The Therapeutic Promise of Molecular Biology[1]

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Abstract

The search for ideal therapeutic agents has been in progress since primitive man discovered that certain trees, plants and shrubs had varying effects upon him after ingestion. The most intensive efforts have been concentrated during the past 30 years and the rewards of such efforts have been great. However, success appears to have met with the law of diminishing returns as novel drugs have become increasingly rare. Fortunately, advances in basic biological research provide new areas of exploitation. An adventure in one of these, molecular biology, which has led to the development of a novel inhibitor of reverse transcriptase, is described.

Introduction

The eternal search for ideal therapeutic agents has come a long way since that day when God, according to ancient legend, used powdered root from the European mandrake plant to anaesthesize Adam while He removed the rib which became Eve and thus created the first drug related problem.

A variety of drugs have been discovered during the following centuries as we progressed from single empirical observations that create folk remedies to the point where highly skilled teams of scientists produce drugs that stimulate, tranquilize, calm, expand, constrict, destroy to the tune of 782 million prescriptions each year, a 5 billion dollar industry. The history of the United States Pharmacopoeia (USP) provides an excellent example of the progress made in the past 150 years. The prototype of this compendium appeared in 1820. In USP I, published in 1830, 70% of the drugs listed were of vegetative origin; in USP X (1924), 30%; in USP XVII (1964), 10%. Natural drugs that predate the USP, but remain with us even today, include opium, rauwolfia, belladonna, digitalis, strychnine, emetine and curare. The cumulative number of organic chemicals included since its inception exceeds 300—from morphine and quinine in USP I to griseofulvin and cyclophosphamide in USP XVII (9).

But there is trouble in paradise. Despite all-out effort, useful results in terms of new drugs are rarer and rarer. It takes an average of 11.25 years to develop a functional drug from the inception of chemical research to approval for marketing (4). Inherent high costs of research and shrinking profits on old products make it imperative that new approaches be tried in the art and science of new drug development if our best efforts are to lead to clinically useful drugs. The scope

[1] This is an overview. The experimental details are due to both the theoretical and experimental abilities of members of the Molecular Biology Department, in particular Dr. J. Colbourn for enzyme chemistry; Dr. R. Erickson for biological approach and relevant data; and Dr. M. Kotick for the theoretical and experimental aspects of the organic chemistry.

of therapeutic research has been extended beyond those disciplines of science which are traditionally regarded as belonging to medicine. The evolutionary process has resulted in widening the range of talents which now contribute to the advancement of medical knowledge and which progressively adds tools for its practice. We think the time is right to commit *our* talents and resources to new directions.

Molecular biology focuses on the structure, function and biosynthesis of the macromolecules that initiate, sustain and maintain living organisms. Since breakdowns in such vital cellular functions are what we recognize as disease, an understanding of life processes in terms of molecular phenomena should bring us a new generation of therapeutic agents. Molecular biology's most important component, the structure and function of nucleic acids, is a major concern of my department.

Specifically, our nucleic acid research program is concerned with 1) viruses and 2) neoplastic disease and the means by which these disease states may be controlled or, more hopefully, cured. Success in the treatment of either syndrome has been disappointing. Effective antiviral agents have not appeared at the same pace as was the case with antibacterial agents. Drug toxicity continues to complicate cancer therapy.

Virology

Exposure of a cell to viral stress may result in: 1) the induction of enzymes required for viral growth; 2) the induction of enzymes required for the protection of the cell; 3) integration of the viral genome into the cellular genome; 4) the production of substances which interfere with the normal regulatory mechanisms—cell surface changes included; 5) production of substances which result in the destruction of the cell; 6) production and/or release of substances which are harmful to other cells—histamine, serotonin and other mediators; and 7) production and/or release of substances which inhibit normal response —such as immunosuppression by murine leukemic viruses.

For the selective inhibition of viruses we must somehow block a step unique to the viral replicative cycle in a cell supporting a myriad of other metabolic events. General metabolic inhibitors, such as Actinomycin D which interferes with the formation of DNA directed RNA, not only affect DNA viruses but the cell processes as well. On the other hand, iododeoxyuridine, which inhibits thymidine kinase, is effective in herpes conjunctivitis since the virus multiplies, but the cell does not.

So where does one start? We do know that the viral life cycle permits us to choose from several possible steps a target for viral inhibition. 1) Direct extracellular destruction of virions—immunization is a common way of achieving viral inactivation outside of the cell. Its usefulness is limited to certain viruses, however, by the prophylactic character and antigenic specificity of the immune response. 2) Prevention of viral absorption and penetration into the cell—Amantadine is the only known agent which effectively blocks the absorption-penetration

step. 3) Inhibition of viral replication within the cell may be blocked at any one of several stages: viral uncoating; replication of the viral genome; viral protein synthesis; assembly of new virions. Several agents are available which are known to inhibit the various steps. Guanidine and 2α hydroxybenzyl benzimidazole inhibit replication of the viral genome, but viruses mutate to dependence. Isatin β-thiosemicarbazone inhibits late viral mRNA so that no late protein and no mature particles are formed—only defective ones. Iododeoxyuridine, fluorodeoxyuridine and bromodeoxyuridine inhibit thymidine kinase and sometimes cause the synthesis of empty viral particles, but natural resistance develops. Arabinosyl cytosine inhibits reduction of cytidylic acid to deoxycytidylic acid. The most potent inhibitor is interferon. Interferon inhibits viral replication by blockade of the synthesis of viral protein (translation). However, some evidence is available which suggests an effect at the transcriptional level (inhibits mRNA synthesis). Poly I:C is an effective inducer of interferon. 4) The release of mature viral particles from the host cell may be prevented by rifamycin.

The discovery of a nucleic acid polymerase which is capable of reversing the usual flow of genetic information has added a new dimension to the problems of chemotherapy. While this enzyme has been found in RNA tumor viruses, its role is not yet completely understood. However, the presence of a template specific DNA polymerase in all oncornaviruses and its general absence in non-oncogenic RNA viruses suggests a specific role of this enzyme in carcinogenesis by RNA tumor viruses.

There are three important differences in the life cycle of viruses containing this enzyme. Such viruses are non-lytic and tend to have long lasting eclipse or latent phases. More important is that they produce new DNA which leads to some real problems.

The new DNA formed by this reaction may: 1) remain as free dormant intracellular (intranuclear) DNA; or 2) replicate with or independently of cell mitosis; or 3) transcribe into products serving to complete new virion production; or 4) transcribe into products expressing information that converts normal cells into cancerous ones (1).

All of these possibilities have potentially significant biological implications in viral life cycles, gene amplification, embryo differentiation, response to antigens, gene transplantation, and, of course, cancer etiology.

Cancer apparently results from the inability of the cell to turn off its replicative processes; its pleiotypic control coordinator is asleep at the switch. The coordinator either 1) does not receive the signal; or 2) receives a distorted signal; or 3) does not perceive the signal; or 4) does not respond to the signal; or 5) responds inappropriately; or 6) any or all combinations.

Our concern, for the purposes of this paper, is limited to those forms of cancer that involve viral agents, *i.e.*, the signal is modulated by some virus or another.

Polynucleotide Chemistry

The key role played by nucleic acids in both viral and neoplastic disease suggests that synthetic analogs of nucleic acids and polynucleotides may be used to influence the course of these processes. We do know polynucleotides have several definite actions on biological systems. They 1) induce interferon; 2) have an adjuvant effect upon the immune response; 3) antitumor activity; 4) inhibitory effects upon enzymes; and 5) are involved in the release of chromatin restrictions for DNA synthesis.

Synthetic polynucleotides differ from the naturally occurring nucleic acids in that their compositions are dictated and specified by the components used in their preparation and by the particular techniques employed for their enzymatic preparation. Ribopolynucleotides are formed by the action of polynucleotide phosphorylases from bacterial sources on ribonucleoside 5'-diphosphates. Polydeoxyribonucleotides are most commonly prepared with deoxynucleotidyl terminal transferase in a primer dependent reaction using deoxyribonucleoside triphosphates as substrates.

An infinite number of polynucleotides could be prepared if a relatively new approach termed "fragment coupling" proves successful (7). This method involves the condensation of suitably protected, preformed oligonucleotide blocks, having 5' phosphomonoester end groups, in such a manner that the chain length should be approximately doubled at each condensation step. It is quite obvious what variety might be expected from such methodology.

To date, most of the synthetic polynucleotides have been derived from substitutions or replacements in the heterocyclic portion of the nucleotide. Modifications of this type have mainly been limited to n-alkylated compounds and several 5-substituted pyrimidine nucleosides. Substitutions of these functions for the oxygens of the pyrimidine base or phosphate groups have been reported to give compounds which show unusual physical properties and interesting biological activity. The preparation of purine and pyrimidine containing polymers on a metabolically stable polymethylene backbone has received much attention (8).

Until recently, the only sugar modified polynucleotides prepared were those containing 2'-O-methoxy substituted ribonucleosides. An area which appeared appropriate for investigation was alteration, substitution and replacement of various functional groups in the carbohydrate moiety of nucleosides. Modifications of the sugar component appear to alter the pucker of the furanose nucleoside ring which results in subtile changes in the conformation of the polynucleotide. The relationship between pucker and biological activity is not understood but differences in pucker of this type may be related to the basic differences between the structure and functions of DNA as compared to RNA.

Consideration of the structures of a typical homopolynucleotide indicates that a number of features must be retained for recognition of the polymer as a polynucleotide. A phosphate substituted moiety in a position approximating the 5'-hydroxy methyl group must be present as well as an 'acceptor' for formation of a phospho-diester bond.

The C-N bond is not necessary for the enzymatic polymerization of a nucleotide phosphate. For example, the purine nucleoside antibiotic, formycin, in which the C_8 and N_9 are interchanged, has been polymerized and the polymer found to exhibit many unusual properties. The art of synthetic nucleoside chemistry has only recently advanced to the stage where C-glycosyl compounds have become available. Many developments in this area are expected shortly; the compounds resulting from this work will be applicable to the preparation of nucleosides for use in polynucleotide synthesis.

The twist about the C-N bond—*anti* and *syn* denote the two stereochemically preferred ranges in the orientation of the heterocyclic base portion of nucleosides about the covalent nucleosidic bond linking the base to the sugar moiety. Although there may be a considerable energy barrier between the *syn* and *anti* ranges, the conformational energies between these states may not differ greatly. Several workers have suggested that the conformation about the nucleosidic bond is correlated with the pucker of the sugar ring.

Most nucleosides crystallize in the *anti* conformation and this is also the conformation observed in the DNA double helix and other multistranded polynucleotide complexes. Hydrogen bonding of the Watson-Crick type is dependent on the heterocyclic base portion being in the *anti* conformation. It has recently been observed that purine nucleosides substituted with a bulky group in the 8-position crystallize in the *syn* conformation. The triphosphates of these nucleoside analogs cannot be polymerized by the Qβ replicase or *E. coli* transcriptase enzymes. Evidently, the altered stereochemistry of these 8-substituted nucleoside triphosphates makes polymerization difficult (presuming that polymerization requires substrates being in the *syn* conformation).

Formycin, the purine nucleoside antibiotic previously mentioned, has also been shown to exist in the *syn* conformation in the crystal state. Evidence indicates that this nucleoside analog also exists in the *syn* conformation in the homopolymeric state whereas the situation may be reversed in heteropolymers as well as in double stranded complexes.

The configuration at the C-1'-carbon atom of the sugar may also be modified. Only nucleosides with the β- configuration at C 1' of the sugar have been polymerized. Will the enzymes which carry out the polymerization of nucleotides be able to polymerize the α- anomers?

The synthesis of cytosine and uracil nucleosides under potentially prebiotic conditions has been studied by L. E. Orgel and co-workers at the Salk Institute (3). An unusual point has arisen from this work; *viz.*, that the reaction of *D*-ribose with prebiotic precursors, such as cyanamide, cyanoacetylene, gave α-*D*-ribofuranosylpyrimidines whereas reaction of *D*-arabinose gave arabinosyl nucleosides. It was further demonstrated that irradiation of aqueous solutions of the above nucleosides resulted in anomerization and epimerization. Studies on the chemistry of prebiotic nucleoside synthesis have yielded an efficient synthesis of the antitumor agent, arabinosylcytosine.

Why do all the nucleic acids investigated to date contain only β-*D*-nucleosides when an efficient method exists for the synthesis of

α-*D*-nucleosides under primitive earth conditions? Since most enzymes are known to exhibit rigid substrate stereospecificity, we are faced with the problem of which came first: β-*D*-nucleoside phosphates or an enzyme capable of polymerizing only nucleosides of the β-configuration. Also, the α-anomer of 6-thioguanine deoxyriboside is a compound of current interest in experimental tumor therapy. The proposed mechanism of action of this compound is by incorporation into nucleic acids.

A logical extension of this work is the preparation of C 1'-α-purine nucleosides whose activation and polymerization would be studied. Double stranded complexes of these α-nucleoside purine-pyrimidine pairs might possess unusual stability with regard to polynucleotide degrading enzymes.

Turning now to C 2' of the carbohydrate moiety of polynucleotides, the major difference between DNA and RNA resides at this position. DNA has a hydrogen at C 2' whereas RNA has a hydroxy group in the ribo- or down-configuration.

The role of the 2'-hydroxyl group in polynucleotide structure is an intriguing question. The methylation of the 2'-hydroxy groups in poly rA results in an increase in the thermal stability of the double stranded, acid structure of poly rA, while no significant effect on the single stranded conformation is observed. Methylation of the 2'-hydroxyl groups had virtually no effect on the thermal stability of the double stranded 2'-*O*-methyl-poly rA:poly rU complex. This result indicated that the presence of an unsubstituted 2'-hydroxyl group in poly rA is not essential for the stabilization of the double stranded conformations of poly rA. The polymerization of 2'-*O*-methylcytidine-5'-diphosphate has been reported (6). The polymer, as might be anticipated, has increased stability to ribonucleases which hydrolyze ribonucleotide linkages *via* 2', 3'-cyclic phosphate intermediates. Studies on these polynucleotide analogs indicated that the involvement of the ribose 2'-OH as a hydrogen bond donor in an intramolecular hydrogen bond need not be considered in the formation of either single stranded stacked structures, or the twin-helical forms. However, the replacement of the 2'-OH in poly rU by 2'-*O*-methyl leads to a pronounced stabilizing effect on complexes with poly rA or poly dA. These results again reflect the view that hydrogen bonding is not important in the stability of poly-2'-*O*-methyl rA or poly-2'-*O*-methyl rC. It appears that the source of the observed stability effect is related to modifications in the conformation of the carbohydrate moieties of the polynucleotides.

To date, one of the more interesting series of unusual polynucleotides prepared in our laboratory is the series of 2'-halogeno-2'-deoxy-polyuridylic and -cytidylic acids (5). In these polymers, the 2'-hydroxyl group of polyuridylic or cytidylic acids has been replaced by either a fluorine or chlorine atom in the down position at C 2'. While these polymers possess many unusual properties, such as resistance to the action of several polynucleotide degrading enzymes, the double stranded complex with the complementary purine polynucleotide has been shown not to induce interferon.

The chemistry of the penultimate position of the carbohydrate moiety of nucleosides—C 4' -, and most carbohydrates in general, has been relatively little studied with regard to chemical modification. The synthesis of *L*-adenosine represents the first attempt to prepare ribonucleosides for biological and physical investigation of enantiomorphic nucleic acid components. Work in the deoxyribonucleoside series, represented by the preparation of 2'-deoxy α- and β-*L*-adenosines and -guanosines and *L*-isomers into RNA- or DNA-like fragments, would provide exciting information concerning helical structures and properties of nucleic acids. The finding that α-*L*-adenosine acts as a substrate for adenosine deaminase suggests the potential biological activity of stereoisomers of nucleosides, a possibility borne out in the case of the selectively toxic nucleoside, 2'-deoxy-α-thioguanosine.

The effect of the sugar ring oxygen on the ability of a nucleotide analog to be polymerized is as yet unknown. In the single stranded random coil form of polyribouridylic acid, as well as in the twin stranded helical form, the conformation is such that the 2'-OH of the uridine residues hydrogen bond to the ring oxygen of a neighboring nucleotide residue. Light could be shed on this point by the preparation of compounds wherein the ring oxygen is substituted by sulfur, nitrogen or methylene. Compounds of this type are known in nature—the nucleoside antibiotic aristeromycin differs from adenosine only in the replacement of the carbohydrate-ether oxygen by a methylene group.

Inhibition of Reverse Transcriptase by Polynucleotides in a Cell-Free System

Starting with the observation that poly uridylic acid (poly U) inhibits the DNA polymerase of Rauscher murine leukemia virus, we set out to determine whether alterations in the structure of that polynucleotide could influence inhibitory activity, specificity of inhibition, and *in vivo* drug potential. Our initial approach to the problem was twofold. First, to acquire as many analogs of poly U as possible and given the appropriate test system, begin to gather clues as to what alterations affected activity. Second, to study the mechanism of inhibition in detail and to use the data obtained to design new polymers.

The cell-free test system was of obvious critical importance. Since we were approaching this problem from its molecular aspect, a well defined system was required. The following was used.

Avian myeloblastosis virus (AMV) is purified by differential and zonal ultracentrifugation, disrupted with non-ionic detergents and the DNA polymerase is isolated by column chromatography. The system measures the incorporation of thymidine triphosphate into poly dT. The template for the polymerization reaction is dT_{10}:poly A and the reaction takes place in a defined buffered salt solution.

Our results concerning the mechanism of inhibition were reported in detail (2, 5) and have provided us with many clues as regard future polymer design. Briefly summarized, we found: 1) kinetics of inhibition show poly U interacts directly with the enzyme in addition to obvious

template interactions; 2) inhibition by poly U is competitive; 3) the inhibition is independent of the nature of the divalent cation; 4) the inhibition is not influenced by actinomycin D; 5) the polymer must be single stranded; 6) the inhibitory polymer must have a chain length greater than 200 residues/molecule; 7) inhibitory activity is abolished by the presence of RNase; and 8) poly U does not interfere with the RNase H activity that co-purifies with the polymerase.

What do these results tell us? First, poly U is a competitive inhibitor of the template binding site on the viral DNA polymerase. Hence, we are approaching viral chemotherapy from a novel aspect. Most other chemotherapeutic agents attack this enzyme reaction from the substrate binding site since their design rationale is based on altered, toxic DNA (or RNA) precursors. Our approach offers a greater opportunity for specificity of inhibition as template binding sites must be specific to insure genome replication while most viruses utilize substrates identical to those of the host. In addition, they may be non-toxic to the host organism.

Second, these results suggest the structure of a good enzyme inhibitor. It must be single stranded and larger than 200 residues/molecule. The studies also tell us that a nuclease resistant polymer *may* be a better *drug* than poly U since by adding a contaminant (RNase) to our defined system we can destroy our inhibitor.

The initial screen of available polymers revealed that both the base and sugar were important for activity. Substitutions at the 4- and 5- positions of the ring significantly altered activity. Poly(2-oxy-4-aminopyrimidine) is an active template but an inactive inhibitor while poly (2,4-deoxypyrimidine) exhibits just the opposite activity. Making the compound resistant to RNase (*e.g.*, poly U—poly dU) decreased activity. Addition of a bromo or methyl group in the 5- position increased inhibition. Most significantly, substitution of the 2′-hydroxyl function by halogeno groups (*e.g.*, poly dUfl, poly dUcl and poly dCcl) greatly

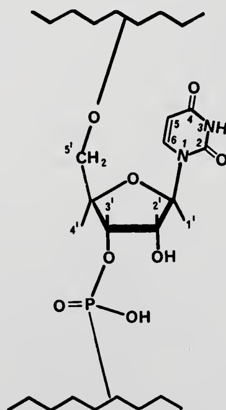

FIGURE 1. *Fluorine is substituted for the 2′-hydroxyl group in poly 2′-fluoro-2′ deoxyuridylic acid (poly dUfl).*

enhanced activity (Fig. 1). These compounds are nuclease resistant and have been chosen for *in vitro* and *in vivo* test compounds to establish model systems. We have established the specificity of poly dUfl (Table 1) and all our polymers will be checked in this manner.

Other leads, provided by this structure activity relationship, are also being pursued. We know base-pairing can destroy activity and poly A exists in most eucaryotic cells. We predict that an analog of poly dUfl that cannot base-pair would be a better antiviral agent. This prediction is now being tested. Other areas include charge distribution of the polymer and the possibility of designing polymers possessing functions that could destroy the active site on the enzyme.

TABLE 1. *Effects of poly 2' fluoro-2' deoxyuridylic acid (poly dUfl) on selected nucleic acid polymerases.*

Inhibition	No Inhibition
RNA-dependent DNA Polymerases	DNA Polymerases
Avian Myeloblastosis Virus	Escherichia coli
Rous Sarcoma Virus	Micrococcus luteus
Feline Leukemia Virus	Calf thymus
Murine Sarcoma Virus	Normal Human Cell
Mason-Pfizer Monkey Virus	(Polymerase II)
Wooley Monkey Virus	
RNA-dependent RNA Polymerase	
Vesicular Stomatitis Virus	
DNA-dependent RNA Polymerases	
Escherichia coli	
Micrococcus luteus	
DNA Polymerase I	
Normal Human Cell	

Antiviral and Antitumor Activity

The logical extension of these studies involves testing for *in vitro* and *in vivo* inhibition of both viral replication and viral activity expressed as cell transformation and tumor development. To date, our test systems have not been satisfactory. While considerable information has been acquired concerning cell-polymer interactions, no definitive data on inhibition has been obtained.

In vitro assay methods that depend upon the measurement of new progeny are prone to error when the synthetic step to be inhibited occurs early in the replicative process. Viruses whose synthesis has proceeded past that point will develop into mature virus particles. On the other hand, a compound may inhibit cellular processes and give the impression of specific antiviral activity.

Problems also arise when attempts are made to assay inhibitors of viral induced transformation by focus assay. In transformed cells, the property of contact inhibition is diminished so that when such cells are plated in solid medium discrete colonies (foci) of piled up cells appear. If normal cells are plated with a transforming virus, transformation is determined by the appearance of foci on the plates. A compound

interfering with transformation would diminish or eliminate foci as compared to a control plate without inhibitor.

In this specific case, we have had considerable variability in our results. We have traced the problem to the extreme sensitivity of our host cells, chick embryo fibroblast, to enviromental manipulation. We have switched to UCl-B cells, a line derived from Balb/313 cells, that transform in response to a murine leukemia virus. An established cell line may provide more consistent results than primary cell cultures.

The *in vivo* inhibition of virus replication is now being tested in what we believe is the best animal-model system available, the AKR mouse-Gross mouse leukemia virus (MLV) interaction. These mice carry the virus in a latent form and at an age of approximately 8 months develop leukemic disease. This system is advantageous in that it not only allows the testing of cytotoxic drugs used in cancer chemotherapy (*e.g.*, methotrexate, 5-fluoro-uracil, cyclophosphamide, chlorambucil, etc.) but also combined chemotherapy (*e.g.*, a cytotoxic agent in combination with a drug to prolong the state of remission.

Our initial tests have been designed to assess the ability of poly dUfl to affect the maintenance of remission. We have chosen this course of investigation since many cytotoxic drugs are available to induce the initial state of remission, but few drugs can prolong this state which lasts approximately 6 weeks in the AKR mouse.

Poly dUfl is non-toxic to conventional mice at levels up to 20 mg/kg administered 3 times/week for 3 weeks. Histological sections were prepared from spleen, thymus, lymph node, Peyers patch, lung, kidney, adrenal and pancreas. The only prominent abnormalities were enlarged germinal centers in the spleen, lymph node and Peyers patch.

Since we plan to use cyclophosphamide to induce remission, these tests will have to be carried out under germ-free conditions as this compound destroys the animals' immunological defense mechanisms.

We have initiated studies with a small number of AKR mice in germ-free conditions to "iron out any wrinkles" that could develop in the system. At present ($\simeq$ 6 months of age), one mouse has developed what appeared to be a large lymphoid tumor of the thymus, showed a high WBC count and was placed on cyclophosphamide (100 mg/kg) for 8 consecutive days. This animal is now on poly dUfl treatment. Other mice have received 3 injections of poly dUfl. We have 105 young AKR mice (2-3 months of age) bred and raised under germ-free conditions in our laboratories which will be utilized in a more statistically significant test.

One of the unexpected bits of information obtained from these studies is that poly dUfl is a potent immunological adjuvant when administered intravenously to mice. This work was initiated by the late Dr. W. Braun and is now being conducted by Dr. O. Plescia at Rutgers University. These data were unexpected because it had been thought that poly A•poly U was a good adjuvant because its double stranded nature protected the poly A and delivered this component to

the cell's active site. Poly dUfl is single stranded. This is a very important aspect of the central problem and is to be pursued "in-house" by the immunology research group of our department. The effects noted in the toxicity experiment—enlarged germinal centers in the spleen, lymph nodes and Peyers patches—may be related to the adjuvant activity.

The development of poly dUfl and other related polymers represent our first steps in an adventure that should produce new therapeutic agents for those syndromes related to failures in cellular nucleic acid metabolism. Viral disease, metabolic disease, congenital defects, aging and cancer are intimately related to inherited or acquired abnormalities in the information processing events that comprise the path from DNA to protein. The development of compounds that would correct mistakes in this information processing system and provide simple, frequent and reliable information would be a contribution that a scientifically guided industry should be able to make—a truly revolutionary advance in therapeutics.

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