causes of Cancer

But if there are no cellular genes that are converted to cancer genes by somatic mutations, cancer would have to be caused by normal

cellular genes. Perhaps a cell could become transformed by gross numerical imbalances of normal genes, e.g., via chromosome abnormalities, just as a computer could be rendered uncontrollable by deleting, duplicating, and misplacing intact chips, or by altering the operating software. To test this hypothesis, it would be necessary to determine how probable such abnormalities are compared to cancer and whether abnormalities exist that are cancer-specific.

Indeed, chromosome abnormalities are the oldest, and, as yet, the only consistent observation made on cancer cells. It was postulated by Boveri in 1914, prior to the discovery of DNA and point-mutations, that cancer would be caused by abnormal chromosomes (*194, 336*). The clonal origin of tumors, the stemline concept predicted by Boveri and defined by Winge in 1930 (*336*), is the strongest support for the view that clonal chromosome abnormalities are the causes, rather than consequences, of carcinogenesis.

This abnormal chromosome–cancer hypothesis would explain why chromosome abnormalities are consistently found in tumors with or without mutated cellular oncogenes and with or without latent viruses.

The hypothesis predicts that diploid cancers that differ from normal cells only in mutated oncogenes or anti-oncogenes are not observed, because certain chromosome abnormalities instead of somatic mutations of specific genes are carcinogenic. Tumor progression would be a consequence of further discontinuous chromosome abnormalities. The hypothesis would readily resolve the paradox that all "viral" tumors presumably caused by HTLV-I, HBV, HPV, HSV, and MMTV have clonal chromosome abnormalities. By contrast, all virus–cancer hypotheses would have to make the odd assumption that only cells with preexisting chromosome abnormalities are transformed by these "tumor" viruses.

Our hypothesis also explains why ". . . despite intensive efforts to transform normal human fibroblasts or epithelial cells with varying combinations of activated cellular oncogenes, the results have been uniformly negative" (*269*). In addition, the hypothesis explains why mutated proto-*onc* genes and anti-oncogenes do not distinguish tumors by their presence. According to our hypothesis, accidental somatic mutations generated by chromosome translocations, such as rearranged proto-*myc* or proto-*abl* genes, would be as irrelevant to carcinogenesis as other mutations of specific genes, such as point-mutated *ras* genes. Further, the hypothesis would explain why transgenic mice with activated oncogenes are breedable and why retinoblastoma cells remain carcinogenic for mice, even if they are infected by a retrovirus that overexpresses its presumed suppressor, *rb* anti-oncogene (see Section IV). Our hypothesis would also resolve the discrepancy be-

tween the rather high probability and incidence of mutation or "activation" of proto-*onc* genes compared to the much lower probability and incidence of cancer (see Section IV) (*37, 337*).

We have previously proposed another alternative to the oncogene hypothesis. It holds that cancer genes are generated by substituting the normal promoters of proto-*onc* genes via rare illegitimate recombinations by strong heterologous promoters from viruses or from cellular genes (37). As yet, the retroviral oncogenes are the only proven examples of this hypothesis (*40, 43, 44*). The relevance of this hypothesis to virus-free tumors depends on whether the cell contains promoters that are as strong as those of viruses.

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