THE UNDERSTANDING OF CANCER, ITS CAUSES, AND TREATMENTS

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by

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FOREWORD

Only through the combined efforts of several individuals has it been possible to complete this research project. Many people have offered advice, consolation, encouragement, and their time to me during my research. Although space and time would not permit me to thank each person individually, there are a few that deserve special recognition.

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THE UNDERSTANDING OF CANCER, ITS CAUSES, AND TREATMENTS

CANCER! When you first read that word, what enters your mind? Death, pain, gloom, suffering, agony, etc? Maybe not, but one thing I am sure that enters your mind is a wary, mysterious doubt and at least a slight sense of fear. That word, probably more than any other, represents more pain, misery, and despair than any other word that has been known to man over the centuries or since its discovery. Although it has existed possibly as long as man, and certainly for the last 2500 years[1], cancer still has a way of seeming new and mysterious each time a person is told, "You may have cancer". Throughout this paper, I hope to briefly discuss what is known about this dreaded disease and the existing therapeutic techniques and to discuss my work on another possible treatment for cancer.

Although we know so much *about* cancer, what really *is* cancer? Several good definitions could be given. Most legitimate definitions, though, should go something like "a disease in which cells of the body proliferate in an abnormal and unbridled way".[2] In other words, a legitimate definition of cancer needs to, in some way, include the idea of uncontrollable and aberrant cell growth. Although scientists are as of yet unable to agree on whether cancer is a single disease or a group of diseases that share common characteristics such as uncontrollable cell growth, cancer will be largely considered to be one disease.

Cancers are often classified in several ways. One way is according to the site of origin and growth. For example, lung, breast, or intestinal cancers are known as *solid tumors*. If cancer occurs in a body system such as the circulation system or lymph system, it is called a leukemia or a lymphoma respectively.[3]

Cancer may also be classified according to the tissue affected. *Carcinomas* affect epithelial tissues on external surfaces, while *sarcomas* are found in connective tissue like cartilage, muscle, and bone.[4]

Cancers are sometimes classified at the most basic level, i.e. the type of cell affected. If they affect basal skin cells, they are called basal-cell *carcinomas*. *Squamous cell carcinomas* affect squamous cells of the skin or lung.[5]

Over the years, there has been a marked increase in the number of new cancer cases. Lots of reasons can be given for the marked overall

increase in cancer cases. One is that the average life span of man has increased; therefore, there is more time for cancer to develop. Also, the population of the U.S. has shown a great increase. Thus, there has been an increase in automobiles and other conveniences which has led to increases in pollutants, many of which are proven to cause cancer. Diet has also been found to be a possible cause of cancer. Today, Americans eat probably more fat, nitrited foods, and junk food than ever before. Many constituents of such foods are known to cause cancer. Bulky, fibrous foods, like cabbage, corn, collards,etc. were very much a vital part of our forefathers' diet; the cancer rate was also lower then than today. Although this does not absolutely prove that our diet today causes cancer, nor that the latter diet is instrumental in preventing cancer, such a relationship seems to exist. Whatever the cause for this drastic increase in cancer rates, one thing is certain and will be shown later in the paper: oftentimes, the development of cancer can be controlled by monitoring the activities and environment to which an individual exposes himself or herself.

Since the very nature of cancer is based on abnormal cellular differentiation and reproduction, it is of vital importance that we understand normal cellular differentiation and reproduction. In other words by understanding the life cycle of a normal cell, scientists are more able to distinguish between a cancerous cell and a normal cell.

Cells reproduce by division of a mother cell into two daughter cells. These daughter cells in turn divide into two cells, each and the process is continued until some cellular signal causes temporary or permanent termination of the process. Therefore, normal cellular division is described as a cyclic process. Since most somatic cells possess the same genetic material and are of relatively the same size, it logically follows that there must be some duplication of the contents of the cell, i.e cytoplasm and organelles as well as the chromatin, before cellular division occurs.

The cyclic process described above consists of four main periods G1, S, G2, and D. These periods are known to exist in the normal cells as well as the cancerous cells of higher organisms. Stages G1, S, and G2 all comprise what is known as *interphase*, in which the components of the cell are duplicated. The S period (referring to DNA synthesis) is the primary period for chromosomal replication. G1 and G2 are the two periods of interphase directly preceding and following the S period. D refers to the cell division period, the last period of the four.

Some human cells have a cycle time of 16 hours. However, this

time is uncharacteristic of most normal human cells, which have much longer cycle times. However for a normal cell with a cycle of 16 hours, G1 covers approximately 5 hours; S covers close to 8 hours; G2 about 2 hours; and D about 1 hour.[6]

We shall take a more in depth look at these four stages beginning with the D stage. During interphase the chromosomes are so stretched out in the nucleus that they are invisible even to light microscopy. However when these chromosomes become thickened, coiled, and, shortened the cell is prepared to divide into two daughter cells. The human nucleus characteristically contains 46 chromosomes, 23 identical pairs, randomly placed in the nucleus. Each chromosome is composed of a double strand of DNA. This doubleness is a result of the duplication of nuclear chromosomes in the S period of interphase. During the middle of the D phase, the chromosomes migrate from their random state to the middle of the nucleus. Spindle fibers, which are anchored to two cellular organelles called centrioles, one on each side of the chromosomes, attach to the duplicated chromosomes. One strand (chromatid) of each duplicated chromosome is then pulled to each side of the cell by the spindle fibers. As the chromosomes are separating to opposite poles of the cell, a furrow begins to form in the center of the cell. Upon complete migration of the chromosomes to opposite poles and complete formation of the furrow, the chromosomes in the two newly formed daughter cells begin to lengthen and decondense. A nuclear envelope encloses the chromosomes, and the D phase is complete.[7]

It is well worth mentioning that the two daughter cells formed under normal conditions are genetically identical and all of their progeny will likewise be identical. The amazement is added to when one realizes that about 10 million cells divide every second in the human body. However, mistakes in division do occur, although they are rare.[8] These exceptions will be discussed in more detail later.

Once daughter cells have formed in the D phase, they enter the G1 period of interphase. This period is very, very important in relation to the study of cancer as will be evidenced later. G1 activities vary for different cell types. However, cells that are "genetically programmed" to differentiate into certain types do so in the G1 period and may remain dormant in G1 for their life time. Cells that reproduce grow throughout this period in preparation for the S period.[9]

The S phase basically is the period in which the chromatin (nuclear genetic chromosomes in interphase) is duplicated. Following the S

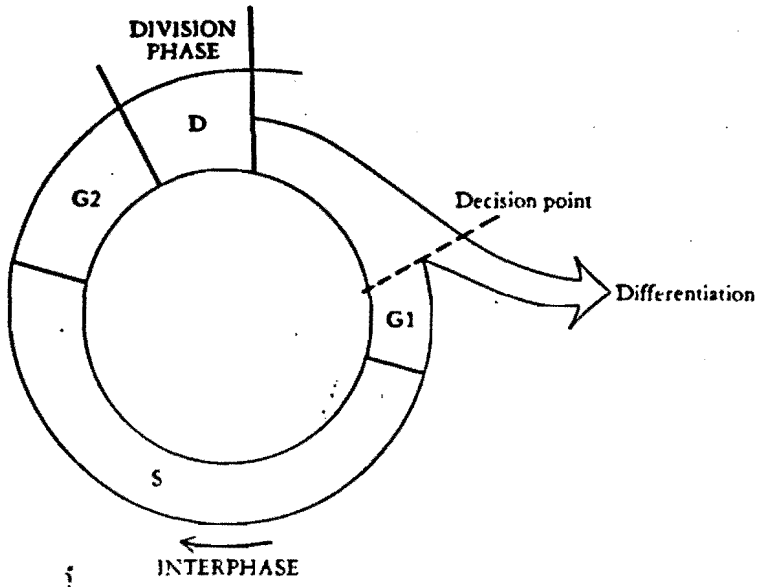


FIGURE 10 Outline of the cell cycle for a typical mammalian cell. A newly formed cell grows steadily until it divides, but it synthesizes DNA only during a period called the S phase. The entire cycle occupies at least 8 hours and typically lasts about 24 hours in adult tissues. G1 is the most variable phase, ranging up to hours or even days. Cells that no longer divide are permanently arrested in G1. Cells usually differentiate after becoming arrested in G1.

phase, the cells continue to grow during G2.

One vital point to know about normal cellular reproduction is that cells are only produced, or turned over, as they are needed. In other words there is a close equilibrium between the number of cells that die or differentiate and the number produced at any given time. This cellular replacement is regulated essentially in the G1 period, which varies from cell type to cell type.[10]

Cells that reproduce fast have a very short G1; conversely, cells that reproduce slowly have a very long G1. S and G2 times are very nearly the same for most cells.[11]

An example of this G1 phenomenon can be seen in the development of an embryo. The cells of the zygote, which initially goes through rapid cell division, have essentially no G1. As development continues and formation of body parts begins (differentiation), there is a lengthening of the G1 period.[12] Just as the time of the G1 period can be altered in embryological development, this period can be radically changed to fit varying conditions that the cell is placed in. For example, esophageal cells have a normal G1 time of 170 hours. If part of the esophagus was to be removed, cellular reproduction in the esophagus could be greatly accelerated to replace the lost cells. The G1 would necessarily decrease phenomenally. However when repair of lost cells is reached, the G1 period should return to normal-170 hours.[13]

Researchers are not quite certain how this G1 arrest phenomenon is regulated, but this regulation is believed to occur in the transition from G1 to S. It is known that once the G1 period is passed, completion of the rest of the cycle is normally inevitable. Therefore, normal cells are able to "decide" whether to have an arrest of division, how long it should last, and when, if at all, to resume the cycle. Scientists are still uncertain as to the technicalities of the process.[14]

At this point, it should be reiterated that it is of utmost importance that the normal cellular division process be understood in order that the erratic cellular reproduction characteristic of cancer will be more clearly understood.

Now that cellular division has been briefly discussed on a more or less macroscopic level, we will turn to cellular division and other cellular operations on a molecular level. It could be said, and correctly so, that cellular division must be understood at the molecular level in order that the dreaded disease of cancer be better understood.

First of all, it is widely held among scientists that cancer results, at least in some cases, from a genetic problem. Therefore, the chrom-

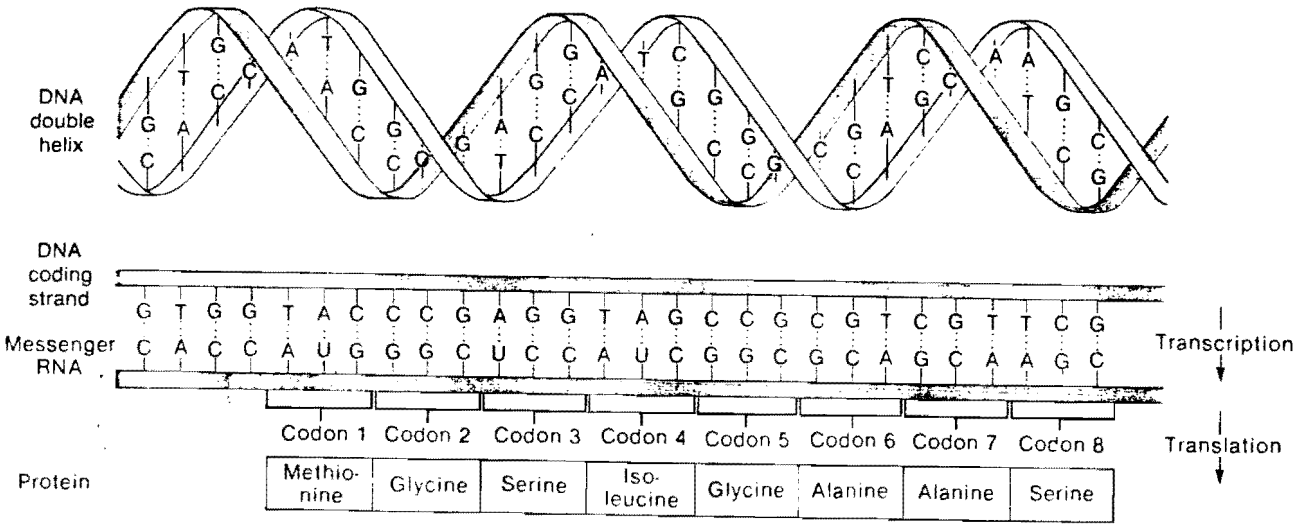
osomal material is implicated.

Chromosomes, which are housed in the nucleus of the cell, are composed of strands deoxyribonucleic acid, abbreviated DNA. These strands of DNA coil around each other to form what is preferably called in interphase *chromatin*-which is very thin and long. When this chromatin thickens and shortens in the D phase, the strands are called chromosomes.

DNA and RNA (ribonucleic acid), which is the other type of nucleic acid, are composed of individual subunits called nucleotides. A normal DNA nucleotide consists of three individual parts - a nitrogenous base (adenine, guanine, cytosine, or thymine), a deoxyribose sugar, and a phosphate. RNA (ribonucleic acid) nucleotides differ from DNA only in that ribose replaces deoxyribose as the sugar, and uracil replaces thymine as one of the bases. In both RNA and DNA, individual nucleotides are joined by phosphate-sugar bonds to form nucleic acid strands.

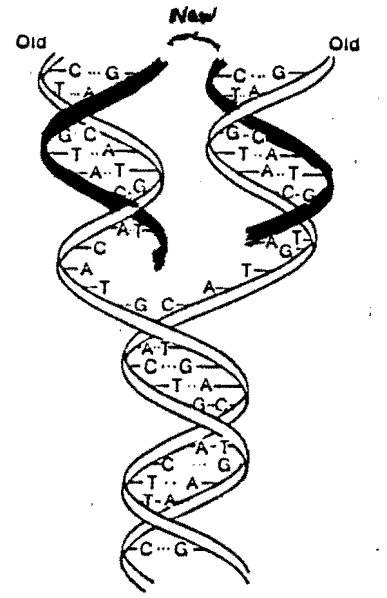
Although both RNA and DNA function in cellular mechanisms, only DNA is responsible for the genetics of the cell. DNA exists as two complementary strands. This complementarity is afforded DNA by the intermolecular hydrogen bonds, which are weak electrostatic forces, between the following complementary bases: thymine=adenine and cytosine≡guanine (the horizontal lines represent hydrogen bonds between complementary bases). These hydrogen bonds give double-stranded DNA a very stable nature compared to the relatively unstable nature of RNA. An example of a DNA strand with its hydrogen bonds is illustrated below.

Figure 3.20. The genetic code is first transcribed into base triplets (codons) in mRNA and then translated into a specific sequence of amino acids in a protein.



Each such strand of DNA is twisted into what is known as a double helix molecule, which resembles a slinky toy.

Replication, put simply, is the synthesis of new and identical DNA from the template, or original strand of DNA, also sometimes called the sense strand. This replication characterizes the S phase of interphase described earlier. This process begins when the two strands of a DNA helix are separated from each other by a dnaB protein, a member of the helicases. DNA polymerase holoenzyme (*Pol III*) then begins replication of each strand of the separated DNA helix.[15] At the completion of DNA replication, which I am greatly simplifying, a duplicate copy of DNA has been created. For example, a DNA sequence of -TACG- would replicate a complementary sequence of -ATGC-. Each original strand has served as a template to produce a new strand complementary to it. Each final duplex consists of an original strand and its newly formed complementary strand. This type of replication is called *semi-conservative replication.*



Proteins are of utmost importance to cellular existence. They are present in the cell membrane and many cellular organelles, including the nucleus. Possibly most importantly, they can function as enzymes. Enzymes act in almost every cellular process imaginable as "catalysts that rapidly perform fairly specific biochemical reactions."[16] Put simply, enzymes cause chemical reactions within the body to occur much faster than they would in a flask. Enzymes may accomplish this task by lowering the activation energy of a reaction and/or by aligning molecules in the proper orientations. They are said to increase the rate

of reaction by as much as 10^9![17]

Just how are these catalysts produced? Since DNA houses all genetic information needed by the cell, it follows that DNA must somehow possess the "knowledge" necessary for protein synthesis. By taking a closer look at DNA, scientists found that certain sequences of nucleotides possess the information needed to produce certain proteins. Such a sequence is known as a *gene*. Although all cells of a person's body have the same set of genes (the same genetic material), not all genes are expressed in every cell, meaning the whole potential for protein production is not always utilized. Therefore, there is differentiation of cell types. In normal cells, duplication of genetic material is held in check by the expression of genes. In cancer, these genes evidently are not expressed and DNA duplication occurs wildly.[18]

A gene codes for the production of a certain protein, but the question remains "how is a protein made?" Part of the process is due to transcription, which is the forming of complementary RNA from DNA.[19] RNA exists as single strands. RNA may be one of three types: messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA). Transcription initiates when a DNA molecule begins to separate at an active gene site, i.e. one that is expressed. An mRNA sequence is assembled which is complementary to the active DNA gene. This transcribing of DNA is facilitated by RNA polymerase. Thus, a DNA sequence of -CATG- would code for an RNA sequence of -GUAC-. When copying of a DNA strand is complete, the mRNA is detached and the complete DNA helix is reformed. Transcription is complete. The genetic information housed in the strand of DNA template is now housed in the mRNA.[20]

Tranlation is the process in which the protein is actually made. The mRNA produced leaves the nucleus and enters the cytoplasm where it attaches to ribosomes, cellular organelles made of rRNA and proteins. Each three nucleotide sequence of mRNA, called a codon, codes for one amino acid. Amino acids are the building blocks of proteins. Each tRNA has two attachment ends. Based on its inherent nucleotide sequence (in groups of three called *anticodons*), each tRNA recognizes one or more specific amino acids. The amino acids are attached at the 3'-OH end of tRNA. The anticodon of tRNA pairs with the complementary codon of mRNA on the nucleotide. The amino acids are deposited on the ribosome, and successive amino acids join to eventually form proteins. At termination of such a protein production, the ribosome dissociates into tRNA, mRNA. This process, which is

also much more complicated than depicted here, is known as *translation*.

Thus, one can gather just from the brief and very much simplified discussion given above that normal cellular reproduction and protein formation is quite involved. Now with a basic understanding of normal cellular function, attention will now be turned to the cancer cell and how it differs from this normalcy.

It is quite difficult to develop a single all-encompassing definition for cancer since so little is known about the disease in comparison to other many other diseases. However, one possible definition might be that cancer is a disease resulting from the improper division and differentiation of a cell. While this definition fails to mention many aspects of the disease, it, nevertheless, entails two of the basic characteristic of cancer, i.e., faulty cell division and faulty cell differentiation. We will now take a closer look at the disease.

Cancer is initiated when one cell becomes cancerous. These cells, interestingly enough, are usually rather healthy but possess a magnified cell division rate and improper differentiation. Cancer cells may be described as somewhat immortal because they do not die off nearly as fast as normal cells. To digress, as discussed earlier in some detail, normal cell reproduction is closely regulated by the G1 period of interphase. Differentiation, discussed more briefly earlier, deals with the specialization of cell types for different functions.[21] Recall, that all cells possess the same genetic information, but all of it is not expressed in each cell, allowing for differentiation when different genes are expressed. Therefore, it would logically seem that cancer cells have a problem in the G1 period and/or in gene expression.

The failure to differentiate properly is exemplified in certain leukemia types. Normally, lymphocytes of the immune system circulate in the human blood stream attacking and destroying foreign bacteria, and then they die after a few days. However in these certain types of leukemia, the lymphocytes produced by the bone marrow fail to mature and, moreover, they do not possess the ability to destroy bacteria. They also do not die as fast as normal lymphocytes. They, in time, accumulate and use up the nutrients available to the normal lymphocytes, contributing to their early demise. The patient is in turn more susceptible to viral and bacterial diseases. Thus, oftentimes leukemia patients die from these secondary infections instead of cancer.[22]

Knowing that differentiation and division are distorted in cancer, the

question arises, but why this distortion? Many researchers have turned to the cell membrane for answers. In the late 1940's, James A. Miller and Elizabeth C. Miller of the McArdle Laboratory "'postulated that cancer might result from the alteration or loss of proteins essential for the control of growth but not for life [of the cell.]'" [23] In fact researchers have observed significant differences between the membranes of normal and cancerous cells (transformed cells). Studies by Sach, Inbar, and Shinitzsky show the fluidity of lipid is more pronounced in leukemia cell membranes. Further studies showed that alterations in the normal membrane moiety may in turn relay certain biochemical messages to the inner cell causing changes in cell division and/or differentiation.[24]

Furthermore, researchers found that there existed a link between cAMP and cGMP and cell division. Usually low cAMP and high cGMP levels, or an optimal ratio of the two, exists during cell division . Ira Pastan and colleagues from the National Cancer Institute in Bethesda, Maryland set out to determine a possible cause of the relationship between low cAMP levels and increased cell division. The level of adenylate cyclase, which catalyzes cAMP production, was found to decrease in cells transformed by the RSV, which is an oncogenic RNA virus. Similarly, they tested the cAMP level and found it to also decrease. The team postulated that since adenylate cyclase is a membrane enzyme, changes in the membrane glycoproteins and lipids produced by transformation inhibit adenylate cyclase activity, which in turn inhibits cAMP production. Further studies by Pietro Gullino and Yoon Sang Cho-Chung at NCI show that administration of dibutyl cAMP (an AMP analog) reduced cancer growth until the treatment was terminated. growth until the treatment was terminated. Gullino theorized that the cAMP derivative functioned to break down tumor cells since the activity of acid ribonuclease, which is a lysozyme that denatures RNA, doubled. He also noticed that the reduction of certain tumors coincided with increases in several other such enzymes.[25]

Given that the cancer cell membrane differs from the normal cell membrane, the next question is what causes the membrane to drastically differ? To answer this question requires a look at the genetics of the cancerous cell. Realizing that abnormal cell division and differentiation are held as main culprits of cancer, genetics must also be responsible for these abnormalities. Since normal cell division and normal differentiation is due to proper genetic regulation, it logically follows that improper differentiation is due to improper

genetic regulation. This improper genetic regulation often results from mutations in the normal genetic makeup. Mutations may be described, molecularly speaking, as "an alteration in a number or sequence of nucleotide pairs in part of a cell's DNA."[26] that may produce significant changes in the cell life cycle.

As described earlier, normal cells receive signals originating from the genetic material that causes termination of cell division. If there is a mutation in the gene(s) that cause(s) termination of cell division, then it can be seen how normal cell division can transform into cancerous cell division. Similarly, it has been found that cancer cells that reproduce fast are less differentiated than those that reproduce more slowly. An intrusion of genetic mutation seems to be also responsible for this phenomena. Clinicians have discovered that mutations in genes that regulate early on in the differentiation pathway produce these highly malignant cancers that are poorly developed. Also, they have found that mutations in genes responsible for regulation further down the pathway cause less strongly malignant tumors since the cells are more normally developed.[27]

Mutations may result from many agents, several of which will be discussed later. The agents may break the DNA in two. Although cells have repair enzymes to fix such breakages, certain normal nucleotides are often left out in the refurbishing process. Sometimes extra nucleotides are included in the DNA strands. Such deletions are called *frameshift mutations*. These deletions exist as mutations and are thus present in subsequent replicated DNA and RNA. Thus, daughter cells will carry this mutated DNA [28]

Another type of mutation, called a *point mutation*, results when one nucleotide pair is substituted by another. For example, a -TG- is substituted with a -CA-. This type of mutation may also detrimentally affect normal cell function. Although most mutations occur due to environmental agents like chemicals and radiation, they also sometimes occur spontaneously. However, this occurs only about once in every 10 billion nucleotides added to DNA.[29]

Mutations, as described above, may cause the production of altered mRNA. This distorted mRNA causes faulty transcription and translation in addition to faulty replication. Thus, proper protein function is damaged. Mutations, therefore, can lethally affect cells by affecting the complete loss of essential enzymes. In fact, many researchers believe that mutation of genes responsible for proteins that regulate cellular differentiation and division is "the basis

of most human cancer".[30]

Cancer, whatever the cause(s), often forms tumors. A tumor is "a swelling due to the abnormal growth of cells." Cancers are often characterized by tumor growths. It should be noted though that not all tumors indicate cancer. Some cells transform without normal differentiation and division to form solid-tumors that possess fibrous capsules. Some of these tumors suddenly stop growing and no cancer results. "Growths that remain enclosed and do not spread, whether they stop growing or continue to enlarge, are called benign tumors."[31]

Although many tumors are benign in nature with no spread of the abnormal cells, many tumors result from the spread of cancer cells in the body. This spreading of cancerous cells to parts of the body from the site of initial growth is called *metastasis*. In many cases, cancers are not discovered until metastasis has occurred. In such cases, the victims often die since the complete treatment of the disease is often impossible. Tumors with cells that spread to other body sites are called *malignant tumors*.[32]

Tumor names are often quite tricky and complex to understand if one does not understand the proper nomenclature. In most cases, the prefix describes the location of the origin of the tumor and the suffix describes whether it is benign or malignant. Benign tumors usually end in *-oma*.[33]

Malignant tumors are usually of two types *sarc* (fleshy) and *carcino* (crablike). Sarcomas, defined earlier in my paper, are "solid tumors growing from derivatives of embryonical mesoderm such as connective tissue, cartilage, bone, muscle, and fat." Likewise, carcinomas are "solid tumors derived from epithelial tissues."[34] Mesodermal tissues produce sarcomas, like osteosarcomal and liposarcomas, while endodermal and ectodermal tissues produce carcinomas (e.g. liver cancer - hepatocarcinomas, cancers of pigment skin cells melanocarcinomas.)[35]

Metastasis involves several steps. Normally, it is initiated with cells or aggregates of cells detaching from the primary tumor and moving to various locations. Interestingly enough, unlike normal cells, cancerous cells have very little affinity for each other; thus, these malignant cells possess the ability to detach from the tumor. The reason for this lessened cell-to-cell attraction is due to the aforementioned alteration of the cell membrane caused by transformation.[36]

The mode of spread of these invading cells is often via the

circulatory and lymph systems. As the heart pumps blood out through the arteries and capillaries, it delivers oxygen to tissues and removes carbon dioxide and other wastes via veins to the kidney and lungs. Motile malignant cells can penetrate the walls of the blood vessels and thus spread to all body areas in a very short time.[37]

The lymph system carries lymph back toward the heart. Lymph is residual fluid from tissues that collects in lymph vessels and empties into the bloodstream immediately prior to the blood reentering the heart. Lymph nodes, situated along the lymph vessels, are aggregates of lymphocytes that filter out viruses and bacteria and destroy them. Cancer cells, primarily those of carcinomas, are often trapped in these lymph nodes. These cells often metastasize from these nodes so other lymph nodes and body organs. Thus during surgical removal of a tumor, doctors often check lymph nodes "upstream" from the primary tumor for metastasis.

Remember, cancer results from an abnormal rate of cell division. Cancer, therefore, by definition must occur in tissues that possess the ability to undergo cell division or the ability to resume cell division, and this is exactly what is found. Therefore, tissues that lose their ability to divide should not readily form cancers, and this is also found to be the case. Nerve cells, voluntary muscle cells, and heart muscle cells, which all cease division, very rarely become cancerous.[38]

Normal cellular division is regulated by the G1 period of interphase. Thus, it is a defective G1 period that also leads to cancer. It should be emphasized and understood that the cancerous cell possesses G1, S, G2, and D periods just as a normal cell does. Furthermore, a cancer cell possessing a duplication time of 16 hours will have basically the same period times as a normal cell with a duplication time of 16 hours.[39]

The deviation of the cancer cell from the normal cell in relation to the G1 period, however, lies in the fact that the normal arrest mechanism of G1 is suspended and/or inhibited. Most cancerous cells do have at least a vestige of a G1 period, though; however, they fail to properly differentiate, possibly as a result of the abnormally short G1 stage. As a result, there is an "incessant and relentless reproduction of incompletely differentiated, malfunctioning cells that are often immortal".[40] Cancer results.

Now that the nature of cancer has been discussed, the next question might be what causes cancer? Although there appears to be a multitude of cancer-causing agents (carcinogens), there exist several such agents

that are given primary consideration. These primary agents include chemicals, radiation, viruses, and oncogenes.

For quite some time, the environment has been linked to cancer. In fact in 1761 Dr. John Hill, a London doctor, said concerning snuff as a nasal cancer agent,

> With respect to cancer of the nose, they are as dreadful and as fatal as any others....It is evident therefore that no man should venture upon [s]nuff who is not sure that he is not so far liable to a cancer: and no man can be sure of that.[41]

In the eighteenth century, the British physician Percivall Pott studied the incidence of scrotal cancer in men who were chimney sweeps as young boys. It was found that soot got embedded in the skin and, therefore, gave rise to scrotal cancer.[42] Since that time, it has been found that components of cigarette smoke, radon gas, soot components, gasoline components, asbestos and many, many more substances are environmental carcinogens or suspected carcinogens.

Many of these environmental carcinogens are chemicals. Cancer results from these chemical carcinogens with various exposure times. It may be as long as 40 years or as short as four months. The long period exists because the development of cancer occurs in several steps divided into two stages called initiation and promotion. "Inititiation occurs when cells are exposed to a limited dose, even a single dose of a carcinogenic substance." Although initiated cells do not further develop into a cancer cell immediately, this initiation is irreversible and subsequent daughter cells acquire this irreversible state. For these reasons, it is widely thought that this initiation is really a mutation caused by the exposure to the chemical(s). Promotion into cancer cells occurs when these initiated cells are further exposed to either the same initiation chemical or another initiator. Complete carcinogens are defined as those that act as both initiators and promoters.[43]

For example, asbestos is believed to be a promoter, but not an initiator. Cigarette smoke, which possesses several initiators and promoters, can initiate a cell. If a worker exposed to asbestos is a cigarette smoker, the promoters from the asbestos and cigarette smoke may combine to compound the promoting effect of cancerous cells that were transformed by cigarette smoke initiators. This combined exposure was found to accelerate the rate of lung cancer 8-fold. Asbestos, which in the past has been widely used, has been shown to be

present in over 80 percent of all Americans. Asbestos promoters can also promote cancers that have been initiated by sources other than cigarette smoke.[44]

It was discussed earlier that cancer is thought to result from mutations. It has been found that most carcinogens also produce mutations in bacteria. (Chemicals that cause mutations are called *mutants*.) Most chemicals that are mutagenic to bacteria are also mutagenic to humans. Three conclusions have been made based on this similarity:

(1) About 90 percent of the chemicals that are carcinogenic initiators in animals act as mutants in bacteria.
(2) Chemicals not acting as mutants in bacteria, usually do not initiate animal cells.
(3) Bacterial mutagens virtually always act as complete carcinogens in animals.

From these conclusions, it can be generalized that most initiators also act as mutagens and virtually all mutagens probably act as carcinogens.[45] Not a whole lot is currently known about the mechanistic actions of carcinogens' ability to cause cancer. But it is accepted that carcinogens must in some way alter, i.e. mutate, the preexisting DNA, RNA, or protein of the cell. Several carcinogens, like B-propiolactone and uracil mustard alkylate (i.e., add a $-CH_3$ group to) the nucleosides and/or amino acids. But most proven carcinogens are paradoxically unreactive chemicals like polycyclic aromatic hydrocarbons, aromatic amines, and nitrosoamines.[46]

However, research by James A. Miller and Elizabeth C. Miller of the McArdle Laboratory has shown that carcinogens contain a reactive electrophilic center. That means they must possess an electron deficient atom that can readily attack the electron-studded centers of DNA, RNA, and proteins. Some electrophilic groups are carbonium ions, free radicals, epoxides, and the nitrogen in esters of hydroxylamines and hydroxamic acids. All accepted carcinogens that are not electrophiles are believed to be altered by the body's normal metabolism into electrophiles.[47]

The metabolism of these carcinogens into electrophilic derivatives is coordinated, paradoxically, by enzymes that normally act in the "detoxification of and disposal of foreign chemicals"! Oxidizing enzymes known as microsomal mixed-function oxygenases oxidize functional

groups of these foreign chemicals, thus making the molecules more polar and more soluble. The foreign chemical can then readily react with sugars and other molecules, making the chemical more soluble.

For example, the normal detoxification route of 2-acetylaminofluorene (AAF), a possible insecticide and proven carcinogen, was studied by James and Elizabeth Miller. Any of the ring carbons can be hydroxylated by the oxygenases. Inert ring-hydroxy-AAF gluronides are formed when the above product is reacted with glucoronic acid.

However, one of theses hydroxylases can hydroxylate the amide functional group of AAF and produce N-hydroxy-AAF. This compound when acted upon by sulfotransperase is converted to a highly electrophilic sulfate ester, which is thought to be a major carcinogen. The outline of this reaction is shown below. Epoxide derivatives and several other groups have been identified as carcinogens.[48]

Protection from carcinogens might be developed by studying these and other related enzyme systems responsible for the detoxification of foreign chemicals. Remembering that oxidation of certain moieties of foreign molecules is the normal route of detoxification for many of them, stimulation of this mechanism might add an element of protection against chemical carcinogenesis. Furthermore since epoxides can lead to carcinogenic derivatives, inhibition of this process would also add an element of protection. However, the enzymatic detoxification process is very complex, and it would seem a very difficult task to safely and positively alter such mechanisms without simultaneously producing detrimental physiological effects.[49]

Not a great deal is actually known about chemical carcinogenesis. Theorized genetic modes of carcinogenesis include the following: (1) modification of existing DNA or of RNA, which is transcribed into mRNA and (2) alteration of proteins that regulate the copying of DNA. The strongest theory is the latter one. Another theory emphasizes the alterations of proteins in cells or in the immune system might

selectively allow for the growth of preexisting cancer cells. After all, many carcinogens have proven to be immunosuppressants as well.[50]

Taking a closer look at compounds known to cause cancer, we find several groups. Only the major groups will be discussed. One such group is composed of polycyclic aromatic hydrocarbons. These compounds are believed to be activated into carcinogenic epoxides, or metabolites of epoxides that possess carcinogenic and mutagenic powers.[51]

Nitrosoamines, over 100 of them, have been proven to be carcinogenic. These compounds are present in beer, whisky, cheese, cooked bacon and tobacco smoke. They are thought to be active as derivatives of the parent compound, such as in hydroxyls, aldehydes, alkyl diazonium hydroxides, and/or carbonium ions.[52]

Aromatic amines, amides and nitro compounds have all been shown to cause cancer also. Aromatic amines, which form N-hydroxy carcinogenic derivatives, have been shown to cause urinary bladder cancer in industrial workers.[53]

Many nitrofurans, which are used as antibacterial and antiprotozoan agents and in human and veterinary medicine and animal food preservatives, have been shown to cause cancer. Nitroreduction to form electrophilic intermediates seems to be the route of carcinogenesis for nitrofurans.[54]

Many alkylating agents act as carcinogens without having to be activated through metabolism. Being already electrophilic, they readily react with nucleophilic areas in cellular molecules. These agents include anti-tumour drugs (to be discussed later), strained lactones, some epoxides, amines and halogen derivatives. These agents alkylate DNA to express their carcinogenic powers.[55]

Finally, a number of inorganic compounds have been identified as human carcinogens. These include arsenic, chromium, and nickel compounds. Beryllium, cadmium, chromium, nickel, and lead have been shown to negatively affect DNA polymerases *in vitro*.[56]

Although just how these chemicals exert their carcinogenic powers on cells is not fully understood, covalent bonding of these compounds to DNA entails alteration of the nitrogen bases. Also some agents react with the phosphodiester linkages. Although reactions at several positions of each base do occur, the most reactive sites are the guanine and adenine nitrogens.[57]

Thus we see that chemicals do possess a wide spectrum of carcinogenic power, much of which is poorly understood. What is more, there

seems to be no unique chemical structure(s) characteristic of all carcinogens that might help in the identification of carcinogens. Thus research continues in chemical carcinogenesis.

Another proven mode of cancer development is by radiation. Radiation is a form of energy that may exist in many forms including light rays from the sun, cosmic rays from outer space, emissions from soil, X-rays, microwaves, etc. X-rays, which are ultraviolet (UV) rays, are definitely carcinogenic,[58] while it is still uncertain if microwave radiation definitely causes cancer.

Ultraviolet light is the number one cause of skin cancer. Over 400,000 new U.S. skin cancer cases are reported each year. The cure rate is over 95 percent though since skin cancer can be detected early on.[59] The sun, a major source of UV light, is thus a main culprit of skin cancer.

X-rays, another form of radiation, have also been proven to cause cancers. Since the risk of cancer developing is proportional to the dosage, large doses of X-rays, such as those used in the treatment of illnesses mean high risks. Since X-rays have been used extensively in the head and neck regions to treat skin disorders, an outbreak of thyroid cancer has resulted. Before X-rays were used, thyroid cancer was not a big problem in the U.S. A striking statistic shows that in women treated with X-rays, the rate of breast cancer is 10^{12} times higher than the rate in the normal population. Children who were exposed to X-rays while they were in the fetal stage of development have a much higher leukemia rate than children not exposed to them.[60]

Although the existence of radiation carcinogenesis has been known for some time, it, like chemical carcinogenesis, is not fully understood. If radiation could pass through the human body without leaving behind energy, there would really be no serious problem. However, radiation, made up of photons (X- and γ-rays) or electrons, neutrons, protons, etc.; deposits enough energy in body cells to cause ionization. From certain types of ionization, free radicals, which are proven carcinogens, can be formed.[61]

Radiation effects may be somatic or hereditary. Somatic effects occur in normal somatic (body) cells. These may appear a very short time following exposure due primarily to a cessation of mitosis brought on by the radiation. Hereditary effects result from sublethal dosages of radiation that may form mutations in DNA that are inherited in each cell generation.[62] It is known that large doses of radiation can cause cleavages and rearrarngements of DNA. However, such large doses also

kill the cells, so cancer is not usually initiated in this way. Low levels of radiation, on the other hand, probably serve to mutate normal DNA. As the dosage increases, the number of point mutations increases. Although such mutations may not be readily evident at first, with time and several generations the evidence of such mutations becomes increasingly clear.[53] In time, cancer often forms as a result of these mutations.

The final major cause of cancer deals with viruses and the related topic of oncogenes. A virus is not a cell, but it is an intracellular parasite that has only one or a very few strands of nucleic acid molecules (either DNA or RNA, but not both) surrounded by a protein coat. Viruses are extremely small and can only be seen with an electron microscope. It is vitally important that it be understood that a virus can only replicate by using the duplicatory machinery of its host cell. Once a virus invades a cell, the virus uses the host cell's DNA to transcribe mRNAs that translate viral proteins. The virus uses the host cell's mitochondrial energy as well as its amino acids to form proteins and viral nucleic acids for its viral progeny. When the progeny are formed in the host cell, the cell may *lyse* (burst) releasing the new viruses or, depending on the type of virus, the progeny may seep through the membrane.

Oncogenic viruses (*onco*, tumor; *genic*, forming),[54] like other viruses may be classified according to some basic characteristics like the nature of their nucleic acids, the shape of the protein coats, and the kinds of host animals infected and how they are affected. DNA viruses may or may not cause cancer and are thus often used in the lab in order to study cancer growth. Three such groups of DNA viruses are the papovaviruses, the adenoviruses, and the herpesviruses.[55]

Papovaviruses are of medium size and replicate in cell nuclei. Certain types of papovaviruses have been discovered in some types of human cancer. Certain papovaviruses, called polyoma viruses may actually produce multiple kinds of tumors. For example, one such virus was found to cause 20 different kinds in mice, including lung, kidney, liver, blood vessel, and skin tumors.[56]

One mouse polyoma virus actually causes more than 10 types of tumors, depending on the tissue type invaded. This finding led to the confirmation by some scientists that cancer is actually a family of related diseases rather than separate diseases. Mouse polyoma virus, however, is not believed to be a carcinogen to man.[57]

Adenoviruses (*adeno*, gland) proliferate in the nuclei of their host

cells. Over 30 various adenoviruses, which are often found in the respiratory tract have been found in humans. While this group of viruses causes cancers in rodents, they are known to only cause respiratory and intestinal diseases in humans.[68]

Herpesviruses, which are DNA viruses, are responsible for such human diseases as chicken pox, cold sores and, infectious mononucleosis. While herpes simplex Type I causes cold sores and cankers, they do change normal human cells *in vitro* into cancer cells, but it is not known if these viruses cause cancer *in vivo*. Herpes simplex II, the major cause of venereal disease., are known to cause some kinds of cancer in humans.[69]

RNA viruses usually proliferate in their host cell's cytoplasm. They infect plants, insects, birds, and mammals. Polio, mumps, and the flu are caused by these viruses. One such virus called the Bittner (B-Type) virus is known to cause mammary cancer in rats. Most female young that feed from an infected mother develop cancer, while the males do not get cancer. However, neither young males nor females from parents who are cancer-free or cancer prone when fed by an uninfected female develop significant cancers. Therefore, it is believed the virus passed on through the mother's milk is responsible for the mammary cancer. Although there is no definite proof that they cause cancer in humans, similar RNA viruses are also found in humans.[70]

C-Type RNA viruses cause cancer in several domestic animals as well as woolly monkeys, baboons and gibbons. They are often transmitted from generation to generation in the gametes and rarely cause any disease. However, these "hidden" viruses are sometimes activated by hormones, radiation, etc. to cause leukemias, lymphomes or sarcomas.[71]

Once a C-Type RNA virus is activated, it may act in several ways. It might transform a normal cell into one that is cancerous, where the virus may continue to grow. Or, the virus may proliferate in a cell with no cancerous results. Lastly, the virus may be transferred form one animal to another through saliva, urine, etc.[72]

The action of oncogenic DNA viruses is quite interesting. After a DNA virus infects a cell, the viral DNA is transcribed into mRNA. The mRNA, through translation as described earlier, forms viral proteins, many of which stimulate normal cellular DNA replication. This then leads to cell division. Of course, the virus continues to reproduce as well.

After several weeks of this division, most infected cells

terminate their reproduction and die (crises stage). The infected cells that survive are transformed by the virus and will grow indefinitely *in vitro*. Some such cell cultures have been kept for more than two decades with no apparent loss of reproduction power. These cells develop into cancer when implanted into hosts.[73]

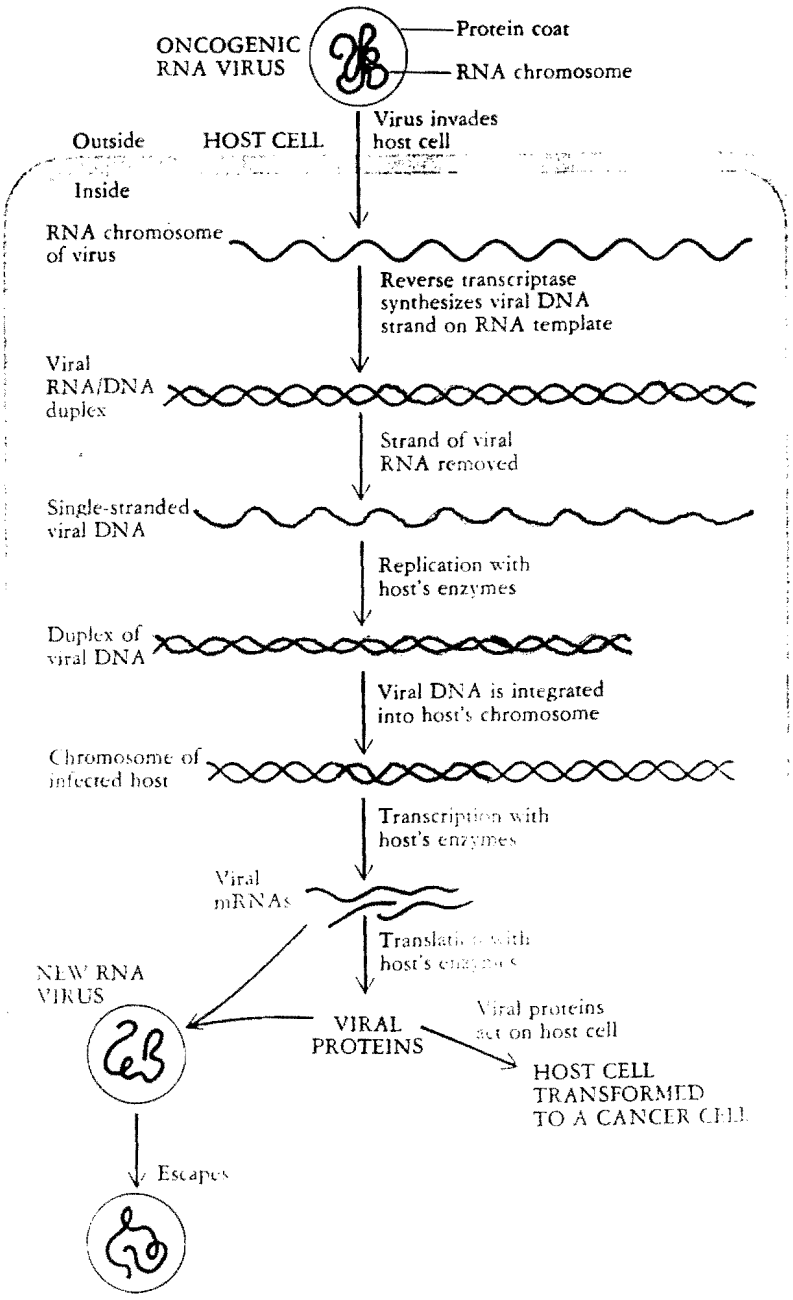
A necessary element of this transformation is the permanent incorporation of viral DNA into normal cellular DNA. Thus, the viral DNA is replicated just like normal DNA. And this incorporation helps explain why viruses transform a normal cell into a malignant cell that does not form more viruses.[74]

Once incorporated, some of the viral DNA continues to direct the action of viral proteins, which transform the infected cell into a cancerous one. Only a small part of viral DNA needs to be incorporated into a normal cells genome in order to transform it. "Indeed it appears that only *one* particular gene is needed, and this codes for a *single* protein whose activities bring about transformation."[75]

Oncogenic RNA viruses transform normal cells in basically the same way as oncogenic DNA viruses, but with a little twist. Remember that in order for the DNA virus to produce viral proteins it had to be incorporated into the normal cellular DNA. So, the RNA viral message must be incorporated into the normal cellular DNA. Thus, from the RNA message a complementary DNA strand must be made.[76]

As the diagram illustrates, this is an almost total reversal of normal transcription. First, an enzyme called reverse transcriptase (which is coded for by the viral RNA) activates the synthesis of the DNA strand complementary to the RNA strand. A double strand (one viral RNA attached to synthesized viral DNA) results. The RNA strand is removed by an enzyme. The remaining DNA strand acts as a template for the production of a new DNA strand. This new viral DNA duplex is incorporated into the normal cell's DNA and acts as the template for the production of viral mRNA. This mRNA in turn produces viral proteins through translation. Also, the new viral RNA from transcription is identical to the RNA chromosomes, thus allowing viral reproduction to occur.[77] Such RNA viruses are called retroviruses. Advanced Immune Deficiency (AIDS) is a common retrovirus.

The reverse transcriptase mentioned above is only found in oncogenic RNA viruses. Thus, the existence of reverse transcriptase in a cell may mean one of two things: (1) a RNA virus is in the cell, or (2) a DNA copy of the RNA from an oncogenic RNA virus has been


ONCOGENIC
RNA VIRUS
Protein coat
RNA chromosome
Virus invades
host cell
Outside
HOST CELL
Inside
RNA chromosome
of virus
Reverse transcriptase
synthesizes viral DNA
strand on RNA template
Viral
RNA/DNA
duplex
Strand of viral
RNA removed
Single-stranded
viral DNA
Replication with
host's enzymes
Duplex of
viral DNA
Viral DNA is integrated
into host's chromosome
Chromosome of
infected host
Transcription with
host's enzymes
Viral
mRNAs
NEW RNA
VIRUS
VIRAL
PROTEINS
Viral proteins
HOST CELL
TRANSFORMED
TO A CANCER CELL
Escapes

incorporated into the cell's DNA. The finding of reverse transcriptase in some human cancer cells helps support the of among some scientists that at least some cancers, including some types of leukemia, result from oncogenic RNA viruses.[78]

Further evidence that certain cancers may be caused by oncogenic viruses is seen in cancer of the uterine cervix in females. Most females with cervical cancer were also found to possess the herpes simplex II virus described earlier. This, virus, commonly found in prostitutes, has also been found to transform normal human cells *in vitro* into cancer cells. Although this does not prove the seemingly forgone conclusion that cervical cancer is caused by herpes simplex II, it sure adds strong circumstantial evidence to this hypothesis.[79]

Epstein-Barr virus, EB virus, often causes infectious mononucleosis in humans. However, this disease is relatively short-term and is over in a few weeks. A variant disease that resembles mononucleosis, called lymphoma, will eventually kill its victims should they go untreated. EB viruses are believed to also cause nasopharyngeal carcinomas in southern China. Thus, there seems to be some variation in the expressive effects of this virus.[80]

To summarize, we see that the major causes of cancer as held by scientists today are radiation, chemicals, and oncogenic viruses. Chemicals and radiation seem to cause cancer by mutation of genes important in the regulation of cell differentiation and reproduction. However, ongcogenic viruses seem to produce cancer by interfering with the cell's gene products that regulate normal differentiation and reproduction, not by causing mutations. From a bird's eye view, one may reasonably conclude that the ultimate cause of cancer lies in the genetic material, i.e., the DNA, of the cell. Having discussed the molecular and genetic foundations of cancer as well as accepted causes of cancer, the next question in one's mind is probable "So, how do we treat cancer?" There are several methods used to treat cancer although no best way has been found yet.

Although methods exist for treating some cancers, the overall outlook still seems gloomy. In fact for most types of cancer, the norm is not to discuss cures, but *5-year survival rates*: "the proportion of patients who survive for at least 5 years after diagnosis without clinical evidence of their disease." Over the last thirty years or so, around 30 percent of all cancer victims have been completely cured, while approximately 70 percent finally die from cancer (excluding nonmelanoma skin cancer, which has a very high occurence and cure rate.)[81]

Conventional therapies have thus given nominal results in the treatment of cancer. These methods are basically to (1) excise cancer cells using surgery (2) to kill the cancer cells using radiation and (3) to kill cancer cells or inhibit reproduction of them by using drugs and/or hormones.

However, common to all these methods is the problem of metastasis. Unless every cancerous cell is abolished, the threat of cancer still remains. So recently, scientists have turned to the field of immunotherapy in order to use the body's normal immune system to fight cancer.[82]

Surgical removal is fairly successful in cases where cancer is diagnosed early on. For example, skin cancers are normally detected early on, and nonmelanoma skin cancers do not metastasize. Thus these skin cancers are fairly curable by surgical procedures.[83]

Cancers that invade internal tissues are normally less treatable with surgery, since they are normally detected only after metastasis. In other words by the time these types of cancer, e.g. lung cancer, are detected, metastasis has often already occurred, and it is too late to try to isolate and/or remove the cancerous cells.[84]

Radiation is often used to treat certain kinds of testicular cancer, Hodgkin's disease, skin cancer, laryngeal cancer, and some kinds of bone cancer. In the forms of X-rays and radioactive materials, like cobalt 60, radiation therapy is often effective on highly localized areas.[85]

"The intent in using radiation against cancer is to kill a maximum number of cancer cells while killing a minimum number of normal cells."[86] And may I add, this is quite a task. Since both normal and abnormal cells are constantly dividing, it is definitely a challenging and probably impossible task to selectively eradicate all cancer cells without affecting the normal cells using when using radiation therapy.

However for several reasons, cancer cells do tend to be more effectible by radiation than a lot of normal cell types. Reproducing cells tend to be killed easier than those halted in the G1 period. Remember, normal cells halt reproduction in G1 while cancerous cells continue reproduction. Secondly, the faster a cell divides, the more sensitive the cell is to radiation damage. This is probably due, at least in part, to the fact that the probability of catching these quickly dividing cells in the S phase and D phase with radiation is a lot higher than it would be for slower dividing cells.[87]

The negative side of this is that there are normal cells that

reproduce at faster rates than most cancer cells, e.g. bone marrow cells. They are thus more sensitive to radiation than the transformed cells. So, when cancer patients are given radiation therapy, often they become anemic, meaning their red blood cell count becomes abnormally low. (NOTE: The bone marrow is responsible for the production of red blood cells.)[88]

Even if a method to selectively increase the sensitivity of cancer cells to radiation has developed, problems with radiation therapy would still exist. First although radiation patients may benefit from radiation therapy, the risk of new cancers developing from the radiation is enhanced. Also, it is virtually impossible to destroy every cancer cell. Therefore, one remaining cancer cell can reform the cancer. Furthermore, sometimes cancer cells stall in the G1 period periodically and proceed from it months or years later. Radiation, therefore, would prove worthless on such cells.[89]

Although chemotherapy is the third of these conventional methods of treatment, I choose to discuss the new field of immunotherapy here and chemotherapy last since my research project is in the chemotherapeutic arena. As was mentioned earlier, immunotherapy is a relatively new method of cancer therapy whereby a patient's own immune system is activated to fight cancer.

The immune system consists of two divisions involving white blood cells called lymphocytes. These lymphocytes, which are found in the blood, compose most of the spleen, the thymus gland, and the lymph nodes.[90]

Lymphocytes are divided into two main groups: B-lymphocytes and T-lymphocytes. B-lymphocytes produce antibodies that are specific for certain antigens, which are foreign proteins, on invading bacteria, viruses, etc. These antibodies bind to the foreign agents via the antigens and thus help remove the foreign elements. This is commonly known as *humoral immunity*. T-lymphocytes are white blood cells that bind themselves to foreign bacteria, viruses, etc. and then release chemicals that may aid in destroying the foreign body or in stimulating the immune system.[91]

Two groups of observations seem to support the immune system as the main line of defense. Among children born with immunodeficiency diseases, an unusually high number of them develop cancer. Also, people, such as transplant victims, who are given immunosuppresants, tend to develop cancer at an abnormally elevated rate. However, only the rates of certain types of cancers are elevated with high

frequency in both cases, thus suggesting that the immune system may not necessarily be important in the protection against all cancer cell types.[92]

The introduction of vaccines into humans have shown some promise in the fight against cancer. Vaccines from Bacillus *Calmette Guerin* and *Corynebacterium* have been shown to inhibit the growth of human melanomas. Although precursor molecules can be administered to bacteria and these bacteria metabolize molecules into the anticancer agents, there is a potential drawback. The agents formed are no doubt detrimental to the bacteria themselves.[93] Therefore, the use of bacteria for anticancer drug production is limited.

Interferon, a product of white blood cells, inhibit the multiplication of viruses. Some evidence indicates that it may inhibit cancerous cell growth.[94]

Finally, there is chemotherapy, a method of cancer research in which chemicals are used to try to alleviate cancer. This branch of cancer treatment is indeed an exciting one, and it is the method of cancer treatment considered by some to offer the most promise at this time. Of course, the aim of chemotherapy is to selectively knock out the cancerous cells without detrimentally affecting the healthy, normal cells.

Chemotherapy is often used in combination with surgery and/or radiation to try to remove all the cancer from a patient. The rationale is that if surgery and/or radiation can remove most of the cancerous cells, then chemotherapeutic agents can be used to eradicate the remaining cancer cells.[95]

Like the other methods of treatment, chemotherapy also has its drawbacks. By forming mutations, cells often establish resistance to these drugs. Since almost all chemotherapeutic agents are mutagens, they could even possibly increase the amount of mutant cells. In fact, these drugs may aid the mutant cells by ridding them of the competition with cancerous cells. Therefore, the drug may cure one cancer and help to initiate another. Also, drugs do not selectively kill only cancer cells; they kill normal cells also. Also, some cancer cells mysteriously arrest in G1 period, and drugs become ineffective since most affect the S period.[96]

It is necessary to have a deeper understanding of chemotherapeutic agents and their mechanisms of action in order to understand and appreciate my individual synthesis project. Granted that the following material may seem quite complex, I will attempt to simplify the

TABLE 1. *Cytotoxic chemotherapeutic agents in clinical use*

- Noncell-cycle–specific alkylating agents (standard)
  - Short, acute acting (1 to 3 weeks)
    - nitrogen mustard (Mustargen®)
  - Medium acting (2 to 4 weeks)
    - Thio-TEPA, i. v. or i. m.
    - Chlorambucil, oral (Leukeran®)
    - Melphalan (Alkeran®-phenylalanine mustard-L-sarcolysin), oral
  - Long delayed (4 to 6 weeks)
    - (Liposoluble, aqueous-insoluble nitrosoureas)
    - Carmustine (BCNU)
    - Lomustine (CCNU)
    - Semustine (methyl-CCNU)
    - Streptozotocin
  - Atypical alkylating agent
    - Busulfan (Myleran®)
    - Hexamethylmelamine (HMM)
  - Atypical phosphamidase activated (2 to 4 weeks)
    - Cyclophosphamide (Cytoxan®)
- Cell-cycle-specific antimetabolites
  - Antipyrimidines, antipurines
    - Methotrexate
    - 5-fluorouracil (5-FU)
    - Cytosine arabinoside-cytarabine (Ara-C®, Cytosar®)
    - Thioguanine (6-TG)
    - 6-mercaptopurine (6-MP); azothioprine (Imuran®)
    - Hydroxyurea (Hydrea®)
- Acute mitotic inhibitors—plant alkaloids
  - Cell recruitment by mitotic delay
    - Vincristine (Oncovin®)
    - Vinblastine (Velban®)
    - Podophyllotoxins (VM-26, VP-16)
- Antibiotics (various mechanisms)
  - Protein inhibitors, synchronizers
    - Dactinomycin
    - Mitomycin
    - Adriamycin
    - Daunorubicin
    - Bleomycin
    - Mithramycin
- Metal complexes—alkylators
  - Cis-platinum—Platinol®, DDP
- Enzymes
  - L-asparaginase (Elspar®)
- Miscellaneous agents
    - Procarbazine (Matulane®)
    - Imidazole carboxamide (Dacarbazine®, DTIC)

material as much as possible.

The ideal cancer drug, or any drug for that matter, is to selectively destroy the target problem (in this case, cancer) without hurting the normal and correctly-operating system (in this case, normal cells). With cancer, this amounts to finding differences (biochemical, morphological, etc.) between cancer and normal cells. Although a very few differences have been found, not many have been found that are significantly different enough to allow for the production of drugs that will selectively home in on the cancer cell without affecting the normal cellular population.

Out of the approximately 500,000 compounds tested since 1955 in the U.S. by the National Cancer Institute for anticancer treatment, only around 30 have been approved for usage in the U.S. Half of these 500,000 are synthetic and half natural products[97] (i.e., they are extracted from natural sources like plants, bacteria, etc.). The synthetic compounds are divided into two major classes: alkylating agents and antimetabolites.[98]

Alkylating agents (RX, R=alkyl group, X=leaving group) are essentially compounds that react with nucleophilic (nucleus-loving) parts of molecules (Y) to form covalently bonded, alkylated products (RY).[99] For example, $RX + Y^- \longrightarrow RY + X^-$. The discovery that such compounds might possess anticancer potential came about when it was discovered that alkylating agents used in chemically warfare in 1940's destroyed lymphoid tissues and blood-forming organs.[100]

Alkylating reagents normally react with target molecules (the nucleophiles) through an S_N1 mechanism (Substitution, Nucleophilic, 1 molecule) or an S_N2 mechanism (Substitution Nucleophilic 2 molecule). Explanation of these organic mechanisms would require a pretty involved discussion of organic chemistry. Therefore, for my purposes in this paper, it should suffice to say that most epoxides, aziridines and alkanesulphonates work by the S_N2 mechanism.[101]

These alkylating agents commonly react with electronegative elements, like oxygen, nitrogen, or sulphur, in nucleophilic molecules. Reaction with water (H_2O) serves as a detoxification of the alkylating agent. These agents can also react with DNA, which contains oxygen and nitrogen in great abundance, and proteins containing -SH groups.[102]

Alkylating agents may be cytotoxic (cell-killing), mutagenic (mutation-causing), and/or teratogenic (effecting fetuses) due to their alkylating powers of nucleophilic molecules. Cytotoxic alkylating agents understandably induce a rapid reduction in DNA synthesis, loss

of cell replication, and production of abnormally large cells.[103]

It has been shown that there is a direct relationship between the number of alkylating groups per agent and the amount of cytoxicity and anticancer potency expressed by the drug. Therefore, scientists have postulated that alkylating agents may work by "cross-linking two nucleophiles in complementary strands of the DNA helix", thus resulting in the witnessed blocking of cell division. As a matter of fact, *in vitro* studies of commonly used alkylating agents (e.g. chlorambucil, melphalan, phosphoramide mustard, busulphan, CCNU [1(2-chloroethyl)3-cyclohexyl-1-nitrosourea]) have shown the formation of inter- and intra- strand DNA cross links. Furthermore, alkylating agents are not phase-specific; therefore, they can be effective at any stage of the G1, G2, S cycle.[104]

By chemically altering the alkyl groups of these agents, scientists have hoped to be able to increase the uptake by tumors of these agents in order to increase their effectiveness. This has resulted in an array of alkyl derivatives, many of which express anticancer potential. Briefly discussed below are several of these drugs.

Mechlorethamine, a member of the group of agents called nitrogen mustards, was the first alkylating agent used to treat cancer. It is often used in combination with other drugs for treatment of cancer. Melphalan has an R group that closely resembles phenylalanine, a normal amino acid of the body. Chlorambucil, another commonly used alkylating agent, is metabolized *in vivo* by oxidation to phenylacetic mustard which also acts as an alkylating agent.[105]

Cyclophosphamide, the most commonly used nitrogen mustard, is activated by the cytochrome P-450 mixed function oxidase system. The metabolism of cyclophosphamide is shown in the following diagram. Cyclophosphamide is initially hydroxylated at the $-CH_2$ groups located adjacent to the N atoms in DNA. Oxidation of the ring gives 4-hydroxycyclophosphamide (11) in equilibrium with aldophosphamide (12). Aldosphamide is further broken down into phosphoramide mustard and nornitrogen mustard (14) which seem to be the active alkylating metabolites of cyclosphamide. As a by-product, acrolein (15), which is believed to be bladder toxic and the causative agent of haemorrhagic cystitis brought on by cyclophosphamide treatment, is produced. Now, mesna (18) (sodium mercaptoethane sulphate) is used in combination with cyclophosphamide in order to protect the bladder. Mesna protects the bladder by reacting with acrolein to alleviate its toxicity.[106]

Methane sulphonates, aziridines, and epoxides are used as alkylating

agents, but are not as commonly used as the nitrogen mustards. They act mostly by reacting with -SH groups in proteins. Busulphan, a methane sulphonate, is by far the most widely used of the group.[107]

Triazenes, a third group of alkylating group of alkylating agents, have only one alkylating group. Dacarbazene, the only triazene clinically in use, has been shown to produce methylated nucleic acids. Treatment with this drug is highly toxic though, possibly because of the diazonium compound (22) produced as a result of light catalysis.[108]

Figure 10.3 Metabolism of 5-(3,3-Dimethyltriazeno)imidazole-4-carboxamide (DTIC, 5)

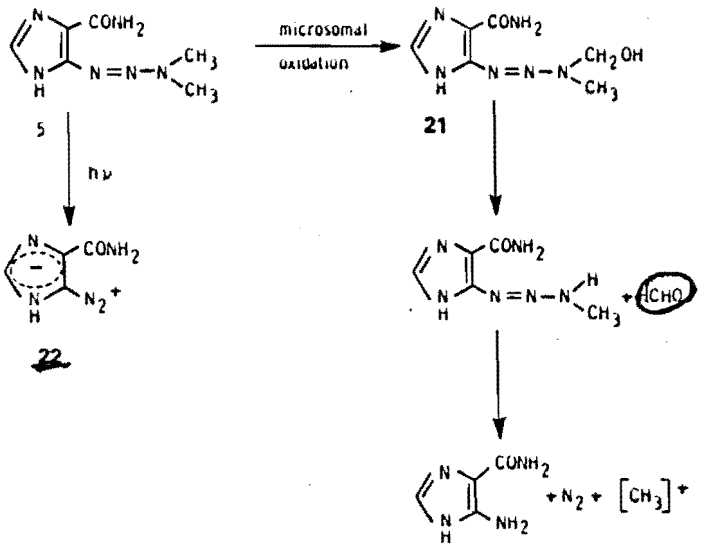


Figure 10.1 (a) DNA Interstrand Guanine-guanine Cross-link Produced by a Bifunctional Alkylating Agent; (b) Hypothesised Production of a Cross-link Between the *N*-7 Positions of Two Guanine Residues in DNA by a Nitrogen Mustard (Phosphoramide Mustard)

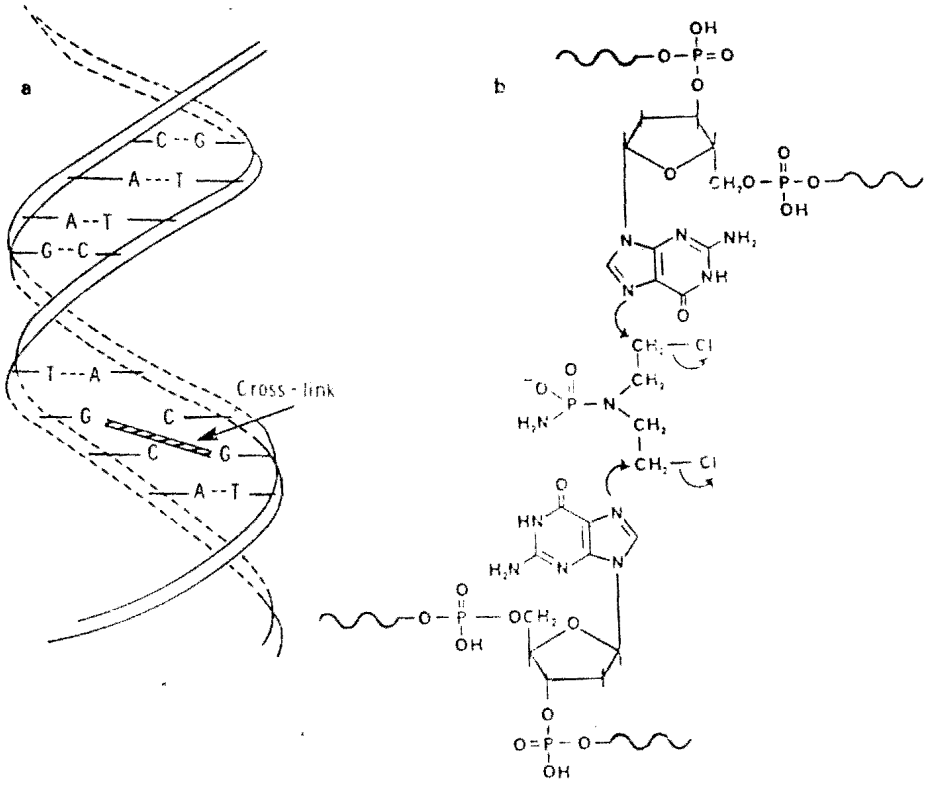
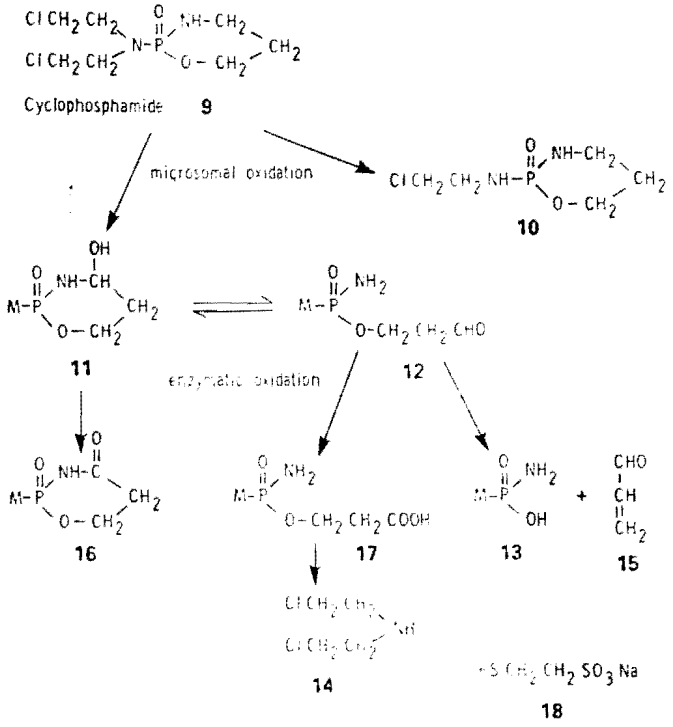


Figure 10.2 Metabolism of Cyclophosphamide (9) [M represents the nitrogen mustard group, $N(CH_2CH_2Cl)_2$]



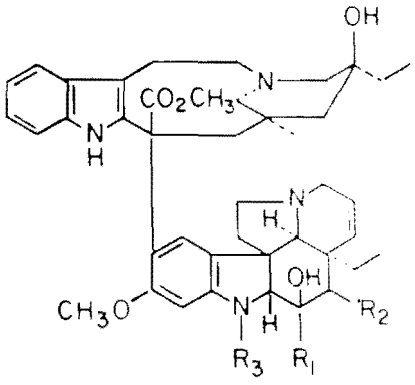
Nitrosoureas, the final group of alkylating agents, act by producing a $ClCH_2CH_2^+$ (chloroethyl carbonium ion) which makes intra- or inter-DNA strand cross links two carbon atoms. In addition, a carbamoylating agent is produced when this drug is metabolized. This agent is believed to inhibit certain enzyme systems by carbamylating the enzyme constituent of the system rendering them inactive.[109]

Certain natural products, which are those isolated from plants, bacteria, etc. are also used as anticancer agents. These agents are often very chemically complex, and thus their mechanisms of action are overall poorly understood. Vincristine (36) and vinblastine (37) are two such agents. They are known to act during the metaphase, which is the phase of mitosis in which the chromosomes align themselves in the central axis of the cell to prepare for division. Normally, spindle fibers form during this phase and attach to the chromosomes in order to pull them back to opposite sides of the cell during anaphase. However, vincristine and vinblastine seem to inhibit or destroy the formation of these spindle fibers. Thus, cellular division is inhibited.[110]

Bacterial products known as antibiotics are also used in cancer chemotherapy. Actinomycin D (dactinomycin) binds strongly to double-stranded DNA, thus interfering with transcription.[111] Anthracyclines binds in between DNA base pairs and forms radicals; methramcin binds to DNA and inhibits RNA synthesis.[112]

The final major class of anticancer drugs is called antimetabolites, a group that I like to think of as the "ultimate intruders". An antimetabolite is really a compound that closely chemically resembles a normal body metabolite. This resemblance allows an antimetabolite to compete with the endogenous metabolite for an enzyme.[113]

Methotrexate, one such antimetabolite, selectively inhibits dihydrofolate reductase. This inhibition in turn blocks the production production of tetrahydrofolate, which is a precursor molecule for N^5,N^{10}-methylene tetrahydrofolate. The latter transfers a carbon unit from deoxyuridine monophosphate (dUMP) to thymidine monophophate (TMP) by an enzyme called thymidylate synthetase. Normally, methylene tetrahydrofolate is reduced by NADPH back to dihydrofolate. However, methotrexate interferes with this process and DNA synthesis is interfered with. Because these folates are needed to form coenzymes responsible for the synthesis of inosinic acid, a precursor of purines, this lack of reduced folates also knocks out the production of purines. Protein synthesis and amino acid production is also interrupted.[114] Therefore for cells affected by methotrexate,



	VINDESINE	*VINBLASTINE*(37)	*VINCRISTINE*(36)
R_1	$CONH_2$	$COOCH_3$	$COOCH_3$
R_2	OH	$OCOCH_3$	$OCOCH_3$
R_3	CH_3	CH_3	CHO

FIG. 7. Common structural formula shared by three vinca alkaloids.

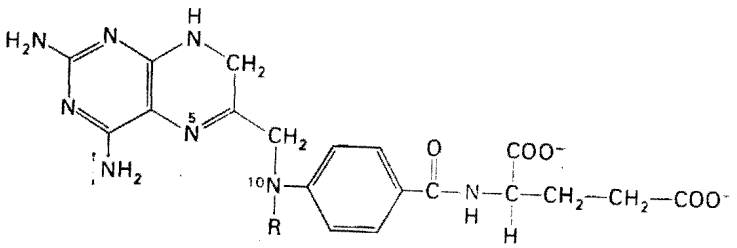
no purines + no proteins + no amino acids = **eventual cell death**

Although methotrexate has no selectivity for action on cancerous cells over normal cells, its cytotoxic ability is dependent on cellular-reproduction, i.e. it is phase-specific for the S-phase[115] This means that cells that are in the S-phase upon administration of methotrexate stand a stronger chance of being killed than those that are not. As mentioned earlier, cancer cells often, but not always, have a higher rate of proliferation and thus have a greater propensity to be affected by cytotoxic drugs than their normal counterparts have.

Purine antimetabolites are synthesized compounds that closely resemble the normal purine constituents of the body but differ enough structurally that they are able to interfere with normal cellular metabolism. 6-mercaptopurine is one such drug. Although the exact mechanism of toxicity is unknown, it is known that it functions best on rapidly reproducing cells. What is known about the metabolism of this drug is that through a very complex set of reactions, it is able to produce "a number of inhibitory effects on purine biosynthesis and purine nucleotide interconversion". Metabolized 6-mercaptopurine (known as 2'deoxythioguanosine) is also incorporated into DNA, and this may further serve as a means of inhibition of DNA production.[116]

Lastly, there are the pyrimidine antimetabolites. Similarly to the purine antimetabolites that resemble normal endogenous purines, these pyrimidine antimetabolites are synthesized compounds that closely resemble endogenous purines. However, they differ enough chemically to interfere with normal cellular metabolism.

The discovery of pyrimidine metabolites is quite interesting. It was found that rat hepatoma cells used uracil in nucleic acid synthesis faster than normal liver cells. Charles Heidelberger synthesized a uracil derivative that would be mistaken by cells for normal uracil. This derivative would differ only by replacing a hydrogen atom with a fluorine atom at the C- 5 position[117] as shown below. Fluorine is known to have a similar atomic radius to that of hydrogen, and might go unrecognized, at least for a while, by the cell.
When 5-fluorouracil is enzymatically transformed into 5-fluorodeoxyuridine monophosphate (5-FdUMP), this "foreign" compound binds and ties up the enzyme thymidylate synthetase. This inhibition prevents the enzyme from reacting with the normal deoxyuridine monophosphate to form thymidine monophosphate, which is a precursor to normal DNA thymidine triphosphate. Similarly, 5-fluoro-2'-



Structure of aminopterin (R = H) and methotrexate (R = CH_3)

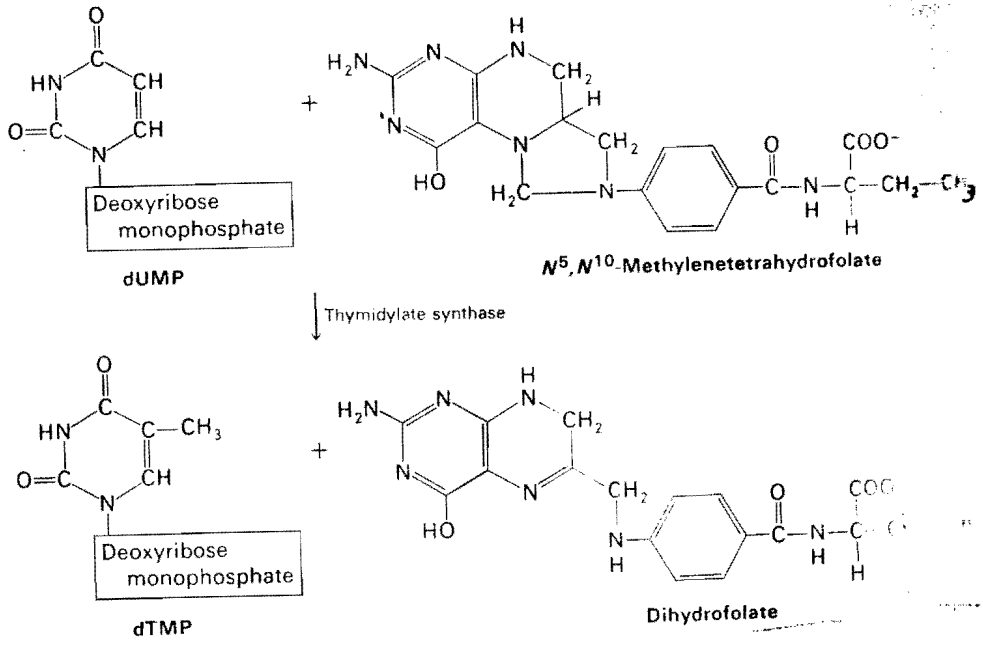


Figure 25-15
Synthesis of dTMP from dUMP.

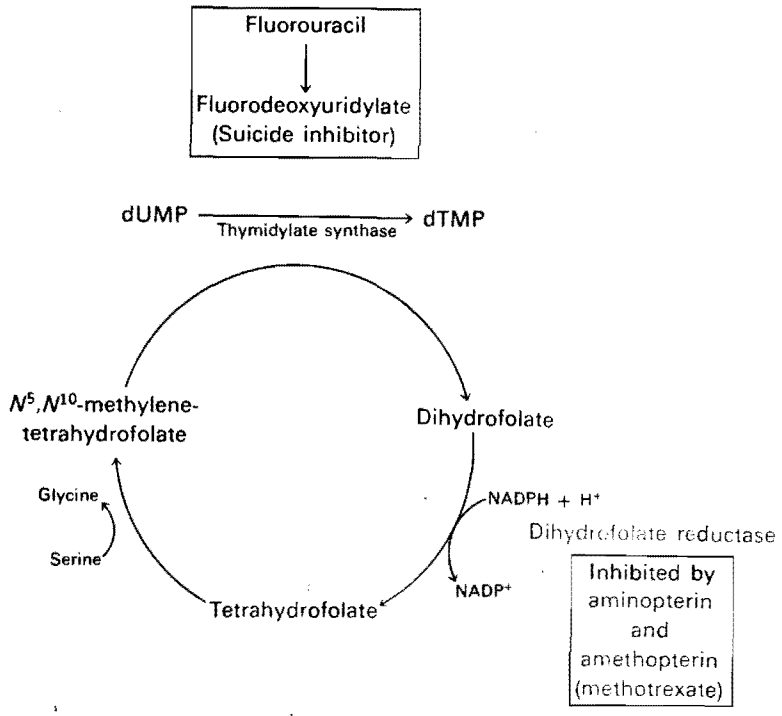
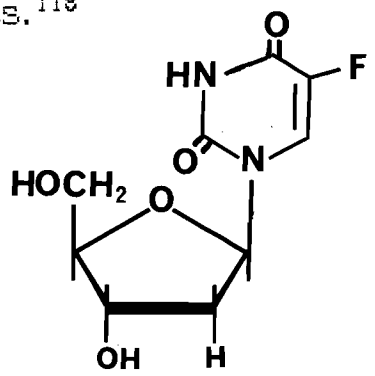


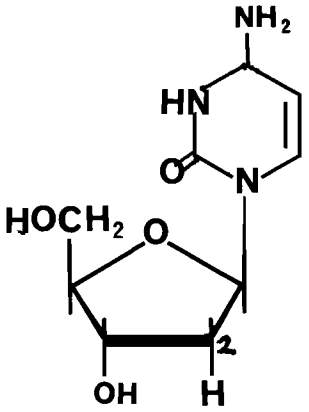
Figure 25-16
Thymidylate synthase and dihydrofolate reductase are target enzymes in cancer chemotherapy. Fluorodeoxyuridylate inhibits the methylation of dUMP. The folate analogs aminopterin and methotrexate block the regeneration of tetrahydrofolate.

deoxyuridine, shown below, may be given in place of 5-fluorouracil with similar results.[118]



To digress for a moment, one subtle, but interesting, statement can be made here. It seems that anticancer drugs are actually carcinogenic. That is, they cause abnormal cellular functions in cancer cells. This is beneficial to the cancer victim. The development of successful anticancer drugs is a good example of how scientists have used nature's natural functions to benefit mankind.

5-fluorouracil is also incorporated into RNA,[119] and can thus interfere with proper protein production (transcription and translation). Remember, uracil is unique to RNA.



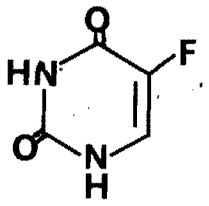
Another prominent pyrimidine metabolite is cytosine arabinoside (1 β Arabinofuranocylcytosine, or araC). This drug differs from deoxycytidine only at the 3-position; the OH is *trans* to the 2-position OH rather than being on same side.[120] Deoxycytidine does not possess an OH in the number 2 position, as shown at the bottom of the last page. DNA constituent sugar is deoxyribose, not arabinose. Yet, once araC is phosphorylated by deoxycytidine kinase to arabinoside cytidine triphosphate (ara CTP), the latter is incorporated into DNA just as normal deoxyribose cytidine triphosphate.[121] AraCTP also inhibits DNA polymerases, and it is also incorporated into RNA, though not as greatly as in DNA. Ara CTP also inhibits ribonucleoside diphosphate reductase, which is responsible for the deoxyribonucleotide needed for DNA.[122]

Much research has been done on ara C, some of which was conducted by Dr. Barry Goz, of the UNC-CH Department of Pharmacology. Upon further study of araC, and its mechanisms along with advice from Dr. Goz and Dr. Harold Teague, Pembroke State University, Physical Science Department, I decided to attempt the synthesis of an antimetabolite that might possess anticancer biological activity.

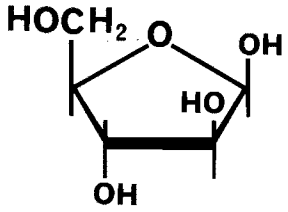
From research and past studies, it was agreed that the most effective antimetabolite possessing anticancer potential would be a nucleotide that first of all closely resembled a normal nucleotide. In fact the resemblance would have to be so close that it would be mistaken for the normal nucleotide by the cell, at least in initial cellular processes. Secondly, the proposed compound(s) would have to be altered in one or more of three molecular positions -the nitrogenous base, the sugar moiety, and/or the phosphate ester- enough to alter vital cellular processes.

After studying the mechanisms of other drugs, it seemed that all or most existing cancer agents homed in on one major mechanism of action. In other words if a cell recognized the primary structural difference between the antimetabolite and the normal metabolite before the drug acted, the drug was ineffective. Thus, it seemed logical that the more unrecognizable structural differences in a compound, the greater the number of targets accessible to the compound for interfering with the cell. In other words, the more unrecognizable structural alterations one can slip by the cell, the greater the chance for successful interference. If the cell recognized one structural modification, there still would remain the possibility of the cell not recognizing the second modification. At least, the compound would have

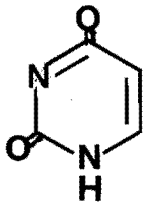
modification, there still would remain the possibility of the cell not recognizing the second modification. At least, the compound would have a chance to do more cellular damage before total recognition as a foreign compound. Thus, we decided to attempt to synthesize a compound with two slight but important molecular distinctions from their normal nucleotidic counterparts. More specifically, 5-fluorouracil was chosen to be the base and arabinose to be the sugar moiety.



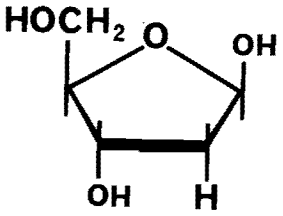
5-fluorouracil



arabinose

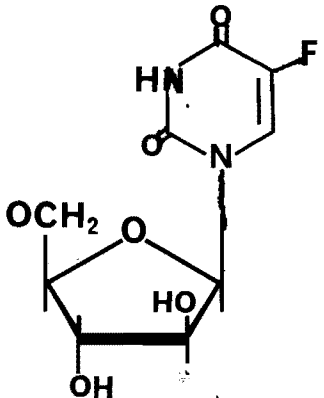


uracil



deoxyribose

Combining 5-fluorouracil to arabinose would produce an antimetabolite with a structure as shown below. Our hypothesis is that since 5-fluorouracil as well as araC are proven anticancer agents, the



combination of the arabinose and 5-fluorouracil moieties should produce the ara-5-fluorouridine compound that may also be an anticancer agent.

Since the arabinose seems to function as deoxyribose normally does in DNA, and 5-fluorouracil is active just as uracil is in RNA synthesis, this proposed derivative may give a double blow to the cancer cell duplication. In other words, this compound might possibly act in inhibiting the *de novo* synthesis of thymine from uracil by inhibition of thymidylate synthetase or some other enzyme vital to this pathway. Also, it may be possible that the synthesized compound might be metabolized into the arabinose and 5 fluorouracil moieties in the cell. The arabinose could then be used in the further *de novo* synthesis of other DNA and/or RNA nucleotides; likewise, the 5-fluorouracil would be free to incorporate into RNA. These nucleotides could interfere with cancer cell replication, transcription, and translation.

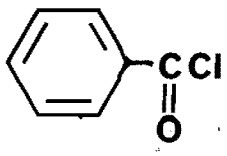
If the cell was to recognize the trans -OH of arabinose or either the -F atom substitution on uracil, the drug would still possess the ability to be incorporated into RNA, thus interfering in transcription and translation (the production of proteins). This double modification would seem to enhance the chance for effective termination of the cancer cell. It is difficult to be sure about the effectiveness of this or any other compound without appropriate pharmacological studies.

While this compound seems promising for possible anticancer potency, the selectivity of such a compound for only cancerous cells is a question. Therefore, we cannot assure that this drug will selectively affect cancer cells and not normal cells. However, selectivity remains a problem with virtually all anticancer drugs used today. It should be remembered, though, that some types of cancer do express abnormally fast rates of cell division. Therefore, they would spend much more time in the S-phase than normal cells do. From all previous research on ara C and 5-fluorouracil, it would also seem that the mechanism of action of ara-5 FU would be to inhibit DNA replication and proper transcription and translation. Since uracil is a component of both araC and ara-5 FU, it would also seem that ara-5 FU should work through similar mechanisms.

The synthesis of this proposed molecule was decided upon as the central part of my research project. It was decided early on that, considering the limited amount of time that I had and my available resources, I would not be able to do any testing for biological activity. Therefore, the synthesis, purification and characterization was decided upon as a suitable project.

The proposed compound would consist of the 5-fluorouracil moiety and the arabinose sugar. My basic goal was to chemically inactivate all reactive functional -O- groups on each molecule except for the -O- attached to carbon 1. Next, a protocol designed for adjoining the two constituent groups, i.e. the 5-fluorouracil group and the arabinose group, in exactly the correct orientation was needed. (This correct orientation -*the tricky part*- would be that the 3-N of the 5-fluorouracil would bond with the carbon 1 -O-constituent of the arabinose.) Next, separation, purification, and characterization would be done.

The initial procedure used was from A Textbook of Practical Organic Chemistry by Vogel pp. 698, 699. 3.0 grams of arabinose were mixed with 6ml of benzoyl chloride and 15.6 ml of 10% NaOH. (The benzoyl chloride, structure below, is a very big and bulky group. Therefore, it should provide adequate steric hindrance to the molecule and appropriately tie up the active -O- groups of the arabinose molecule, also shown below.) Three flasks were set up with each one containing the reactants described above. The reactants were stirred for approximately 72 hours.

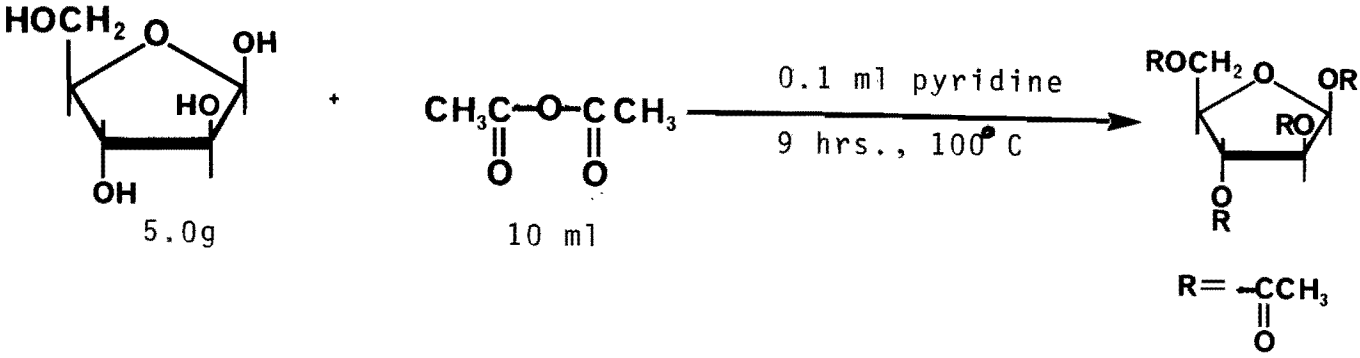


After the reaction was complete, small white spherical solid products remained. The spheres were pulverized and subjected to a thin layer chromatography test (TLC). 95% ethyl alchol was found to be an appropriate solvent for the product, which was hypothesized to be either arabinose tetrabenzoate, arabinose tribenzoate, arabinose dibenzoate, arabinose monobenzoate, or a mixture of the three. Since this synthetic mechanism was old and efforts to find the complete reaction mechanism proved futile, this procedure was abandoned, and another procedure was used.

The next procedure attempted was an alteration of a procedure developed by Abraham Ollapally at Florida A & M University. Approximately 5.0g of arabinose were added to 10 ml of acetic anhydride and 0.1ml pyridine. This solution was stirred for

approximately 9 hours and then placed in a 100°C water bath. A white precipitate formed and later dissolved.

Next, the solution was filtered through silica gel to remove impurities and remixed with ethyl acetate. The solvent was then drawn off by then using the rotavapor. No crystals formed, but a brownish, yellow viscous liquid remained. An infrared spectrum of the liquid was run to determine whether or not the arabinose tetraacetate remained. Although impurities seemed to remain in the product, at least some of the desired product, arabinose tetraacetate, was believed to be present. The spectrum seems to show some of the catalyst pyridine still present.



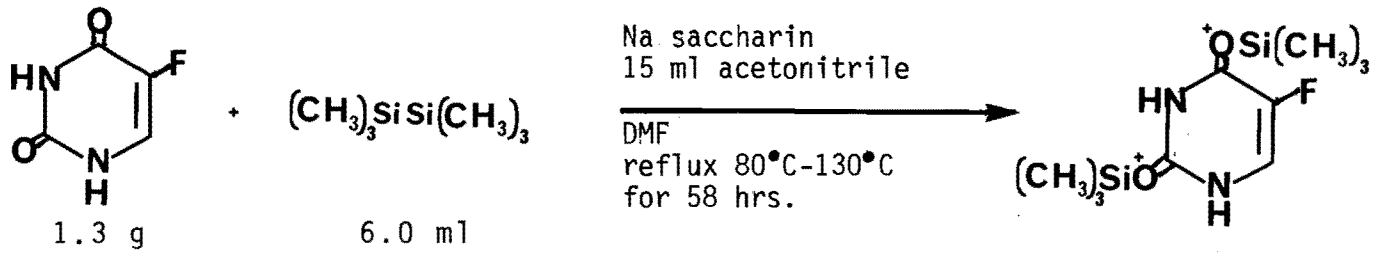
The next important reaction was designed to inactivate the C-2 and C-6 -O's- . Hexamethyldisilane was used for this step. This mechanism was also borrowed, but altered somewhat, from Dr. Ollapally at Florida A & M. To 1.3g of 5-fluorouracil was added 4.0 ml of hexamethyldisilane, approximately 15 ml acetonitrile, and a small amount of sodium saccharin as a catalyst. The solution was refluxed for about 2 hours with no observable reaction occurring. Dimethyl formamide was then added to try to increase dissolution of the reactants. Dimethyl formamide is also a very good solvent for aqueous as well as organic phases. Dissolution occurred and an increase in reaction temperature was noticed from 80°C to 90°C. After refluxing at a temperature between 80°C and 90°C for approximately 11 hours, the acetonitrile and was drawn off with the rotary evaporator. The remaining solution was yellowish-brown and oily. The flask containing the product(s) was then placed in an ice-water bath to promote crystallization. A solid formed. Methylene chloride was used to

dissolve the solid. The methylene chloride was removed with the rotary evaporator, and a yellowish-brown liquid remained.

The yellowish-brown liquid was again refluxed for approximately 12 hours, and the solvent was removed. White crystals and a small amount of oil remained after removal of the solvent. A melting point test showed that the crystals had a melting point between 200°C and 245°C. 5-fluorouracil has a melting point of 280-282°C.

After running this reaction again, a product of large crystals remained as before. There was not enough of this product to work with; therefore, 2 ml of hexamethyldisilane, and a small amount of dimethyl formamide (DMF) were added to the crystalline product above and refluxed at 130°C for about 20 minutes. The temperature was then reduced to 112°C and allowed to reflux for 17 hours. No observed reaction had occurred after this time, so the temperature was increased to 130°C for approximately 6 hours. The temperature was then decreased to 30°C for refluxing overnight. There was no visible change the next day.

The solvent was then drawn off and a whitish-yellow solid remained. This solid was not readily soluble in acetonitrile.
An infrared spectrum of this product is shown. Although spectral analysis of the product has proven inconclusive at this point, the product does not seem to be the original 5-fluorouracil. The product is believed to contain mono- and/or 2, 6 di-trimethylsilo-5-fluorouracil, which is the desired product. The reaction just discussed is shown below.



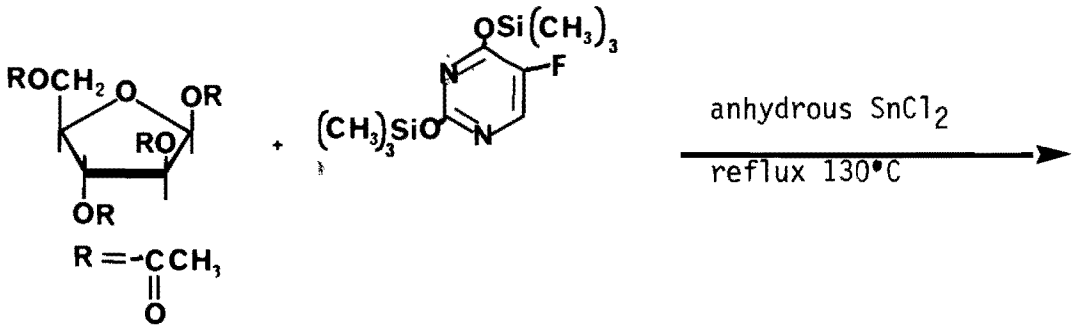
1.3 g 6.0 ml

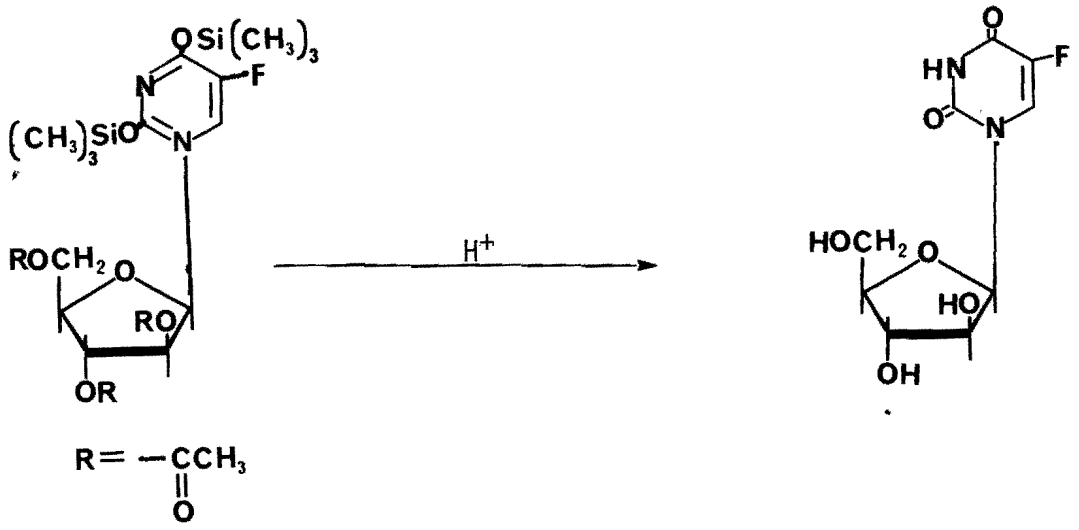
Should further characterization testing prove that the above two products described have been successfully synthesized, one more step remains in the synthesis of my proposed compound - the joining of the two constituents. This reaction, although it has not been completed, seems to be the easiest of the three. The two constituents are refluxed with anhydrous $SnCl_2$ at 130°C. After they are joined (N-1 of the 5-fluorouracil derivative to the C-1 of the arabinose derivative), the acetyl and trisilane groups used to tie up the reactive -O- groups are removed. This reaction is shown below.

The project is still continuing and will hopefully yield some positive results. Dr. Barry Goz has expressed interest in pharmacologically testing the ara -5-FU for biological activity upon successful isolation of the compound. Should I attend attend graduate school in medicinal chemistry, there seems to be a very good chance that I could complete this project and do some work on several other compounds that I have in mind.

Looking toward the future should I decide to attend graduate school, I plan to continue work in drug development. I have several more ideas of possible anticancer compounds that look promising. One such compoound is very similar to the one I worked on for this project. It is ara- 5 fluorocytidine. Since ara C has been proven a successful anticancer agent, my hunch is that by exchanging an H atom in the 5-C position of cytosine with an F atom the cell will not initially recognize the difference, but the difference will cause normal cellular interference later on in the normal proces of cellular metabolism.

Cancer is quite a perplexing disease and one that should be feared. Yet, cancer should not be feared to the point of utter dismay. Without hope, man is most miserable in any endeavor sought after. Therefore, it is the goal of current research and, I hope, future research that a selective cure will be discovered for one of the most dreaded scourges to have ever existed in this world, namely cancer.





R = $-\overset{\overset{O}{\|}}{C}CH_3$

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[32] Braun, pp. 49, 50.

[33] Prescott and Flexer, p. 37.

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[99] Farnert, p. 262.

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APPENDIX

NO. 007-1202

Infrared Spectrum 4-3-89

REMARKS

Pure compounds

ORIGIN

PURITY

PHASE

CONCENTRATION

THICKNESS

DATE

OPERATOR

PERKIN-ELMER

MODEL 700

SPECTRUM NO. Arabinose + pyridine + Ac_2O

SAMPLE 1 Arabinose tetra-acetate

SAMPLE 2

SAMPLE

SPECTRUM NO.