ciates at the NCI, looked at the effects of phorbol esters on mouse thymoma cells. Phorbol esters specifically bind to these cells, whereupon the cells produce a growth factor and proliferate.

When Anderson and Sando treated the thymoma cells with phorbol esters, they noticed a dramatic decrease in c-kinase activity in the cell cytoplasm. "As soon as we added phorbol esters to the cells, as soon as we could measure it—within seconds—there was a dramatic decrease in kinase activity," Anderson recalls. And the abilities of various phorbol ester derivatives to decrease the kinase activity correlated with their abilities to bind to the phorbol ester receptor.

The reason Anderson and Sando saw a decrease in kinase activity following phorbol ester treatment is that they were looking for the enzyme in the cytoplasm. Upon the addition of phorbol esters, however, the enzyme becomes tightly associated with the cell membrane. "It is *really* tight," Anderson remarks. "The only way to get it off is with detergent." He believes that the kinase may be loosely associated with the membrane and that the kinase may be the receptor that the phorbol esters recognize. The esters are lipophilic so they glide easily through the cell membrane. Once through, they clamp onto the c-kinase.

In most cases of receptor binding to hormones or viruses or other compounds, the receptor is on the outside of the cell membrane. The phorbol ester system, where the receptor is on the inner surface of the membrane, then is highly unusual.

Researchers in the field believe that the c-kinase normally is activated by some sort of membrane compound that phorbol esters mimic. Whatever this compound is, it most likely is a key to controlling cell growth and differentiation. Nishizuka notes that the membrane compound diacylglycerol, along with calcium and acidic phospholipids, activate the c-kinase. Since phorbol esters are able to replace the requirement for diacylglycerol, he reasons that phorbol esters may stimulate the effects of this membrane phospholipid.

Another open question is, What is the c-kinase phosphorylating? It is known that the kinase phosphorylates a variety of proteins in vitro and it phosphorylates them on serine residues. And once the c-kinase is associated with the cell membrane, cells are much more susceptible to differentiate or to proliferate—depending on the type of cell. Clearly, there are some important clues here to how cells control their growth.

—GINA KOLATA

Interferon Activity Without the Interferon

In the mid-1970's, Ian Kerr, now at the Imperial Cancer Research Fund in London, observed that double-stranded DNA introduced into cells provokes a sharp inhibition of protein synthesis. Several investigators subsequently found that the DNA activates a nuclease that inhibits protein synthesis by destroying the messenger DNA that codes for the proteins. In 1978, Kerr isolated a low molecular weight substance that initiates this process in cells treated with the naturally occurring antiviral agent interferon. The compound was 5′-*O*-triphosphoryladenylyl(2′→5′)adenylyl(2′→5′)adenosine or 2-5A, an RNA analog in which the nucleosides are linked by a 2′→5′ bond rather than the conventional 3′→5′ bond. It was the first naturally occurring compound found to have this type of linkage.

2-5A is an extremely potent inhibitor of protein synthesis, and investigators hoped it would be a useful antiviral and anticancer agent. Unfortunately, the compound is rapidly degraded by a specific phosphodiesterase, so that its half-life in the cell is only about 20 minutes. The molecule is also very polar and does not pass through the cell membrane, and therefore studies must be conducted by direct injection into the cell or in disrupted cells. At the recent meeting of the American Chemical Society (ACS)* two groups of investigators reported that they have to overcome some of the problems.

Paul F. Torrence and his colleagues at the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases have solved the longevity problem. They reacted the terminal sugar of 2-5A with periodate to give a dialdehyde, which was reacted with hexylamine and reduced to give a 2-5A analog in which the sugar is replaced by a substituted morpholine ring. This analog persists in the cell about 15 times as long as 2-5A and is as active as 2-5A at one-tenth the concentration. It still does not pass readily through the cellular membrane, however, and Torrence is working with longer amines to make the analog more lipophilic.

*185th National Meeting of the American Chemical Society, held 20 to 25 March in Seattle.

Opendra K. Sharma and Biswendu B. Goswami of the AMC Cancer Research Center in Lakewood, Colorado, have prepared analogs of 2-5A in which the 3′-hydroxyls of the sugar moieties are methylated. Sharma reported that these analogs strongly inhibit the growth of vaccinia virus in intact cultured cells without affecting cell replication. Corrado Baglioni of the State University of New York at Albany has shown that these analogs are as effective as 2-5A when injected into cells and that they persist longer. It is still not clear, however, whether the analog added to the culture medium is transported into the interior of the cells or if it is degraded to 3′-*O*-methyladenosine, which also has antiviral activity.

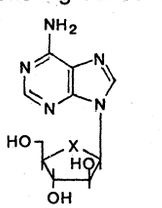
New Agents Active Against Herpesviruses

Herpes has become one of the most feared viral diseases because there is no vaccine to protect against it and no good therapy once it has been contracted. The two best antiviral agents, 9-β-D-arabinofuranosyladenine (known as ara-A or Vira-A) and 9-[(2-hydroxyethoxy)methyl] guanine (acyclovir or Zovirax) have limited effectiveness. Ara-A is too polar to pass through the skin and is not used against herpes. Acyclovir penetrates the skin more readily but it, like ara-A, is easily degraded by an enzyme called adenine deaminase that is found throughout the body. In the cell, both drugs are activated by a viral enzyme, thymidine kinase, which converts them to a triphosphate ester that inhibits a viral DNA polymerase. Viruses that lack this enzyme are resistant to chemotherapy with these drugs.

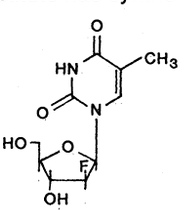
Several investigators reported at the ACS meeting on new ways to overcome these problems. William M. Shannon of the Southern Research Institute, David Baker of the University of Alabama, and William I. Higuchi of the University of Utah School of Medicine have been working with ara-A that has been esterified at one or more of the sugar hydroxyls. Esterification makes the molecule more lipophilic, so that it passes through the cell membrane more easily, and also makes it more resistant to deamination. Shannon reported that the

2′,3′-diacetate (ara-ADA) is relatively easily transported into the cell and is as active as acyclovir against herpes in guinea pigs and mice. Shannon has also used a penetration enhancer called Azone (1-dodecyl-aza-cycloheptane-2-one) that, like DMSO, transports other chemicals through cellular membranes; Azone does not have the side effects of DMSO, however. Azone makes ara-A as effective as acyclovir in mice and ara-ADA more effective.

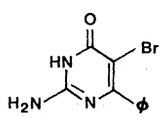
Jack J. Fox of the Memorial Sloan-Kettering Cancer Institute has synthesized a series of halogenated nucleosides that are active against herpes. The two best, he reported, are 2′-fluoro-5-iodoarabinosylcytosine (FIAC) and 2′-fluoro-5-methylarabinosyluracil (FMAU). Raymond Shinazi of the Emory University School of Medicine has tested both against herpes encephalitis in mice and has found that FIAC is "the most potent and effective drug known to date" against this disease. One advantage is that the deaminated drug is as effective against herpes as the parent compound. Fox has conducted a double-blind, randomized clinical trial of FIAC on 35 immunosuppressed patients with herpes varicella zoster and has found that the drug is "therapeutically superior" to ara-A.



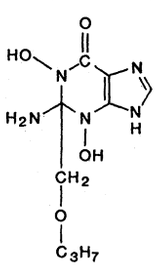
X≡O, ara-A
X≡CH_2 cyclaradine



FMAU



2-amino-5-bromo-6-phenyl-4-pyrimidinone



DHPG

Robert Vince and Susan Daluge of the University of Minnesota, working with Shannon, have synthesized a carbocyclic analog of ara-A called cyclaradine. Cyclaradine has a carbon atom substituted for the ring oxygen in the sugar moiety of ara-A. This substitution protects against deamination; interestingly, it also enables the drug to be activated inside the cell by the cell's own adenine kinase, so that viruses deficient in thymidine kinase are not resistant. Tattanahalli L. Nagabhushan of the Schering-Plough Corporation reported that the 5′-methoxyacetate ester of cyclaradine is more active than acyclovir against herpes infections in guinea pigs and that it is equally active against ara-A–resistant strains.

John C. Martin and his colleagues at Syntex Corporation reported that 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) is 68 times as effective as acyclovir when given orally to mice. A series of analogs with modifications of the acyclic sugar moiety have been prepared and some of these have even greater activity.

Finally, Harold E. Renis of the Upjohn Company reported some unusual results with a series of 2-amino-5-halo-6-aryl-4-pyrimidinones. These compounds have no activity against cultured viruses, but are active against herpes in mice, guinea pigs, and the one monkey strain that has been tested. Upjohn has been studying the compounds as inducers of interferon, but Renis reported that the investigators could not detect interferon near the animal lesions. They did observe increased concentrations of white blood cells near the lesions in treated animals and subsequently observed that antithymocyte serum blocks the activity against herpes but not that against RNA-containing viruses. Pretreatment of the animals with the pyrimidinones before inoculation with herpes protects 90 percent of them against infection, an effect not observed with other antiviral agents.

New Drugs from Plant Tissue Cultures

Many useful drugs originally obtained from plants must be manufactured synthetically because the plants are rare or can be cultivated only in limited areas. James P. Kutney of the University of British Columbia reported that he and his colleagues have been able to produce significant quantities of some of these pharmaceuticals by tissue culture, the growth of individual plant cells in liquid media. By optimizing the conditions of growth in the laboratory, they have also been able to improve the yield without using genetic engineering techniques. They have, for example, produced a five-fold increase in the yield of catharanthine, a potential anticancer agent, and a 20-fold increase in the yield of tripdiolide, another potential anticancer agent. They have also made progress in producing two popular and expensive drugs, vincristine, which is used against leukemia, and vinblastine, which is used against Hodgkin's disease and testicular cancer.

To begin the process, a clipping from the desired plant is sterilized and embedded in a solid, nutrient-rich medium along with special growth hormones. A solid mass of cells, called a callus, grows at the edge of the clipping. Cells from the callus are then grown in a small amount of liquid medium. If tests show that the desired drug is present, the process is scaled up until, in the laboratory, the cells are grown in 15-gallon vats. The process is optimized by varying temperature, light, shaking, and nutrients.

How Many Chemicals Are There?

There are an estimated 63,000 chemicals in common use (*Science*, 13 January 1978, p. 162), but how many are there altogether? One measure is provided by the American Chemical Society's Chemical Abstract Service (CAS), which indexes the world's chemical literature and provides each different chemical with a registry number. On 24 February, the registry listed its 6 millionth chemical, 2-cyclohexyl-3-methyl-4-(pentylamino)-2-cyclopentene-1-one.

The registry identifies some 6000 chemicals each day, about 1000 of which are new. Most of them are synthesized for specific research purposes: fully 75 percent of the 6 million have been mentioned only once. About 97 percent contain carbon. The registry lists 9.2 million names for the 6 million chemicals; the greatest number occurs for polyethylene, with more than 1200 synonyms listed.

The registry covers only papers published since 1965. CAS estimates that an additional 1.3 million compounds are described in papers published between 1920 and 1964.

—*Thomas H. Maugh II*—