Recent Developments Toward a Unifying Concept of Carcinogenesis

DANTE G. SCARPELLI, M.D., PH.D.

Department of Pathology,
Northwestern University Medical School,
Chicago, IL 60611

ABSTRACT

Although considerable progress has been made toward identification of the various factors involved in the causation of cancer, little is known about how they mediate the neoplastic transformation. Recent developments in the molecular biology of tumor viruses and cancer genetics have established that normal mammalian cells contain genes sequences (oncogenes) that are homologous to those in certain oncogenic RNA viruses in mouse, rat, and chicken. These sequences are turned off or expressed at very low levels of activity in normal cells and conversely turned on in the neoplastic state where, in some instances, large amounts of a specific gene product are produced which appears to be linked to the neoplastic transformation. Oncogenes may represent the final common pathway through which chemicals, ionizing radiation, oncogenic viruses ultimately cause cancer.

Although the somatic mutation theory of carcinogenesis has been the accepted dogma for many years, it has not been clear precisely how different agents which are known to induce cancer, such as certain chemicals, viruses and ionizing radiation, ultimately effect the neoplastic transformation. In this communication some general fundamental aspects of chemical, radiation, and viral carcinogenesis will be reviewed and interpreted in the light of recent developments in cancer genetics which promise to bring an understanding of fundamental mechanisms common to carcinogenesis regardless of etiology.

Chemical Carcinogenesis

The implication that "substances" in the environment might be involved in the development of cancer in humans first became apparent in the 18th century through the observations of John Hill in 1761, Samuel von Soemmering in 1795, and Percivall Pott in 1775 who pointed out the development of cancer in persons using tobacco, and in chimney sweeps constantly exposed to flue dust. This was followed by other examples of occupational carcinogenic hazards such as arsenic, and aniline dyes, both of which were established by astute physicians who noted the frequent occurrence of specific cancers in persons working in certain industries.

The involvement of "substances" in the development of cancer remained largely a matter of conjecture until the report of the experimental induction of squamous cell cancer by repeated applications of coal tar to the ears of rabbits by Yamag-
iwa and Ichikawa in 1918. The isolation of a carcinogenic chemical, 3,4-benzpyrene from coal tar, was accomplished 14 years later by Kennaway and his associates. From these early beginnings the number of carcinogenic chemicals has increased to several hundred or so and include different classes of compounds, many of which are synthetic ones derived as a result of our high degree of industrial development. Others are derived from cultural practices such as smoking; finally, there are a relatively small group of naturally occurring carcinogens.

Much progress has been made in our understanding of what happens when carcinogenic compounds enter the body by inhalation, absorption, or ingestion. The results of a series of now classical studies by the Millers and Weisburgers and their coworkers have led to the general acceptance that most carcinogenic chemicals require metabolic alterations by enzymes in the host tissues to forms which are carcinogenic, a process termed activation. The activated form of a carcinogen is much more reactive than the parent compound and, as a consequence, interacts readily and rapidly with a variety of intracellular macromolecules such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and proteins. How such interactions ultimately lead to the induction of cancer has been and continues to be a major thrust of contemporary cancer research. Since the transformation of a normal cell into a neoplastic one involves a permanent, heritable alteration in phenotype, which is passed on from parent to daughter cell, it is not surprising that the neoplastic state has been long suspected of arising as a result of mutation. This notion is supported by the fact that most carcinogens are mutagens. Accordingly, much attention has been focused on the binding and subsequent chemical interaction of carcinogen metabolites with DNA. These metabolites are molecular species which are strong electrophiles, such as carbonium and nitrenium ions. Such ions attack nucleophilic oxygen as well as nitrogen atoms of the bases of DNA and its phosphate backbone. The most reactive groups in adenine, guanine, cytosine and thymine, the four bases of DNA, are the purine nitrogen atoms; in order of descending reactivity these are N-7 of guanine, and N-3 and N-7 of adenine. In addition to the foregoing, chemical carcinogens also interact to a lesser extent, at several other positions in the pyrimidine and purine bases of DNA. These are O-6, N-3, 2-NH₂ and C-8 of guanine; 6-NH₂, N-1 of adenine; N-1, N-3, and C-5 of cytosine; and O-4 and C-6 of thymine. The use of certain chemicals which are methylating agents and carcinogens as model compounds has given some insight into the nature and variety of DNA adducts shown in figure 1 which may be involved in mutagenesis and carcinogenesis in one class of carcinogen.

Despite considerable efforts to dem-

![Figure 1](image-url)
onstrate clear relationships between the sites of adduct formation on DNA bases, mutagenesis, and subsequent carcino­genesis, these have not been unequivocally established. In addition to the classes of chemical carcinogens that are metabolized to active forms, a number of carcinogens have been identified that are not activated by metabolism, which do not damage DNA, and are not mutagenic when tested in vitro. One class of such compounds, consisting of hypolipidemic drugs and industrial plasticizers, has been the recent focus of intensive study. These compounds are potent hepatocarcino­gens which do not bind to DNA or damage it! Reddy and his coworkers suggest that since these compounds stimulate synthesis of enzymes which produce sufficiently high levels of $\text{H}_2\text{O}_2$ and oxygen free radicals to cause extensive peroxi­dation of cell membranes, they could also lead to oxidative damage of DNA and subsequent mutagenesis. There is ample evidence that oxygen radicals are capable of causing mutation as a consequence of DNA damage owing to strand breaks and base alteration. In many ways, this would be similar to injury from oxygen free radicals produced by ionizing radia­tion which is also a potent mutagen and carcinogen. Regardless of the mecha­nism(s) by which DNA is damaged by chemicals, such damage is considered to be of salient importance in the causation of cancer by inducing a loss of growth control of the affected host cells.

It is important to note that experimen­tal and clinical evidence point to the fact that the development of cancer following exposure to chemical carcinogens is a relatively rare event. This can in part be explained by the ability of affected cells to identify rapidly and to excise segments of damaged DNA, and to repair the in­jury by synthesis of identical single strands of the required segment which are then spliced into the defect. This biological property, which appears to be ubiquitously present in living material, is so efficient that it imparts a survival advantage which has allowed life to con­tinue in an adverse environment. The identification of persons with an impaired capacity to repair DNA, as a con­sequence of rare, autosomal recessive, genetic disorders and who have a high propensity to develop cancer at a young age, is clinical evidence which tends to support the somatic mutation theory of cancer. In one such syndrome, xero­derma pigmentosum, in which patients are extremely sensitive to sunlight and are unable to excise thymine dimers induced by ultraviolet light, the incidence of squamous cell cancer of the skin approaches 100 percent by early adult­hood. Despite such evidence supporting the notion that mutation of DNA is central to carcino­genesis, there is evi­dence that epigenetic changes in gene expression may also be involved.

Once mutagenesis has occurred and the cell has been “initiated”, its evolution to a cancer cell is a slow process which, in the case of humans, may take many years. This process, called “promotion”, is capable of considerable modulation by other chemicals to which the host is exposed, hormones and other endogenous growth factors, and cell replication. Since de­tailed discussion of the evolution of chemically induced cancer is not the in­tent of this presentation, promotion will not be considered further.

Radiation Carcinogenesis

The carcinogenic effects of ionizing radia­tion became known within a few years after the discovery of x-rays and radium in 1895 and 1898. By 1902, cases of skin cancer induced in humans, by chronic exposure of physicians to x-rays, were being reported from Europe and the United States. Since then, there have been numerous other examples of radiation carcino­genesis both in experimen-
tal animals\textsuperscript{10} and in humans.\textsuperscript{5,6,14} These include the classical report of Martland\textsuperscript{20} on the development of osteogenic sarcoma in watch dial painters who had chronically ingested minute amounts of radium sulfate paste in their work, and the high incidence of leukemia in the atomic bomb survivors of Hiroshima and Nagasaki.\textsuperscript{15}

The various types of ionizing radiation are classified according to their physical properties into those that are electromagnetic, nonparticulate, and devoid of mass and charge, and those that are particulate, have mass, and may be charged. These are summarized in Table I. The effects of ionizing radiations on cells are initiated with the absorption of energy by cell substance. Absorption of energy by matter leads to the ionization when the energy is sufficient to expel external electrons from component atoms rendering them positively charged. Expelled electrons interact with adjacent atoms, giving them a negative charge which leads to the formation of ion pairs. In the case of x-rays or gamma rays, the ejected electrons are so highly energized that multiple ion pairs are formed. Alpha and beta rays also displace electrons, if sufficiently energetic, and these become secondary ionizing particles. Chemical bonds are broken following the absorption of energy when it is greater than that of the bond. Interaction of ionizing radiation with cell substance excites atoms to form ions, which lead to localized physicochemical disturbances in the cell.\textsuperscript{2}

<table>
<thead>
<tr>
<th>Type</th>
<th>Mass</th>
<th>Charge</th>
<th>Description</th>
<th>Tissue Penetration</th>
</tr>
</thead>
<tbody>
<tr>
<td>X rays</td>
<td>None</td>
<td>None</td>
<td>Electromagnetic &amp; nonparticulate</td>
<td>High (feet)</td>
</tr>
<tr>
<td>Gamma (γ) rays</td>
<td>None</td>
<td>None</td>
<td>Electromagnetic &amp; nonparticulate</td>
<td>High (feet)</td>
</tr>
<tr>
<td>Beta (β) rays (particles)</td>
<td>1/1836</td>
<td>Either + or -</td>
<td>Charged electrons</td>
<td>Very low &lt; mm</td>
</tr>
<tr>
<td>Alpha (α) rays</td>
<td>4</td>
<td>+2</td>
<td>Ionized helium atoms</td>
<td>Intermediate between α &amp; β</td>
</tr>
<tr>
<td>Protons</td>
<td>1</td>
<td>+1</td>
<td>Hydrogen nuclei</td>
<td></td>
</tr>
<tr>
<td>Neutrons</td>
<td>1</td>
<td>None</td>
<td>Neutrons</td>
<td>High (feet)</td>
</tr>
</tbody>
</table>

Research on the radiochemistry of water has clarified the nature of the reactions that probably occur in irradiated cells. These become significant since cell substance is a complex mixture of nucleic acids, proteins, carbohydrates, and lipids, present either in aqueous solution or in suspension. Since the major component of the cell is water, cells absorb energy from ionizing radiation to a greater extent than will solute molecules, which are more infrequent. Interaction of ionizing radiation with water leads to the formation of ionized molecular species such as H\textsubscript{2}O\textsuperscript{+} and H\textsubscript{2}O\textsuperscript{-}, which dissociate to form H\textsuperscript{+}, OH\textsuperscript{-}, which rapidly react with each other, with other water molecules, with their own reaction products, and with other substances in the cell including macromolecules. Such interactions of free radicals are largely dependent on their volume concentration, i.e., how closely together the free radicals are formed. As the energy transferred from ionizing radiations to surrounding matter increases, the number of free radicals formed and the interactions with nearby molecules also increase. Various types of ionizing radiations differ in the amounts of energy they lose as they pass through matter. High penetrating gamma rays lose energy over a long distance and generate very low ion concentrations. On the
A UNIFYING CONCEPT OF CARCINOGENESIS

Summary of Various Reactions that Occur During Irradiation of Water Molecules

<table>
<thead>
<tr>
<th>Initial reactions</th>
<th>Radiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{H}_2\text{O}^+ \rightarrow e^- + \text{H}_2\text{O} )</td>
<td>( \text{H}_2\text{O}^+ + e^- \rightarrow \text{H}_2\text{O} )</td>
</tr>
<tr>
<td>( \text{e}^- + \text{H}_2\text{O} \rightarrow \text{H}_2\text{O}^- )</td>
<td>( \text{H}_2\text{O}^- \rightarrow \text{H}_2\text{O} + \text{e}^- )</td>
</tr>
</tbody>
</table>

Reactions of free radicals:

<table>
<thead>
<tr>
<th>Radiation products</th>
<th>H(^+) + OH(^-) --- ( \rightarrow \text{H}_2\text{O} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{OH}^- + \text{H}_2\text{O} \rightarrow \text{H}_2\text{O}_2 )</td>
<td>( \text{H}_2\text{O}_2 )</td>
</tr>
<tr>
<td>( \text{OH}^- \rightarrow \text{H}_2\text{O}_2 )</td>
<td>( \text{H}_2\text{O}_2 )</td>
</tr>
</tbody>
</table>

Reactions with organic molecules:

<table>
<thead>
<tr>
<th>Radiation products</th>
<th>( \text{HO}_2^- + \text{OH}^- \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{HO}_2^- + \text{H}_2\text{O} \rightarrow \text{R}^- + \text{H}_2\text{O}_2 )</td>
<td>( \text{R}^- + \text{H}_2\text{O}_2 )</td>
</tr>
<tr>
<td>( \text{RH} + \text{HO}_2 \rightarrow \text{HO}^- + \text{H}_2\text{O} )</td>
<td>( \text{HO}^- + \text{H}_2\text{O} )</td>
</tr>
</tbody>
</table>

other hand, radiations with low penetration such as alpha rays give rise to high ion concentrations. X-rays and beta rays and particles with intermediate penetration generate moderate ion concentrations. \( \text{H}_2\text{O}^+ \) and \( \text{H}_2\text{O}^- \) are unstable and dissociate in about \( 10^{-16} \) second; the free radicals formed react with nearby molecules in about \( 10^{-5} \) second.\(^2\) These reactions are summarized in table II.

Irradiation of deaerated aqueous solutions of a small organic molecule such as glycine leads to its decomposition, a reaction that may also occur in irradiated cells.

\[
\text{OH}^- + \text{NH}_3\text{CH}_2\text{COO}^- \rightarrow \text{OHCH}_2\text{COO}^- + \text{NH}_3^+ \quad \text{and} \quad \text{NH}_3^+ + \text{H}_2\text{O} \rightarrow \text{NH}_4^+ + \text{OH}^-.
\]

Irradiation of proteins leads to their denaturation through attack of peptide linkages and oxidation of sulphydryl groups; the latter reaction is enhanced by oxygen dissolved in the water. In addition, DNA is also drastically altered by chemical changes which include rupture of glycoside and phosphate ester linkages and ring opening of heterocyclic bases; these changes are reflected by its rapid depolymerization and decrease in its viscosity.

Experiments with amphibian eggs have shown that introduction of minute amounts of cytoplasm from cells previously exposed to irradiation into nonirradiated cells rapidly induced nuclear injury. These experiments also established that isolated nuclei are very radioresistant, withstanding levels of x irradiation as high as 30,000 roentgens, in contrast to intracellular nuclei which are injured by doses as low as 3000 roentgens. These results suggest that substances that promote radiation injury probably arise in irradiated cytoplasm, and that at least part of the irradiation injury must be indirect i.e., it must be mediated through chemical alterations of the cytoplasm that secondarily affect the nucleus.

Much is known about the effects of ionizing radiations on cells that are focused in the nucleus and more especially affect chromosomes.\(^{19}\) All phases of the cell cycle can be altered by ionizing radiation, depending on their intensity and the duration of exposure. Sensitivity of cells appears to be greatest in \( G_2 \); irradiation during this phase leads to a temporary block of mitosis. Irradiation during mitosis induces a variety of chromosomal aberrations, which differ depending on when the cells are exposed. Irradiation during or after metaphase, and before S phase, induces lesions of the entire chromosome which are expressed at the first subsequent mitotic division. Such lesions consist of chromosomal breaks which may remain broken (deletion) or may be repaired and rejoined. Rejoining may be so complete that no visible lesion is apparent, or it may be grossly abnormal if different broken ends are rejoined which result in a so-called exchange. If exposure to irradiation occurs during late S phase, or early in \( G_2 \), after the chromosomal doubling has taken place, the lesions involve one of the paired chromatids of the duplicated chromosomes. Chromatid lesions may be simple breaks or symmetrical or asymmetrical ex-
changes owing to complex rejoining. Such lesions may lead to mutations because of structural changes in the purine and pyrimidine bases. These may result from the formation of hydrogen bonds between two bases after ionization, or the modification or deletion of bases as a result of their interaction with free radicals. Although these have not been fully studied, some alterations of bases have been identified. For example, bases upon interaction with the free radical OH·and H· are converted to their hydrated forms13; such a reaction involving deoxycytidine is shown in figure 2. A variety of other pyrimidine hydrates and similar products have also been identified. Such "errors" are replicated and the functional disturbance, which more often than not is a negative one such as depletion of an enzyme, is perpetuated in the daughter cells. If the mutation involves the deletion of a critical function or functions, it may be lethal. One of the most serious complications of mutation is the transformation of a previously normal cell to a malignant one. All evidence points to the fact that oxidative free radical species formed by irradiation of tissues play an important role in the induction of mutation and subsequent carcinogenesis.

**Viral Carcinogenesis**

Following the initial observation by Ellermann and Bang6 in 1908 that chicken leukemia could be transmitted by injection of cell free filtrates, the evidence that certain viruses are oncogenic has been steadily mounting. Notable contributions include, discovery of a transmissible chicken sarcoma by Rous33 in 1912, transmissible papilloma/carcinoma of rabbits by Shope37 in 1933, and polyoma virus capable of causing cancer in a variety of hosts such as hamsters, rats, rabbits and guinea pigs by Stewart and Eddy40,41,42 in 1953 and 1958, respectively. Detailed studies of host-virus interactions and subsequent carcinogenesis have of necessity been limited to the interaction of tumor viruses in experimental animals and in cells cultured in vitro.11 More recently, technologic developments have allowed basic studies with human tissues and cells which strongly suggest that some human neoplasms may be caused by viruses.18,31

Oncogenic viruses are classified according to the type of nucleic acid they contain. A common feature of both classes of virus is that they induce cell transformation to a neoplastic phenotype which in some cases includes the ability for autonomous growth, including metastases upon transplantation into animals, behavior characteristic of malignancy. A second common feature is physical integration of virus-specific genetic information into DNA of host cells. In the case of DNA tumor viruses, the viral genome is integrated directly into host DNA,34 whereas that of RNA viruses is first transcribed into DNA by a unique enzyme (RNA-dependent DNA polymerase)44 and the copy of virus-specific DNA is then integrated. An important difference between these two classes of tumor viruses is that RNA tumor viruses are frequently replicated by the cells they transform while DNA tumor viruses are not. It should be evident from these initial introductory facts why the biology of tumor viruses has been difficult to unravel and why progress to establish cause and effect has been so slow and tenuous.

Insertion of the genome of DNA viruses into host chromosomes occurs di-

![Figure 2. Formation of the hydrated form of deoxycytidine 6-hydroxy-5,6-dihydrodeoxycytidine following its irradiation in an aqueous solution.](image-url)
rectly by a process similar to genetic recombination in which crossing over between two DNA strands leads to rearrangements within the genome. The presence of integrated complete SV-40 viral DNA which is cryptic or unexpressed, because it is in non-permissive transformed cells, can be demonstrated by fusing them with normal permissive cells whereupon, SV-40 virus reappears in the hybrid cells. This indicates that under the proper conditions, integrated viral DNA will express itself. Integrated viral DNA can also be demonstrated by hybridizing it with SV-40 complementary RNA. Studies involving fusion of SV-40 transformed human cells with normal mouse cells have shown that synthesis of T-antigen, an SV-40 gene specific product, and malignant behavior remains only if human chromosome 7 is retained suggesting that SV-40 DNA is integrated preferentially in this chromosome. Deoxyribonucleic acid tumor viruses include the following classes: papovaviruses (papilloma, polyoma and SV-40); adenoviruses; herpes-viruses (Mareck's disease of chicken, Lucke frog virus, Epstein-Barr virus, herpes simplex type 2, saimiri monkey lymphoma virus); and poxviruses (Shope rabbit fibroma virus and Yaba monkey virus). Three points merit special mention because of their significant biological implications: (1) that many viruses are not species specific and can cause transformation in cells from a number of species; (2) when cells of a different species are infected, the virus does not replicate, although it is integrated in the host cell DNA and causes transformation, and (3) that integrated virus DNA is passed from parent to daughter cells during DNA replication.

Ribonucleic acid tumor viruses integrate their genome in host cell DNA by virtue of RNA-dependent DNA polymerase (reverse transcriptase), a property which has led to their also being referred to as "retroviruses." The presence of this enzyme is so unusual that its demonstration in a cell can be taken as presumptive evidence that the cell has been infected with an RNA tumor virus. Absence of the enzyme in mutant virus is accompanied by an inability of the virus to integrate, replicate, and transform infected cells. Ribonucleic acid tumor viruses (oncornaviruses) include the following classes: sarcoma viruses (Rous chicken, murine, feline, and bovine sarcoma viruses); leukemia viruses (avian, murine, and feline leukemia viruses); and mouse mammary tumor viruses. Since both DNA and RNA viruses depend on DNA for their integration, replication, and transforming capacities, it appears reasonable to conclude that oncogenesis is a property of viral DNA.

In both DNA and RNA viruses, host cell transformation appears to depend on viral genes and the production of virus-specific gene products. In the case of papovaviruses, virus replication and transformation are associated with the appearance of a new protein (T-antigen) in the host cell nucleus. Its localization in the cell nucleus suggests that it exerts its effects on the host cell by direct interaction with host cell DNA. Removal of the segment of the virus genome, which codes for T-antigen by restriction endonucleases, results in a loss of its transforming capacity. On the other hand, removal of most of the viral genome, save that segment which codes for T-antigen, results in DNA which retains its ability to cause cell transformation. In adenoviruses, transformation appears to depend on only a small amount of viral DNA; in one case, as little as six percent of the viral genome is necessary for transformation. Such fragments are referred to as transformation genes. In oncornaviruses, similar transformation genes and gene products have been identified. The Rous sarcoma virus which is capable of causing rapid cell transformation, has a small gene segment called the src (sar-
coma) gene which appears to be essential for transformation. The src gene codes for a 60,000 dalton protein which some studies suggest is a factor stimulating growth of transformed cells. The evidence for this is indirect and circumstantial, and requires further support and amplification. Further, in addition to producing transformation proteins, there is increasing evidence that retroviruses may also induce mutation as a consequence of their insertion into host DNA. Apparently, this can be either the result of physical disruption of DNA or due to regulatory signals in retroviruses which may adversely affect transcriptional control of adjacent sequences of host DNA.

Oncogenes and a Common Pathway of Carcinogenesis

Thus far, the major means have been considered by which cancer can be induced both in laboratory animals and in humans. All of these appear ultimately to induce alterations in host cell DNA that lead to a loss of cellular growth control which is the most characteristic property of the neoplastic transformation; beyond this generalization, little is known. For example, once DNA is effected, do chemicals, ionizing radiation, and viruses induce cancer by totally separate pathways and mechanisms or by a common one? In 1969, Huebner and Todaro postulated that integrated retrovirus genes are part of the genome of all cells, probably as the result of viral infection early in evolution, and that upon their activation normal cells are converted into neoplastic ones. They suggested this as a common mechanism for carcinogenesis by widely diverse agents, the so-called oncogene hypothesis. This bold and imaginative notion was based on increasing evidence that retrovirus oncogenes are widespread in murine and other species and are transmitted vertically from parent to offspring as part of the genome of apparently healthy cells. As one might expect, this hypothesis stimulated many workers to begin testing its validity. In the short span of 13 years, numerous bits of the puzzle have fallen into place. First, Stehelin and Spector and their coworkers have, by hybridization studies, identified src gene DNA identical to that in Rous sarcoma virus in the DNA of normal uninfected cells of chickens, other birds, fish, and mammals, including man. Its wide distribution across so many species was unexpected and supported Huebner and Todaro's postulate that infection had probably occurred early in evolution. Since the discovery of src gene DNA sequences in normal cells, the DNA sequences of 15 additional retroviral oncogenes of chicken, turkey, mouse, rat, cat, and monkey origin have been found in normal vertebrate cells indicating the magnitude of the phenomenon. The apparently wide distribution of viral oncogene sequences in normal cell DNA has led to the suggestion that perhaps retrovirus oncogenes have copied cellular genes, rather than the other way around. If this is so, the mechanism(s) and biological significance remain to be elucidated.

In an effort to remain focused, further consideration of the oncogene in this manuscript will be limited to the src gene. The fact that the src gene has been conserved throughout the evolution of different species suggests that the gene may either have served, or continues to serve, some vital function in normal cells. Although the src gene in vertebrates is chemically identical to the viral src gene, its structural organization resembles that of a cellular gene. Like other cellular genes, the src protein encoding sequences in normal vertebrate cells are divided into separate segments as contrasted to the continuous segment char-
A UNIFYING CONCEPT OF CARCINOGENESIS

characteristic of viral genes. Appropriately, it is referred to as a \( c\)-\( src \) gene. More recent work has shown that the \( c\)-\( src \) gene is functional in normal cells and produces minute amounts of \( src \)-protein, a gene product identical to the 60,000 dalton protein produced by the viral \( src \) gene and which appears to be obligatory for cell transformation by this retrovirus. This finding posed another question, namely, if the cellular oncogene \( c\)-\( src \) normally produces small amounts of \( src \)-protein, how and why does infection with the Rous sarcoma virus, which leads to the production of \( src \)-protein, cause transformation of normal fibroblasts into malignant ones? Since retrovirus transformed cells produce 10-fold or so more \( src \)-protein than their noninfected normal counterparts, it is now thought that perhaps the neoplastic transformation is mediated by the production of large amounts of \( src \)-protein.

Transcription of genetic information is accomplished by RNA polymerase attaching at a specific point on DNA, the so-called promoter, which then reads the message in a single direction. Studies of gene regulation in simple systems such as prokaryotes have clearly shown that the attachment of RNA polymerase to DNA is regulated by the binding of specific repressor proteins to adjacent DNA sequences known as operators. As long as the repressor molecule occupies the operator sequence, RNA polymerase cannot bind to the promoter region. It is tempting to speculate that perhaps the very low activity of the \( c\)-\( src \) gene in normal cells may be due to such a regulator mechanism, and that its increase in malignant cells is due to a dysfunction of the repressor-operator complex, as shown in figure 3. All of the foregoing evidence suggests that cancer may be the result of excessive production of a normal protein which is involved in the stimulation of growth. Precisely how this is accomplished is not now entirely clear. However, there is emerging evidence that suggests that \( src \) protein may act by phosphorylating tyrosine amino acid residues in a component protein of a specialized structure of the plasma membrane, the so-called "adhesion plaque" which is thought to be responsible for cell to cell contact, and perhaps even growth control which appears in normal cells to be mediated by this phenomenon, a property which is lost by malignant cells.

Perhaps the strongest evidence that cellular oncogenes are indeed tumorigenic has come from the demonstration that DNA isolated from mouse and rat can induce normal cells in culture to become malignant. More recently, this has been amplified by the transformation
of normal cultured fibroblasts by DNA isolated from chemically transformed cells, and a number of diverse human tumors of epithelial and connective tissue cell origin.25 These findings suggest that normal cell oncogenes fully expressed in the neoplastic cell are capable of transforming normal cells by transfection in vitro.

Recently, Tabin et al43 and Reddy et al27 have shown that a minute modification of the bases in the DNA of a normal c-src gene is sufficient to convert it into a transforming gene capable of converting normal cells to malignant ones. Thus, we have come "full circle" because such minute base changes are precisely what happens when chemicals, ionizing radiation or oncogenic viruses induce mutations in host DNA. If such alterations are localized in the c-src gene or any of the other cellular oncogenes which have been identified in the DNA of normal cells, they could be fully activated and previously normal cells would assume a malignant behavior. In other words, the potential for neoplastic behavior appears to exist in all cells, and needs only to be fully activated either directly by a mutation of the cellular oncogene itself, or of a nearby regulatory gene which normally keeps the oncogene operating at a low level of activity, or perhaps even shut down. While these developments are both exciting and significant, this emerging view of carcinogenesis may be much more simplistic than reality, because it does not explain many important aspects of carcinogenesis which involve not only genetic but extragenetic factors as well. In closing, let us be ever reminded of the sobering advice given by Alfred North Whitehead,50 "Seek simplicity and distrust it".

References

24. POTT, F.: Chirurgical observations relative to the cataract, the polyposis of the nose, the cancer of the scrotum, the different kinds of ruptures, and the mortifications of the toes and feet. London, Hawkes, Clarke and Collins, 1755.