RECENT DEATHS

John Allison, 71; professor emeritus of psychology, Frostburg State College; 6 January.

Joseph C. Aub, 83; professor emeritus of medical research, Harvard University; 30 December.

Harry Bakwin, 79; retired professor of clinical pediatrics, New York University; 25 December.

Edmund deS. Brunner, 84; former professor of sociology, Columbia University; 21 December.

Richard S. Burington, 72; former professor of mathematics, Case Western Reserve University; 24 December.

Walter Cohen, 52; professor of psychology, State University of New York, Buffalo; 20 December.

George H. Estabrooks, 78; former chairman, psychology department, Colgate University; 30 December.

Griffith C. Evans, 86; professor emeritus of mathematics, University of California, Berkeley; 8 December.

Ralph H. Fox, 60; professor of mathematics, Princeton University; 23 December.

Joan P. Giles, 54; research associate professor of pediatrics, New York University; 28 November.

Vincent H. Gillson, 52; assistant professor of pathology, Fairleigh Dickinson College of Dentistry; 20 December.

Salvatore M. Marco, 65; professor emeritus of mechanical engineering, Ohio State University; 15 December.

Max Meenes, 73; professor emeritus of psychology, Howard University; 5 January.

Jacob van de Kamp, 69; retired head, Synthetic Organic Preparations Laboratory, Merck Sharp & Dohme; 22 November.

Wolf V. Vishniac, 51; professor of microbiology, University of Rochester; 10 December.

Joseph L. Walsh, 78; professor emeritus of mathematics, University of Maryland; 10 December.

Louis G. Welt, 60; chairman, internal medicine department, Yale School of Medicine; 13 January.

Gerald L. Wendt, 82; chemist and retired head, publication center, United Nations Education, Scientific and Cultural Organization; 22 December.

RESEARCH NEWS

RNA Viruses: The Age of Innocence Ends

Virologists have traditionally been among the most optimistic of cancer investigators, and for many of them the 1960's were an era of relative innocence. Secure in the knowledge that viruses cause tumors in animals, they were confident that these agents would provide an elegantly simple solution to the problem of human malignancies. If only a human cancer virus could be isolated, many virologists argued, a vaccine could be developed and control of cancer would be a reality.

That attitude engendered a tremendous outpouring of research results—a large number of little-recognized successes and a few more highly publicized failures. The investigators developed tissue culture systems for growing large numbers of virus particles, and thus learned a great deal about the biochemistry of oncogenic (tumor-forming) viruses. They discovered many animal tumor virus systems that served as models for what might occur in humans, and thus learned a great deal about the interaction of virus and host. They also isolated several putative human cancer viruses, and thus learned a great deal about humiliation and the loss of credibility as one after another of the ballyhooed candidates proved to be of nonhuman origin.

The age of innocence has slowly drawn to a close, however, as many virologists have begun to recognize that the problem is substantially more complex than they had originally anticipated. Although some still argue that a tangible oncogenic human virus will eventually be isolated, a growing number of investigators have concluded that this approach may be futile and have thus begun to reconsider the fundamental concepts of the nature of viruses and their role in animal biochemistry.

If viruses do play a causative role in human malignancies, these scientists suggest, it is most likely that the active agent is an incomplete or defective portion of one virus—or perhaps of several viruses—whose normal function is beneficial to the host. Research on oncogenic animal viruses, as a consequence, has been somewhat de-emphasized as investigators have pressed the search for virus fragments or information in human tumors. Nonetheless, there has been a continuing strong interest in ascertaining the normal role of oncogenic viruses, particularly those whose hereditary information is contained as RNA.

Oncogenic RNA viruses (also called oncornaviruses and RNA tumor viruses) are generally divided into three main classes, labeled A, B, and C. Type C RNA viruses, the most important class, have been shown to infect a large number of animal species. Most type C RNA viruses are oncogenic, causing mainly leukemias, lymphomas, and sarcomas—all tumors arising in tissues of mesodermal origin, such as bone, cartilage, connective tissue, and lymph nodes. Type B RNA viruses, which are fewer in number, have been associated primarily with certain tumors (carcinomas) of the breast. Type A RNA viruses, which are not infectious, are a very small group of viruslike particles that have not been found outside the confines of cells and that have not been shown to be oncogenic.

A principal difference between oncornaviruses and other animal RNA viruses lies in the size of the genome, the complete set of hereditary information contained in the chromosomes. Oncornavirus genomes have a mass of about 12×10^6 daltons, compared to about 6×10^6 daltons for the paramyxoviruses and about 2×10^6 daltons for poliomyelitis virus. Perhaps as a result of this large genome, oncornaviruses have a more complex internal structure with no clearly observable symmetry. They may also contain more types of proteins and more species of nucleic acids.

Distinctions among the various types of oncornaviruses have been based on morphology, but they can also be made on immunological differences and modes of maturation. The type C RNA viruses consist of a roughly spherical, compact nucleoid (that is, RNA and the associated proteins) surrounded by an electron-lucent lipid layer that gives electron micrographs of the virus a targetlike appearance (Fig. 1E). The nucleoid of the type B viruses is more eccentric in shape (Fig. 1D), apparently because its major internal protein is about two-thirds larger than that of the type C viruses. The glycoprotein surface spikes of the type B viruses are also larger and more regularly spaced than those of the type C viruses.

The type A particles occur in two subtypes, one found in cellular cytoplasm and one found in cisternae, reservoirs for lymph and other body fluids. Those found in the cytoplasm are believed to be immature forms of type B viruses, to which they are immunologically similar, and there is speculation that those in the cisternae are immature type C particles. The morphology of type A particles is similar to that of the other viruses (Fig. 1A), but the type A particles are encapsulated by a protein shell rather than by a lipid-containing membrane.

Contain Reverse Transcriptase

The most important characteristic of the oncornaviruses is that they contain an RNA-directed DNA polymerase, or reverse transcriptase. Reverse transcriptase was discovered in 1970 by Howard M. Temin and Satoshi Mizutani of the McArdle Laboratory for Cancer Research at the University of Wisconsin Medical School, Madison, and independently by David Baltimore of the Massachusetts Institute of Technology, Cambridge. Discovery of this enzyme, which mediates the synthesis of DNA from an RNA template, provided the first biochemical evidence of a mechanism for perpetuation of the viral genome when a cell divides.

After an oncornavirus has entered a cell and shed its protein coat, the first important step in infection, most investigators now agree, is production of a DNA copy of the viral genome by the reverse transcriptase. Several lines of evidence confirm the presence of this intermediate, called the provirus, but perhaps the most conclusive evidence is provided by M. Hill and Jana Hillova of the Institut Gustave-Roussy in Villejuif, France. They found that RNA-free DNA from chicken cells transformed by Rous sarcoma virus in turn transforms uninfected chicken cells and mediates the production of more Rous virus. H. Hanafusa of the Public Health Research Institute of the City of New York has also shown that mutant type C RNA viruses that do not contain a reverse transcriptase are not infectious.

The provirus, once formed, is generally believed to be integrated into the host cell's genome to produce a virogene—a gene that is the template for the production of a virus. (The terms provirus and virogene are often considered synonymous.) Several investigators have shown that the virus contains an endonuclease, an exonuclease, and a ligase, all the enzymes necessary for cleaving cellular DNA, inserting the provirus, and mending the break. There is no evidence that these enzymes actually perform this fuction.

Several Pathways Possible

Once the provirus is integrated, several alternative pathways are possible. Since the host's immune systems react primarily to viral proteins, integration provides a way for the provirus to replicate while remaining shielded from the immune defenses. Proliferation of the infected cell results in transmission of the virogene to the daughter cells without the appearance of viral protein or intact viruses. If the infected cell is a germ cell, moreover, the virogene is also transmitted to the host's progeny. Parental infection of progeny is known as vertical transmission.

Under certain, as yet undetermined circumstances, the virogene can be activated, and the cell will begin producing new virus particles that may infect neighboring cells or other organisms of the same species. This type of transmission is known as horizontal transmission. And finally, if the virus is oncogenic or if it acquires additional information to become oncogenic, the same set of circumstances that initiate virus production—or perhaps a slightly different set of circumstances that activate only part of the virogene—may convert the infected cell into a tumor cell.

This last possibility, which is one manifestation of the oncogene theory developed by Robert J. Huebner and George J. Todaro of the National Cancer Institute (NCI) in Bethesda, Maryland, is still considered rather speculative. But the other alternatives are supported by firm experimental evidence, the foremost of which is the induction of type C RNA viruses from apparently virus-free cells.

This induction was first demonstrated in 1971 by Wallace P. Rowe of the National Institute of Allergy and Infectious Diseases, Bethesda. Rowe cultured cells from a strain of mice with a high natural incidence of leukemia and exposed the cells to certain types of chemical mutagens such as bromodeoxyuridine. After this exposure, the cells began to produce an RNA virus similar to, but distinct from, the murine leukemia virus. Subsequent experiments by several others have shown that such viruses, called endogenous viruses, can be induced in many cell lines, including cells from animals with a low natural incidence of tumors. None of the endogenous viruses induced in this fashion have been shown to be oncogenic, however.

More recently, Robert M. McAllister of the University of Southern California Medical School, Los Angeles, inadvertently discovered an apparently different type of endogenous virus. McAllister injected cells from a human sarcoma into the brain of a kitten and observed the release of a type C RNA virus, called RD-114, that he initially thought might be a human cancer virus. Intensive investigations in his and a half-dozen other laboratories, however, soon revealed that RD-114 was actually a hitherto unknown feline RNA virus distinct from the well-known feline leukemia virus.

A New Class

Unlike the chemically induced viruses, which replicate to a limited extent in the species of origin, RD-114 will not, in general, replicate in feline cells. It was thus one of the first examples of a class now known as xenotropic viruses—endogenous viruses that do not, under most conditions, replicate in the species of origin. It is possible that the chemically induced viruses are merely forms of the xenotropic viruses that have mutated slightly so they can replicate in the species of origin, but there is no firm evidence to support this thesis.

Xenotropic viruses have also been isolated from several other species, including chickens, hamsters, mice, rats, and pigs. It now seems possible, moreover, that each species has a unique virus shared by all its members. Jay A. Levy of the Cancer Research Institute at the University of California, San Francisco, has demonstrated, for

example, that apparently identical xenotropic viruses can be induced from all strains of mice, including wild field mice, that he has examined. The limited evidence yet available suggests that an analogous situation occurs in other species.

There are apparent exceptions to the principle that xenotropic viruses will not grow in the species of origin. Many investigators have observed what they thought to be type C RNA viruses in tissues from a variety of species, but have assumed that the viruses were not infectious because they did not proliferate in cultures of the same tissues. These observations have occurred most frequently in embryonic and placental tissues.

Endogenous Primate Viruses

S. S. Kalter and his associates at the Southwest Foundation for Research and Education, San Antonio, Texas, have obtained electron micrographs of what appear to be endogenous type C particles in baboon and monkey placentas. These putative viruses do not replicate in cultured primate placental cells, but Todaro has shown that those from the baboon will infect dog brain tissues, and several investigators are now attempting to characterize them. Kalter and others have also observed such particles in human placental tissue, but no one has yet been able to find tissues in which these putative endogenous human viruses will replicate. None of the xenotropic viruses have been shown to be oncogenic in any species.

The scenario that has emerged from these findings is thus considerably more complicated—and confusing—than that which was envisioned only 2 or 3 years ago. Whereas virologists were then fairly confident that one virus might be the cause of any particular tumor, they are now faced with the possibility that two, three, or perhaps even more might be involved.

At least one strain of mice, Huebner points out, is already known to harbor three different type C RNA viruses. The first, believed to be an exogenous virus, is the principal virus isolated from mouse tumors (murine leukemia virus) and produces tumors when injected into newborn mice of the same strain. The second, a chemically induced endogenous virus, replicates poorly in embryonic mouse cells in culture and does not produce tumors in newborn mice. The third, also an endogenous virus, does not replicate in cultured mouse cells and does not produce tumors. Even though the last ~~two~~ types do not produce tumors in newborn mice, many virologists think that these types are nonetheless implicated in the etiology of cancer. There is some evidence to suggest that a similar number of RNA viruses may occur in animals of other species, but the extrapolation to humans is still speculative since there is as yet no firm evidence for the presence of complete type C RNA viruses in human tumors.

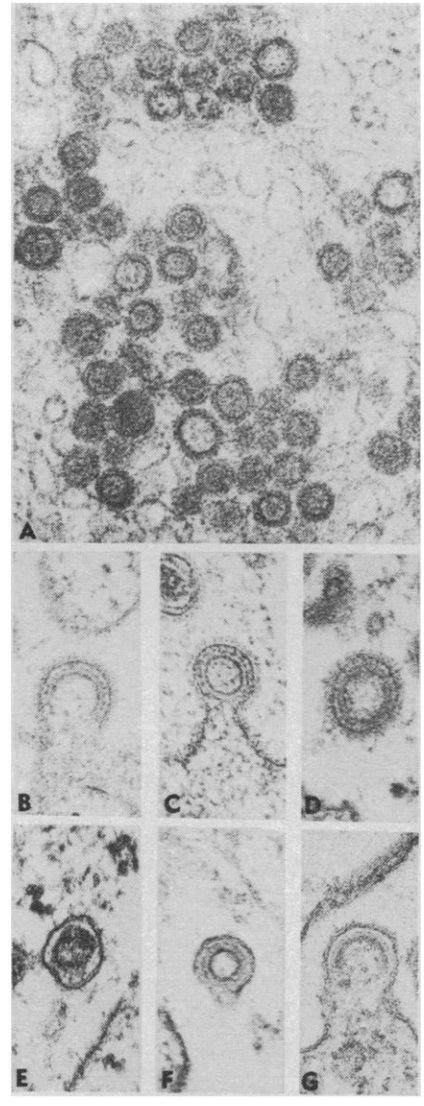

Fig. 1. Electron micrographs of oncornaviruses [magnifications: (A) ~ × 100,000; (B to G) ~ × 140,000]. (A) Group of intracytoplasmic type A particles in a mouse mammary tumor. (B) Type B particle budding from a mouse mammary tumor cell. (C) Late bud of type B particle from mouse mammary tumor. (D) Free, immature, type B particle with spikes on surface, electron-lucent center in the nucleoid, and spokes radiating from outer surface of the nucleoid to inner surface of the envelope. (E) Type C particle in extracellular space. (F) Late bud of type C particle in tissue of human embryo kidney cells infected with a strain of Rauscher leukemia virus. (G) Type C particle budding from human embryo lung cell in tissue culture infected with feline leukemia virus. [Source: Albert J. Dalton, National Cancer Institute]

The situation becomes even more complicated when DNA viruses are brought into the picture. Sol Spiegelman and his associates at Columbia University's Institute for Cancer Research, New York City, have reported that the incidence of Marek's disease, a lymphoma of chickens, increases in fowls exposed to both a DNA virus (Marek's disease herpesvirus) and a type C RNA virus called Rous associated virus type 2. Chickens are also known to have a virogene that can produce at least one endogenous virus that may be implicated in the etiology of Marek's disease.

An even more complex situation is observed in human nasopharyngeal carcinoma, a malignancy of the nasal cavity, pharynx, and oral cavity. By molecular hybridization, Harald Zur-Hausen of the University of Erlangen-Nurenberg, Erlangen, West Germany, has found in nasopharyngeal tumors DNA sequences homologous to those of the Epstein-Barr virus, a DNA herpesvirus that has been tentatively associated with some types of cancer. This finding suggests that the virus—or hereditary information derived from it—is involved in the etiology of the tumor.

Four Different Viruses

Spiegelman, using a similar technique, has reported that nasopharyngeal tumors contain DNA sequences homologous to RNA sequences in a type C RNA virus that causes similar tumors in experimental animals. And Albert Sabin, while working at NCI's Frederick Cancer Research Center in Frederick, Maryland, reported that the tumors contain an antigen (a protein or glycoprotein that elicits an immune response) characteristic of the herpes simplex virus. Many virologists also believe, as is suggested by the observation of placental particles, that humans have a virogene that can produce at least one type C RNA virus. There are, then, four types of viruses that might be associated with this tumor.

Most of the evidence suggesting this complexity has been developed within the last year, and the investigators have had little time to sort out all of the implications. But the steady accretion of unexpected new results has initiated a major rethinking of the nature of

viruses and of their role in the transmission of hereditary information within and between organisms.

In the first place, many investigators are beginning to accept the conclusion that infectious oncogenic viruses are the exception rather than the rule. Strongly transforming viruses such as the Rous sarcoma virus, which transforms nearly all the cells it infects, are very rare. They can be maintained only by passage through laboratory animals, and are generally agreed to be artifacts.

Those oncornaviruses that do cause tumors in nonlaboratory animals are only weakly transforming. These viruses are generally transmitted from parent to progeny, but some have been shown to be infectious also. William Hardy and Lloyd Old of the Memorial Sloan-Kettering Cancer Center in New York City recently demonstrated, for example, that feline leukemia virus is transmitted both vertically and horizontally.

Tumors a Rare Response

In most cases, tumors are a very rare response to infection by oncornaviruses; their efficiencies of transformation are as much as a dozen powers of 10 lower than that of the Rous sarcoma virus. When transformation does occur, moreover, the responsible virus can generally be recovered from the tumor or its presence can be demonstrated in some other manner. Since such viruses have never been definitively demonstrated in human tumors, Temin reasons, it seems likely that the oncogenic animal viruses provide an analogy for human cancers rather than an etiology. That is, experiments with the oncornaviruses are useful in providing information about the formation and expression of genes that might be responsible for malignancies in humans, but it is unlikely that an infectious virus causes cancer in humans.

One of the major conclusions that some scientists have reached from animal experiments is that a virus must be integrated into the host cell's genome before it can transform the cell. A corollary to this conclusion is that transformation is controlled genetically rather than epigenetically; that is, transformation can be caused only by a change in gene structure and not by cellular changes altering gene expression. Many scientists, particularly some of those investigating chemical carcinogenesis, would disagree with this latter conclusion.

But whether the mechanism is genetic or epigenetic, all the evidence indicates that only RNA viruses that contain a reverse transcriptase are potentially oncogenic. (DNA viruses, of course, can be integrated directly and thus do not exhibit this requirement.) Neither the presence of a reverse transcriptase nor integration, however, is sufficient for oncogenicity. Todaro and Wade Parks of NCI have suggested, for example, that syncytium-forming RNA viruses (viruses that cause cells to fuse into large masses) replicate through a DNA intermediate but are not oncogenic, possibly because they replicate in the cell's cytoplasm and are thus physically separated from the cellular DNA. And Harold E. Varmus of the University of California, San Francisco, and Ashley Haase of the San Francisco Veteran's Administration Hospital have shown that visna virus—a sheep virus which has never been demonstrated to cause tumors in animals—replicates through an integrated DNA intermediate.

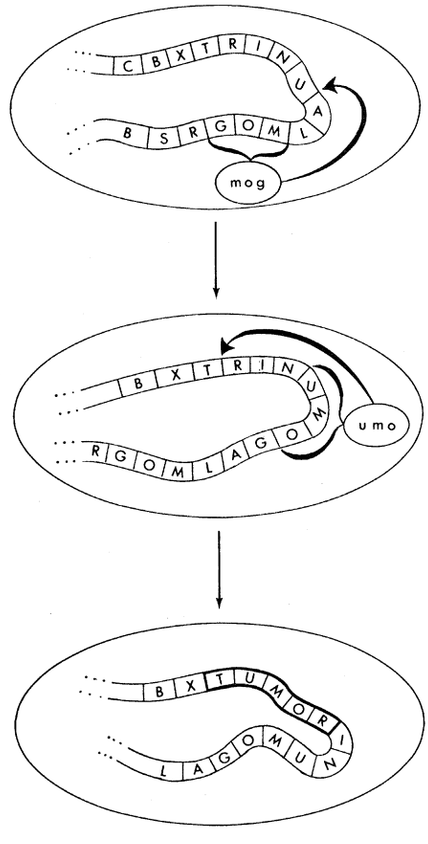

Fig. 2. One method by which the protovirus can realign chromosomes within a cell to produce oncogenic information. The protovirus transcribes a segment of the gene into RNA (lowercase letters), which is then copied into DNA and inserted into the chromosome at a new location. Repetition of this process can, in some cases, produce a side-by-side alignment of the genes (outlined in bold) that are necessary for carcinogenesis.

If it is granted, then, that oncogenic information must be integrated in the host genome at the time of tumorigenesis, there are two main theories about how the information gets there if there is no exogenous infection. The oncogene theory, proposed by Huebner and Todaro in 1969, postulates that the virogene, which is now thought to be present in all cells, contains oncogenic information that is normally repressed by cellular mechanisms. The virogene, according to this theory, thus contains an oncogene, a segment of the genome that contains information necessary for the transformation of cells.

A Mutable Gene

The protovirus theory, proposed by Temin in 1970, postulates that the virogene or a precursor is, in effect, highly mutable, and that oncogenic information is occasionally synthesized de novo. The oncogene theory thus predicts that information for transformation is transmitted vertically through the germ line, whereas the protovirus theory predicts that only the potential for synthesis of the information is transmitted. Neither theory requires the expression of complete virus particles for the initiation of cancer.

The oncogene theory represented a significant extrapolation from existing data. In one sense, however, it might be termed an ad hoc theory: It provides a possible explanation for tumorigenesis, but it offers no normal role for the oncogene or the virogene. In the absence of a normal role, many scientists argue, there would be no evolutionary pressure for maintenance of the gene—it would provide no benefit or advantage to the host organism—and it is unlikely that it would be universally incorporated in animals, as the virogene appears to be.

The oncogene theory also predicts a relatively static genome, whereas much recent evidence suggests that the interaction between virus and genome produces a great deal of change. The oncogene theory is thus falling into disfavor among virologists, although not all who reject it would embrace the protovirus theory.

The protovirus theory, on the contrary, offers a method by which genetic evolution can occur in cells other than germ cells. Temin suggests that the

normal function of the virogene is to provide information transfer between cells or between chromosomes in one cell, and perhaps to provide for the synthesis of new hereditary information. The synthesis of genetic information necessary for oncogenesis could thus be simply a normal—albeit rare—result of the functioning of this system.

In simplest terms, the protovirus theory suggests that the protovirus (virogene) directs the production of an RNA copy of certain segments of the cellular genome and then packages the copy and a reverse transcriptase in a form that is able to enter the nucleus of a neighboring cell. In the infected cell, the reverse transcriptase produces a DNA copy of the RNA, which is integrated into that cell's genome. This process may also occur at a different site on the genome of the original cell.

After integration, the new information may simply remain quiescent in the host's genome, it may alter the biochemistry of the host cell, or it may be repackaged to infect other cells, depending on the state of the host cell's genetic controls. This transfer of information from DNA to RNA to DNA could serve, for example, to recruit and identify cells during the process of embryonic induction (differentiation) and to permit new genetic information to be encoded during the lifetime of a single organism (gene amplification).

Synthesis of New Information

The possibility for generation of new information arises if either the transcription, the integration, or both, are not precise. Consider, for illustration, the hypothetical sequences *abcdefgh* in the genome of one cell and *lmnopqrs* in that of another, and suppose that normal functioning of the virogene would involve transcription of *abcd* from the first and insertion of this fragment between *m* and *n* in the second. If a mistake were made in transcription, the resultant sequence in the second cell might be *lmbcdenopqrs*.

Most of the time, this newly synthesized information (*mb* and *en*) will be either nonsense or irrelevant to the cell, and in some cases perhaps even lethal. But in a small number of cases, the new information will be beneficial to the host organism, and in even a smaller number of cases, the new information may be oncogenic. If the protovirus does not integrate at a predetermined site, this process might also lead to a random selection and interchange of genes; development of the potential for transformation might then simply require the side-by-side alignment of preexisting genes (Fig. 2) without a requirement for synthesis of new information. In either case, once the oncogenic information is present in the cell, transformation can occur without the expression of viral particles.

An analogous type of process has been demonstrated for DNA viruses that integrate into the cellular genome. That process is somewhat different, however, in that the DNA which forms the new virus is physically excised from the cellular genome. Imprecise excision can thus leave some of the viral DNA behind and incorporate host DNA into the new virus. Ernst Winocur and Niza Frankel of the Weizmann Institute in Israel have shown, for example, that polyoma and SV40 viruses contain increasingly greater amounts of host DNA after serial passage through cultured cells. After six passages, they find, as much as 50 percent of the DNA in the virus is of host origin.

Less Data for RNA Viruses

There is much less data to support this possibility for RNA viruses. One important requirement of the protovirus theory is that a reverse transcriptase must be present in healthy cells, and this requirement has been a major stumbling block. Several early reports of reverse transcriptases in normal cells have subsequently been discounted because they were based on results obtained with a synthetic RNA template that has since been shown to be nonspecific for reverse transcriptase. Only Temin has thus far been able to demonstrate conclusively that this situation does occur.

In 1972, Temin and Chil-Yong Kang of the McArdle Laboratory reported reverse transcriptase activity in uninfected chicken embryos and demonstrated that the responsible enzyme is distinct from the reverse transcriptases in avian oncornaviruses. Unlike viral reverse transcriptase, however, the chick embryo enzyme does not accept exogenous RNA as a template, indicating that it has great specificity for a particular cellular RNA. Robert C. Gallo of NCI has produced less definitive evidence suggesting that a similar reverse transcriptase activity may be present in normal human lymphocytes. (This activity, however, is observed only when the lymphocytes are treated with phytohemagglutinin, a chemical that stimulates mitosis.) These reports have provided the first major evidence in support of the protovirus theory.

Temin has, in fact, carried the protovirus theory one step further. Last year, he and Mizutani demonstrated that the reverse transcriptase of a new avian oncornavirus, reticuloendotheliosis virus, is closely related serologically to the normal chick embryo DNA polymerase and to the reverse transcriptases of other avian oncornaviruses (which are less closely related to the normal polymerase). It is quite possible then, that the reverse transcriptase in reticuloendotheliosis virus has evolved from the normal cellular polymerase, and that the other avian reverse transcriptases have also evolved from this source. Gallo has observed the same type of relationship between a human DNA polymerase and reverse transcriptases from primate oncornaviruses.

Oncornaviruses Evolved from Cells

Temin thus suggests that the avian oncornaviruses, and perhaps all oncornaviruses, have evolved from normal cellular components. At some point in history, conceivably, the endogenous viruses have mutated in such a fashion that they were freed from the cell's genetic controls and became able to replicate in other cells. The major evolutionary changes, Gallo adds, may have occurred when the viruses crossed interspecies barriers.

The implication, then, is that genetic controls are more effective in humans than in other species, or perhaps evolutionary pressures are different, so that endogenous human viruses have not been able to escape from cellular control. Although endogenous human viruses may be able to assemble or synthesize oncogenic information in susceptible cells, the virus that has done the assembling has not yet been able to escape and infect other humans.

The cancer virologists have thus gotten results substantially different than those they had originally bargained for. The situation is, in fact, quite analogous to that immediately after World War II when physicists naively set out to build a fusion power plant and instead created the new discipline of plasma physics. The virologists set out to isolate a human cancer virus and instead appear to have created a new discipline of (for lack of a better term) viral genetics.

—THOMAS H. MAUGH II