APPOINTMENTS

Eugene B. Skolnikoff, head, political science department, Massachusetts Institute of Technology, appointed director, Center for International Studies at the institute. . . . **David B. Bates**, chairman, physiology department, McGill University, to dean of medicine, University of British Columbia. . . . **Neal L. Gault, Jr.**, chairman, medicine department, University of Hawaii, to dean, Medical School, University of Minnesota. . . . **Francis G. Brennan**, associate professor of English, St. Louis University, to dean, Graduate School at the university. . . . **Albert R. Haskell**, professor of pharmacy, University of Tennessee, to dean, College of Pharmacy, University of Nebraska. . . . **Arthur W. Brown**, president, Marygrove College, to dean, School of Arts and Sciences, Baruch College, City University of New York. . . . **Asa G. Hilliard, Jr.**, chairman of secondary education, San Francisco State College, to dean, School of Education, at the college. . . . **Robert G. Valpey**, dean, School of Engineering, California State College, Fullerton, to dean of engineering and technology, California State Polytechnic College. . . . **Keith Goldhammer**, dean, School of Education, Oregon State University, to dean, College of Education, Michigan State University. . . . **Edward E. Sampson**, visiting professor of psychology, Clark University, to chairman, sociology department at the university. . . . **Barclay Kamb**, professor of geology and geophysics, California Institute of Technology, to head, geological and planetary sciences division at the institute. . . . **James R. Chandler**, acting chairman, otolaryngology department, University of Miami School of Medicine, appointed chairman. . . . **Franklin H. Epstein**, professor of medicine, Yale University, to head, medicine department, Harvard Medical School. . . . **Emile M. Scarpelli**, associate professor of physiology, Albert Einstein College of Medicine, to chairman, physiology department, The University of Texas Medical Branch. . . . **Anne E. Coghlan**, professor of biology, Simmons College, Massachusetts, to chairman, biology department at the college. . . . **John I. Brewer**, professor of obstetrics and gynecology, Northwestern University Medical School, to chairman, ob-gyn department at the school.

RECENT DEATHS

Howard L. Alt, 71; former professor of medicine, Northwestern University; 12 February.

Theda Bennett, 48; professor of biology, State University of New York College, Buffalo; 9 February.

Harvey C. Brill, 90; professor emeritus of chemistry and former head, chemistry department, Miami University; 11 January.

Ira W. Drew, 94; osteopathic physician and board member, Philadelphia College of Osteopathic Medicine; 12 February.

Frank. J. Eichenlaub, 77; professor emeritus of dermatology, Georgetown University; 13 February.

Charles E. Farr, 96; clinical professor emeritus of surgery, Cornell Medical School; 20 February.

Frank P. Graham, 85; former president, University of North Carolina; 16 February.

Grant O. Graves, 67; chairman, anatomy department, Ohio State University; 7 February.

Albert Hartzell, 80; retired entomologist, Boyce Thompson Institute for Plant Research, New York; 7 January.

Stanley Katz, 51; professor of chemical engineering, City College, City University of New York; 19 February.

Frank Kingdon, 77; former president, University of Newark; 24 February.

Colin M. MacLeod, 63; microbiologist, director, Oklahoma Medical Research Foundation and former White House science adviser; 13 February.

Maria G. Mayer, 65; professor of physics, University of California, San Diego; 20 February.

Henry B. McDaniel, 68; professor emeritus of education and psychology, Stanford University; 20 February.

Edwin M. Miller, 83; professor emeritus of surgery, Rush Medical College, Illinois; 4 February.

RESEARCH NEWS

Cancer Virus Theories: Focus of Research Debate

The realm of the molecular genetics of cancer is populated by three elusive characters: the provirus, the oncogene, and the protovirus, each a theoretical precursor of the putative human cancer virus. There is no incontrovertible evidence that any of them exist in human cells, but the search for that evidence constitutes one of the most active areas of fundamental cancer research.

The three hypothetical agents are each meant to account for the way that the genetic information for cancer is formed and expressed in cells, and they are based on the assumption that viruses do, in fact, cause human cancer. This, too, has yet to be definitively proved. Nonetheless, an impressive corps of investigators is tenaciously pursuing these suspected cancer agents in the belief that the ultimate understanding of cancer will be found in the molecular structure of the cancer cell and the current efforts are likely to succeed in elucidating that structure.

The discovery 2 years ago of reverse transcriptase, or RNA-directed DNA polymerase, an enzyme that catalyzes the flow of genetic information from RNA→DNA in a surprising reversal of the usual DNA→RNA direction of genetic expression, brought molecular virology to the forefront of cancer research. The enzyme was discovered by Howard M. Temin and Satoshi Mizutani of the University of Wisconsin and, independently, by David Baltimore of the Massachusetts Institute of Technology (*Science*, 28 May 1971).

Because most viruses known to cause cancer in animals have an RNA core, discovery of this enzyme was of particular significance to cancer virus studies. It explained, for the first time, the mechanism by which genes in the RNA of a virus can be incorporated into the DNA of a cell, where they might func-

tion like any other genes. These RNA cancer viruses do not kill cells, but when they are incorporated into cellular DNA and then expressed, they can transform those cells into neoplastic ones.

Whether or not reverse transcriptase turns out to be unique to tumor cells—as was initially suspected, but now seems somewhat less certain—there is no doubt that it has advanced research during the last 2 years by permitting previously unfeasible experiments. "Reverse transcriptase is a very powerful tool for analysis of the cancer problem," says Sol Spiegelman of Columbia University, who was one of the first persons to confirm and extend data about the enzyme. "It allows us to ask whether you can find in a tumor cell the genetic information related to the putative etiologic agent; therefore, it will help us solve the question of whether viruses cause human cancer." The enzyme is also a powerful probe with which to explore normal cells for information about their genetic apparatus. Temin, who recently reported evidence of reverse transcriptase activity in normal embryo cells, believes the enzyme activity is critical to processes of differentiation in healthy, developing cells.

Of the three current RNA cancer virus theories, the provirus hypothesis came first. Formulated by Temin in the early 1960's, it states that first an RNA tumor virus infects a cell, which then incorporates the genetic information lodged in the RNA of the virus into its own DNA and thereby acquires the capacity to produce an oncogenic virus and become transformed from a normal cell to a neoplastic one. According to the provirus hypothesis, the genes for making that tumor virus are located next to each other on the same chromosome of the cell. If malignant transformation is examined from this point of view, the critical genetic event occurs when the exogenous virus infects the cell, which then integrates the cancer virus genes with its own genes. The integration takes place using a viral reverse transcriptase that makes a DNA copy of viral RNA. Once this copy becomes integrated with the cell's DNA, the enzyme system is no longer necessary for neoplastic transformation. "At this point," Temin observes, "the provirus model merges with the oncogene model."

Most people accept the provirus idea as an accurate model of what can occur in animals and, now, some are beginning to think that it might describe a

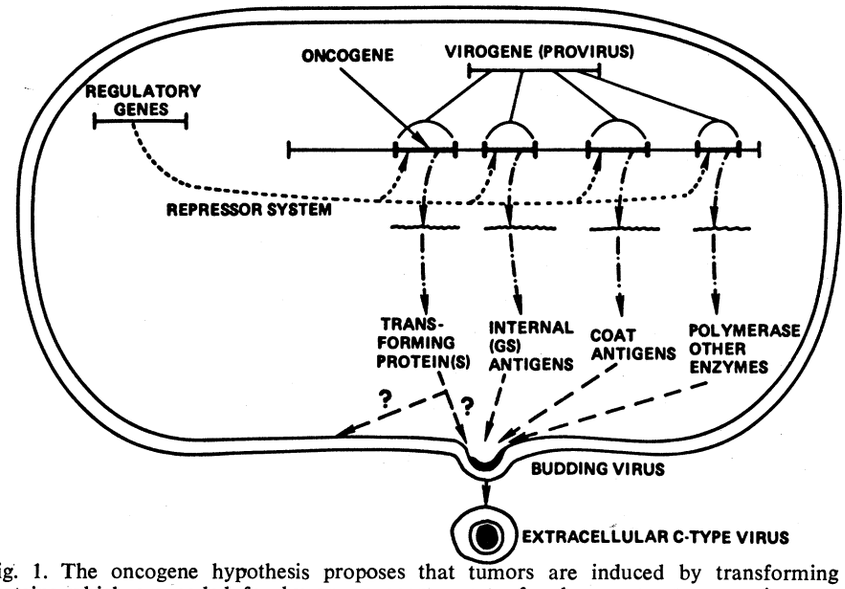

Fig. 1. The oncogene hypothesis proposes that tumors are induced by transforming proteins which are coded for by an oncogene, part of a larger structure, a virogene, which has the capacity to produce a complete tumor virus. Theoretically, oncogenes are turned off in normal cells by regulatory genes that code for a repressor system.

form of horizontal transmission of cancer viruses in man. It is possible, they speculate, that viruses spread from cell to cell within the body.

The oncogene hypothesis, put forward in 1969 by Robert Huebner and George Todaro of the National Cancer Institute, states that the genetic information for cancer already exists in every cell, vertically transmitted from parent to child. According to this model, infection of cells by C-type RNA viruses occurred millions of years ago during the course of evolution. Now, Huebner and Todaro believe, every cell contains an oncogene, a region of DNA that is normally repressed. When it is derepressed, possibly by a virus, a chemical carcinogen, or radiation, it expresses itself by coding for a "transforming protein." When this occurs, a cell may become malignant even though no viruses can be recovered from it.

Todaro explains that the oncogene, as he and Huebner conceive of it, is only one part of a larger structure, the virogene (see Fig. 1). It is the virogene, composed of various bits of genetic information, that must be turned on in order for a complete virus to be made. Thus, the virogene can code for transforming protein, internal and external viral antigens, polymerases, and other enzymes that go into the making of a complete virus. "It is quite possible," Todaro says, "that you do not need complete virogene expression in order to get transformation. In fact, such complete expression may mitigate against cancer, because the body might recognize and destroy the antigens associated with a whole virus while it would be defenseless against the transforming protein of the oncogene."

Recalling the time during which the oncogene hypothesis was formulated, Todaro says, "It was proposed to provide a unifying theory that would counter the view that cancer is really hundreds of distinct diseases with a thousand different causes. Contrary to the assumption that the oncogene theory is pessimistic, in that it says we each carry the seeds of our own destruction, it is optimistic in that it proposes that there is a single mechanism (or relatively few mechanisms) of oncogenesis."

The protovirus hypothesis, which evolved as a logical extension of the discovery of reverse transcriptase, was proposed by Temin in 1970. In ways, it is similar to the oncogene (or virogene) model and, indeed, some scientists see it as a modification of the earlier model. However, the two are distinguishable in certain important ways. The protovirus hypothesis concerns itself with RNA-directed DNA synthesis as a mechanism of information transfer in normal cellular processes, such as differentiation. It is not *exclusively* concerned with oncogenesis but sees the development of cancer as an event that occurs through a "misevolution of protoviruses." The protovirus hypothesis holds that cancer viruses arise from protoviruses—segments of genetic information randomly brought together

Table 1. Outline of the viral cancer theories.

Provirus—1962	Oncogene—1969	Protovirus—1970
1) Genetic information for cancer enters cell through viral infection from outside.	1) Genetic information for cancer exists in every cell.	1) Genetic information for cancer is synthesized *de novo*.
2) Reverse transcriptase is required for that information to be integrated into the host cell.	2) Theory does not postulate that reverse transcriptase is required for cancer production.	2) Reverse transcriptase is required.
3) Proviruses are pieces of genetic information that may be assembled into a whole as predicted by the protovirus theory.	3) Neoplasia, and sometimes whole viruses, arise when pre-existent genetic information is derepressed.	3) Malignancy, and tumor viruses, arise through a process of genetic change in which pieces of genetic information happen to be so assembled that cell transformation occurs through "misevolution."
	4) Oncogene is not continually functioning in normal cells.	4) Reverse transcriptase is present in normal cells where it plays a role in differentiation.
	5) Oncogene is vertically transmitted through the germ line.	5) Only the "potential" for malignant transformation is vertically transmitted.

through a variety of genetic events. (This is in contrast to the oncogene notion of derepression.) Further, the protovirus model maintains that cells do not come into being with a full complement of the genetic information for malignancy (the oncogene) but only with the "potential" for assembling that information. The egg cell, in this view, contains in its chromosomes the "potential" for the genetic evolution of somatic cells that may lead to the *de novo* synthesis of the information for cancer.

As data about the molecular processes of cancer cells accumulate, so does evidence in favor of each of the cancer-virus hypotheses. And, there is increasingly compelling evidence to support the presumption of a viral etiology of the human disease. Yet, as Temin points out, there is still reason to be "skeptical" about it all.

The idea that viruses induce cell transformations characteristic of tumors was originally conceived by two French investigators, A. Borrel and F. J. Bosc, in 1903. Eight years later, Peyton Rous showed that a virus, now known as the Rous sarcoma virus, induces tumors in chickens, and launched the hypothesis that viruses cause cancer. For many years the idea that viruses cause cancer in animals was thought dubious. Today it is clear that Rous was right and the controversy has shifted to the relation between viruses and cancers in man. Many of the principal investigators exploring this relationship presented their latest data early last month when Miles Laboratories, Inc., held its sixth international symposium on molecular biology at the Johns Hopkins Medical Institutions.

During the last few years, several investigators have sought to isolate a whole cancer virus from human tissue and they have found what everyone refers to as "candidate" viruses. Among the newest candidates are the ESP virus, isolated from a human tumor by Elizabeth S. Priori and Leon Dmochowski of the M. D. Anderson Hospital and Tumor Institute in Houston, and the RD 114 virus identified in the laboratory of Robert M. McAllister of the University of Southern California School of Medicine. Whether either of these, or other C-type RNA virus candidates, is really of human origin is moot. Another, somewhat less controversial agent is the B-type virus that has been detected in human milk by Dan Moore of the Institute for Medical Research in Camden, New Jersey, and by others.

Another way to look for human viruses—a way opened by the discovery of reverse transcriptase—is to search human tumor cells not for whole, intact virus, but for viral information. Spiegelman is among those whose research in this area has turned up interesting data during the last year. He presented what he calls "the most convincing evidence yet that a virus is involved in human cancer," early last month at the Johns Hopkins meeting.

Spiegelman's experimental approach relies heavily on a technique known as molecular hybridization. Using it, he has searched for viral information in breast cancer, leukemias, lymphomas, and sarcomas. In the breast cancer study, he and his colleagues took the mouse mammary tumor virus, an RNA virus known to cause breast cancer in mice, and, using reverse transcriptase, made a DNA copy of the virus that contained genetic information from the viral RNA. They exposed that DNA to RNA from human breast cancer tissue to see whether they would get a hybridization reaction. Such a reaction would mean that the DNA made from the mouse tumor virus RNA and the human tumor RNA had some genetic information in common. They found such a homology, indicating that there is a correlation between the oncogenic mouse virus and the genetic messages in human tumor cells.

Similar hybridization experiments were performed using C-type Rauscher mouse leukemia virus, another RNA virus, as the probe. DNA copies of the Rauscher virus RNA hybridize with genetic information in human leukemias, lymphomas, and sarcomas. There is no corresponding hybridization reaction in normal control tissue either in the case of the mouse mammary tumor virus or the Rauscher virus.

These data provide good circumstantial evidence of a correlation between viruses and human cancer but, as Spiegelman himself points out, they do not mean that a viral agent has been identified. More convincing evidence of that came from experiments in which he and his group showed that in cells of many leukemia patients, there exists a reverse transcriptase associated with a high molecular weight RNA and that this RNA is homologous to the RNA of the Rauscher leukemia virus.

In developing these data, Spiegelman showed that the agent he is looking at fulfills three of the requirements that would have to exist to link it closely with known RNA tumor viruses: (i) that it contain a high molecular weight RNA, (ii) that it be associated with a reverse transcriptase, (iii) that it is complementary to a known oncogenic virus.

Many investigators find this evidence convincing, but others believe that it is necessary to get hold of a complete virus to demonstrate the viral etiology of human cancer. There is a similar uncertainty among researchers about the three cancer-virus hypotheses as new data are evaluated (see Table 1).

For example, the oncogene hypothesis, originally derived from the observation that C-type RNA viruses occur naturally in a variety of animal

cells, has gained support recently from experiments in which C-type viruses were induced in normal, supposedly virus-free cells. Wallace P. Rowe of the National Institute of Allergy and Infectious Diseases induced viruses in clones of cells from AKR mice, a strain with a high natural incidence of leukemia, by exposing them to various agents including IdU (iododeoxyuridine) and BrdU (bromodeoxyuridine). Robin Weiss of the University of Southern California Medical School finds he can induce viruses in "clean" chicken cells from normal, healthy domestic animals and from cells of red jungle fowl he trapped during an expedition to the jungles of Malaysia. These latter data, many scientists feel, are indicative of the presence in all cells of the information for tumor viruses because of the assumption that the jungle fowl could not have been contaminated in any way by laboratory viruses. Subsequent experiments by several investigators have shown that viruses can be induced in a number of types of cell lines, including those from animals with a low natural incidence of tumors such as normal 3T3 cells from Balb/c mice.

However, other investigators point out that there is, as yet, no evidence that these inducible viruses are biologically active. Furthermore, they say, induction experiments are also in keeping with the protovirus hypothesis.

Additional data to support the oncogene theory (or the protovirus model, for that matter) would come from the identification of the "transforming protein" that is predicted. As yet, no such protein has been isolated and characterized. One place to look experimentally for these postulated proteins, according to Todaro, is in sophisticated model systems for protein synthesis. Another is in temperature sensitive mutants. Such mutant cells, he explains, make an altered protein (coded for by a virus) which transforms the cells at normal temperatures. However, at high temperatures, these transforming proteins lose their capacity to function and the cells revert to a normal condition. The idea is to find these proteins. "If they are virus-coded," Todaro notes, "they may be part of the virus particle which would be an advantage in that the virus would have done much of the purifying for you. We could isolate them more easily in a virus particle than we could if we have to fish them out of the cytoplasm of the cell."

Another point in favor of the oncogene hypothesis, Todaro says, comes from the fact that viruses derived from cell clones from animals with a natural resistance to cancer do not reinfect the clone from which they come. Viruses isolated from clones from highly susceptible strains, on the other hand, can reinfect. "It is possible," he says, "that susceptible strains can be infected by their own virus because they lack strong repressor systems to keep them in check. Quite probably, this is a phenomenon related to inbreeding. You don't see this in nature. If you did, cancer would be an epidemic disease."

Early in its history, the oncogene hypothesis was broadly criticized as being untestable. Now, with the experimental advances that have come about because of the discovery of reverse transcriptase, that certainly is no longer true if, indeed, it ever was.

The Protovirus Gains Validity

The protovirus theory, which many researchers in the field claim to have difficulty understanding, gained credibility recently from Temin's experiments that indicate the presence in normal embryo cells of a reverse transcriptase activity which appears to be unrelated to any known viral enzyme. (Previous data reported during the last year and a half to the effect that a virus-related reverse transcriptase exists in normal cells must be discounted, many investigators believe. It was based on studies using a synthetic RNA template which has turned out to be nonspecific for the enzyme.)

Temin and his co-workers looked in apparently virus-free chick embryo cells and rat cells infected by Rous sarcoma virus for evidence of reverse transcriptase activity. In both systems, he reports, there is evidence of endogenous RNA-directed DNA synthesis. In both systems, the RNA template of this enzyme activity, he says, is not related to the RNA of Rous sarcoma or other RNA viruses and, therefore, this activity "may represent the activity suggested by the protovirus hypothesis." Although Temin maintains that he has, indeed, found reverse transcriptase activity in normal cells (many of his colleagues are not fully convinced by the data he presented), he as yet claims nothing definitive about its normal biologic role. "Once you show the enzyme activity exists, you can make all sorts of predictions about what it does, but we don't *know* that it does anything."

One question that arises about Temin's finding is whether the enzyme activity he described is really unrelated to any oncogenic virus. Temin's answer is that "There are two ways of looking at it. One can say that the activity is unrelated to known viruses or, if you prefer, that it is related to unknown viruses."

Temin's proposition that reverse transcriptase activity exists in normal cells received unexpected tentative support from work by Robert Gallo and his colleagues at the National Cancer Institute. Extending their own earlier data, they showed that an enzyme purified from the blood lymphocytes of acute leukemia patients has the biochemical properties of reverse transcriptase; this strengthens the link between cancer and viruses.

Then, they went on to report preliminary data indicating that there may be reverse transcriptase activity in *normal* blood lymphocytes that are stimulated to proliferate in vitro. In phytohemagglutinin stimulated cells, they see evidence of an enzyme that carries out endogenous DNA synthesis and is sensitive to ribonuclease, an enzyme that degrades RNA. "Yet, the enzyme," says Gallo, "does not copy exogenous RNA as do the enzymes from tumor viruses and human leukemic cells." Their data are quite similar to Temin's and, as Gallo notes, "lean toward" the protovirus idea. It may be that, if there is reverse transcriptase activity in normal cells, the enzyme has tremendous specificity for a particular RNA and that the cancer enzyme lacks that extra measure of specificity.

Researchers pursuing these various problems at the fundamental level also have in mind possible therapeutic approaches if any of the models, or a combination of them, proves to be correct.

However, with the situation changing as rapidly as it is—new twists in support of each proposition are appearing with great frequency—it may be premature to think of therapeutic approaches tied specifically to one model or the other. It is also premature for anyone to be sure which model will prove to be correct. Some invetigators believe the question will be settled conclusively within the year. Others predict that aspects of each will turn out to be accurate and that there will be a new model, a mutation emerging from the evolution of research.

—BARBARA J. CULLITON