CANCER CHEMOTHERAPY[1]

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In selecting specific topics to be examined in this biennial overview of cancer chemotherapy we have favored those which have clear relevance to the treatment of cancer in man and have not received extensive discussion in the *Annual Review of Pharmacology* in the past four years (1–3). We will examine first the newer information on drugs in current or prospective use and then deal with recently derived concepts that have altered the use of anticancer agents both in laboratory and clinic. We have, of necessity, postponed consideration of many new compounds, as yet untested clinically, which are reported to inhibit growth of transplanted tumors in animals. Because of space limitations selections must even be made among drugs under current clinical assessment. Since that which is judgment to one man may seem bias to a second, the actual choices may not receive universal acclaim. Indeed, a decade hence we ourselves may have reason to regret various inclusions and exclusions. Be that as it may, since the utility, and in fact the perusal, of a review is maximal while it is current, we hope that this effort will provide useful information to those interested in this field over the next four to six years.

POLY I:C

A considerable excitement has been aroused among oncologists by recent reports that interferon-inducing synthetic RNA inhibits growth of certain transplanted tumors in mice. This excitement was based upon the hope that a previously untapped mode of antitumor action had been uncovered. The fact that these agents induced antiviral activity made them particularly appealing because of the widespread belief that viral agents may play a significant role in producing cancer in man as well as in lower animals. The distance that yet remains between the above-stated hope and the experimental support for it will become evident in the paragraphs that follow.

Interest in synthetic double-stranded RNAs as potential anticancer agents derives directly from the reports that poly I : C, (the double-stranded

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complex formed by mixing the homopolymers polyriboinosinic acid and polyribocytidylic acid) inhibited viral oncogenesis (4–6), and possessed antitumor activity against virus-induced and nonviral induced transplanted tumors in mice (6, 7). These observations are one outgrowth of decades of research into the nature of viral interference, a phenomenon originally described by Hoskins (8) and shown by Isaacs & Lindenmann to be mediated by a protein which they called "interferon" (9). The physiologic mechanisms involved in the induction of interferon and in its antiviral effect have been reviewed repeatedly in recent years (10–12); they will be described here only in outline.

Interferon is a collective name applied to low molecular weight proteins with antiviral activity produced by cells in culture or by intact animals of a wide variety of vertebrate species. The phenomenon may even extend to plants and bacteria (12). The antiviral activity of interferon is characterized by narrow species specificity, broad, but not universal, antiviral spectrum, and wide pH stability. Interferon is produced following cell exposure to DNA or RNA viruses, to endotoxin, and to a variety of synthetic anionic polymers; the most active polymers are double-stranded RNAs. According to current concepts interferon induction in the course of virus infection is mediated via formation of double-stranded RNA of viral origin and recognition of its presence by some cellular receptor (10). This triggers a sequence which requires DNA-directed RNA synthesis followed by specific protein synthesis, i.e., formation of interferon. Following this concept the polyanionic inducers of interferon might accomplish this because of, and to the degree of, their physical resemblance to double-stranded RNA. Interferon does not directly affect viruses; rather it stimulates uninfected cells to synthesize a new protein and in some way, not yet understood, prevents the formation of new viral nucleic acid, thereby suppressing virus formation (13).

Because of dosage requirements and the problems of producing interferon in bulk quantity, that protein itself appears to be an impractical tool in the prophylaxis or treatment of human disorders (10). However, with the discovery that synthetic polynucleotide complexes can induce interferon in vitro and in vivo and thereby protect cells in culture and intact animals against some viral infections (14), it has become reasonable to attempt to use the interferon phenomenon therapeutically.

Synthetic double-stranded RNA is formed by the interaction of appropriate single-stranded ribohomopolymers or copolymers when solutions thereof are mixed under proper conditions of temperature and ionic strength (15–17). The single-stranded ribopolymers are formed by the bacterial enzyme polynucleotide phosphorylase using ribonucleoside diphosphates as building blocks (18). The polynucleotides and their complexes are not of uniform composition. The single-stranded polymers may differ greatly in molecular weight and polydispersity. Differences in conditions for bringing together the complimentary homopolymers may cause great differences in the degree and kind of complex formed. In spite of these consider-

ations poly I:C has uniformly proven to be the most active of the synthetic RNAs studied to date with respect to interferon induction and production of resistance to lytic viral infection in cultured cells and intact animals (14, 19–21). As such it is the synthetic RNA that has been most extensively studied in both lytic and oncogenic systems, and in transplanted neoplasms.

Numerous attempts have been made to extend the viral interference phenomenon into viral oncogenic test systems; these studies have given mixed results. Concentrated interferon preparations inhibit or delay oncogenesis by Rous virus in the chick embryo (22) and the newborn chick (23), as well as by Friend and Rauscher viruses in mice (24, 25). Extending their work on viral-induced tumors into effects upon transplanted tumors, Gresser and coworkers have reported therapeutic effects of interferon preparations in mice inoculated respectively with Rauscher virus-induced and carcinogen-induced tumor cell lines (26). Therapeutic regimens were most successful that afforded intimate physical contact between tumor cells and interferon; appropriate controls, including human interferon, had no antitumor effect. These observations have been supported by the reports of Wheelock that either coinfection with Sendai virus, or administration of the interferon-inducer statalon, or mouse interferon itself, would prolong survival in mice infected with Friend virus (27, 28). On the other hand, Vandeputte failed to obtain protection against Rauscher virus-induced spenomegaly in spite of induction of high titers of interferon with Sindbis virus, Newcastle disease virus, endotoxin, or exogenous interferon (29).

Similarly, assessment of the effects of poly I:C on viral oncogenesis in vitro and in vivo has yielded discordant findings. That drug's reported inhibition of murine sarcoma and leukemia virus transformation of cultured cells (4, 5) required drug concentrations several orders of magnitude greater than those commonly used to induce interferon or to protect against lytic virus infections. Since parallel studies were not carried out on the three phenomena employing both the same cell line and poly I:C preparation, the possibilities of relative cell resistance or ineffective poly I:C preparation, or both, cannot be assessed. Poly I:C protected against oncogenesis by murine sarcoma virus (6) but had negligible activity against either the Friend virus in mice (30) or adenovirus 12 or SV_{40} in hamsters (31, 32). In the therapeutic context poly I:C has shown impressive antitumor activity against two murine strains of reticulum cell sarcoma (7, 33) and significant activity against a murine malignant melanoma (34). Its activity against leukemia L-1210 was weak (7, 33); negligible therapeutic effects were seen against Friend leukemia (30) and a SV_{40}-induced transplanted tumor in hamsters (31).

Potential reaction mechanisms for the rather selective antitumor effects of poly I:C have not been explored in detail; the drug is not cytotoxic to any significant degree (19). Although a straightforward interferon mechanism may be operant in some animal systems it is difficult to support this concept in nonviral tumors or in the drug's inhibitory effects on dimethylbenzanthracene tumorogenesis (35). A more plausible hypothesis may be

found in the effects of poly I : C on the reticuloendothelial system. In a number of its pharmacologic effects in animals the polynucleotide complex resembles bacterial endotoxins (36), themselves inducers of interferon (vide supra). Like endotoxins poly I : C is pyrogenic although neither poly I nor poly C causes fever (37). With repeated injections of poly I : C tolerance develops to both interferon induction and pyrogenicity in the fashion of endotoxin. The pyrogenicity of poly I : C complexes is reduced by prior incubation with ribonuclease containing fluids confirming the concept that the effect is produced by the polynucleotide complex rather than a contaminating endotoxin (38). Although the mechanism is obscure and the effects are decidedly erratic, bacterial toxins have been known since the 19th century to produce antitumor effects in animals (39) and man (40).

Since the transplanted tumors are homografts the adjuvant activity of polynucleotide complexes could play a role in the antitumor effect. Poly A : U and poly I : C enhance antibody formation to sheep red blood cells (41, 42) and influenza antigens (43). Poly I : C markedly decreased survival of isografts in mice which crossed but a single histocompatability locus on the Y chromosome (42). This adjuvant effect has been explored by Friedman et al in leukocyte culture from peripheral blood (44). Poly I : C had no effect on macrophage-depleted cultures but it markedly increased thymidine uptake and blast transformation in macrophage-containing cultures exposed to PPD. A similar effect of poly I : C was seen on the response of mixed leukocyte cultures.

Poly I : C has antigenic activity in New Zealand mice strains which spontaneously develop antinucleic acid antibodies and manifest an auto-immune disorder that resembles human systemic lupus erythematosus (45). By coupling injections of poly I : C with large dosages of cyclophosphamide, Steinberg et al have been able to establish immunologic tolerance to poly I : C in these mice (46). The antigenicity of poly I : C may assume clinical importance in view of the observations of Koffler et al (47), and Schur & Monroe (48) that circulating antibodies which cross-react with the synthetic RNA complexes are present in the plasma of some patients with systemic lupus erythematosus.

The dose-limiting acute toxicity induced by poly I : C varies from species to species; in mice it is damage to the intestinal villous epithelium (49), while in the dog it is liver necrosis (33). It is embryotoxic in rabbits (33). In clinical trials in patients with advanced cancer poly I : C has induced circulating interferon and fever (50, 51). Although minor evidence of intravascular coagulation has been obtained with one preparation (51), neither significant anticancer effect nor limiting toxicity have been reported.

Antitumor Antibiotics

Biochemical and pharmacologic data on antibiotics having antitumor activity were last summarized in this series in 1963 (52); subsequently extensive reviews have appeared elsewhere on actinomycin (53), on the mechanism of action of antibiotics (54) and on the biosynthesis of antibiotics

(55). It is useful for this discussion to divide the antitumor antibiotics into two groups, (*a*) drugs which produce biological effects by interacting with DNA, (*b*) agents which damage cells in some other manner. Only the former group of compounds will receive extensive consideration in this review. It includes one agent of established value in the treatment of cancer, actinomycin D; and four other drugs of unquestioned activity, i.e. mithramycin, daunomycin, adriamycin, and bleomycin, which are presently undergoing extensive clinical trial. The antitumor antibiotics which primarily inhibit protein synthesis (cycloheximide, pactamycin) or perturb nucleotide metabolism (tubercidin and related agents) are of considerable biochemical interest but have had little clinical application.

Kersten, Kersten & Szybalski (56) have reported detailed comparisons of DNA-antibiotic complexes formed by actinomycins, anthracyclines, (e.g. daunomycin, nogalamycin), and chromomycins, (most notably mithramycin, now of known structure (57). They concluded that anthracycline antibiotics intercalated between base pairs but that actinomycins and chromomycins did not. Anthracyclines and actinomycins, but not chromomycins, stabilized native DNA to thermal denaturation. Although extensively studied, the precise nature of the actinomycin-DNA complex has not been resolved. The requirement in DNA for 2-amino purine residues (guanine or 2,6 diamino purine) seems absolute but is not, of itself, sufficient to produce binding (58, 59). Models of the actinomycin-DNA complex have been offered which provide for binding of the chromophore by hydrogen bonds to the outside of the DNA helix (60), and, more recently, for intercalation of the chromophore between base pairs adjacent to a GC[2] coupling (61). The chromomycins also require DNA guanine (56, 59, 62); but Mg^{2+} is needed to produce maximal binding (59, 62). While the anthracycline antibiotics can form complexes with DNA polymers which are totally lacking in guanine (56, 63) the extent of binding may increase somewhat with increasing guanine content (56).

The metabolic consequences of antibiotic-DNA complex formation with these drugs uniformly include inhibition of RNA synthesis. Synthesis of DNA may be inhibited also; effects upon DNA synthesis correlate well with the drug's effect on the melting curve of purified DNA. For example the anthracyclines daunomycin and nogalamycin significantly raised the Tm[2] of purified DNA and produced almost equal inhibition of synthesis of RNA and DNA in cultured cells (63, 64). Mithramycin does not affect the Tm and has had no acute inhibitory effect on DNA synthesis (65, 66). Detailed examination of drug effects upon synthesis of the varying functional types of RNA has been carried out only with respect to actinomycin and nogalamycin. With the former drug, chain elongation may be more severely affected than the initiation of new RNA chains (67). At the cellular level, synthesis of ribosomal RNA was selectively inhibited by low concentrations of actino-

[2] GC: Guanine: Cytosine base pair; curve.
Tm: Temperature at the point of inflection in a melting curve.

mycin (68, 70) and nogalamycin (71); in both instances this was associated with progressive deterioration of nucleolar stucture (71, 72). It is tempting to attribute the selective inhibitory effects of actinomycin on ribosomal RNA synthesis to the relationship between actinomycin binding and DNA guanine content. Since ribosomal RNA has a high C + G: A + U ratio the DNA that codes for ribosomal RNA (rDNA) presumably is high in guanine. However, a similar biochemical effect was produced by nogalamycin which can bind to dAT copolymers; comparable nucleolar lesions were induced by daunomycin (73), as well as by proflavin and ethidium bromide (73, 74), which intercalate between base pairs in DNA without regard to specific base pairs.

Recent studies with daunomycin and phleomycin suggest that DNA-antibiotic interactions may have biologic consequences independent of drug effects on nucleic acid synthesis. In synchronized populations of mammalian cells daunomycin (75) and phleomycin (76) interfered with mitotic events at low drug concentrations which did not inhibit precursor incorporation into nucleic acids. Phleomycin produced similar effects on meiosis and mitosis in plant cells (77). The drugs altered mitotic (and meiotic) events even when added shortly before prophase at a time when DNA synthesis could not be detected and peak synthesis of RNA during G2 had already occurred. The daunomycin effect seemed dependent upon that drug's capacity to complex with DNA (75).

The availability of tritium-labelled actinomycin-D and daunomycin has led to extensive studies of drug entry into and retention within cells in culture and tissues in intact animals. The data provide a positive correlation between tissue levels of both drugs and acute inhibitory effect on synthesis of RNA (64, 79, 80), and between prolonged tissue retention of actinomycin and cytotoxic effect (79, 80). Cytotoxicity may be accompanied by extensive loss of cellular RNA (81). Studies on spontaneous and drug-induced resistance in bacteria (82–84) and mammalian cells in culture (85–87) have, with a single exception (87), confirmed the concept that cellular resistance and impermeability coincide. In mammalian cell cultures the decrease in permeability is not specific for actinomycin but encompasses a number of compounds which are similar to that antibiotic in molecular size and charge (86).

In spite of their similarities at the molecular and cellular levels the three antibiotic types differ significantly in their clinical effects. Both daunomycin (88) and the 14-hydroxylated derivative of daunomycin, i.e. adriamycin (89), have significant antileukemic activity in man (90, 91); actinomycin and mithramycin are not therapeutically useful in leukemia. All of these antibiotics are myelosuppressive, but mithramycin seems unique in its capacity to lower serum calcium and induce hemorrhagic phenomena. The mechanism by which mithramycin produces hypocalcemia is not understood; clinical studies suggest that the drug interferes with calcium resorption from bone (92). This pharmacologic property may have therapeutic application in the management of Paget's disease of bone (93) and the hypercal-

cemia of malignant disease (94). The hemorrhagic toxicity of mithramycin appears due to a combination of several effects: damage to the terminal capillary bed, hepatotoxicity with reduced formation of clotting factors, as well as the previously mentioned bone marrow suppression resulting in thrombocytopenia and morphologic abnormalities in circulating platelets (95). Daunomycin may produce delayed cardiac toxicity in some patients (88, 96). The drug-induced cardiac damage is as yet only a clinical entity with an incidence of 5 to 10%. It becomes manifest weeks to months following initiation of drug therapy and is more common following total dosage in excess of 20 mg/kg. A pathologic lesion has not been identified on light microscopy nor has the syndrome been produced in lower animals.

Bleomycin is of importance because of its considerable activity against epidermoid cancer in man (97, 98). Although its biologic activity appears to be mediated, at least in part, via drug interaction with DNA, the nature of the interaction is sufficiently different to commend its discussion as a separate entity. Bleomycin is the generic name for a group of closely related sulfur-containing glycopeptide antibiotics occurring in a partially saturated chelate with copper in cultures of *Streptomyces verticillus* (99). The bleomycins have been separated into twelve fractions by chromatography on paper and on Sephadex (100). The most abundant single fraction, bleomycin A_2, yielded seven ninhydrin positive products following acid hydrolysis (101), including a chromophoric amino acid, 2′-(2-aminoethyl)-2′,4′, zole-4-carboxylic acid (101, 102). On alkaline methanolysis of bleomycin A_2 two sugars were obtained, 1-gulose and 3-o-carbamo-1-α-mannose; these are presumably linked to the remainder of the molecule by glyosidic bonds (103). The bleomycins possess broad antibacterial activity (104); their antitumor activity is most apparent when administered intraperitoneally to tumors growing in the ascites form (105, 106). The toxicology of bleomycin is remarkable in that the drug causes extensive injury to skin but produces minimal if any damage to bone marrow
tic (97, 98) and toxic (107) effects upon epidermal tissues suggests preferential drug localization therein; this has been confirmed in drug distribution studies (105, 108).

Biochemical studies of the effects of bleomycin upon bacteria and mammalian cells have shown the copper-free antibiotic to be more active than the chelate (109). The drug inhibited synthesis of DNA under conditions where synthesis of RNA and protein were apparently unimpaired (109–111). However, progression to mitosis was inhibited at drug concentrations that did not alter isotope incorporation into DNA, and by initial drug exposure late in G2 (110). In this respect the effects of bleomycin resemble those of daunomycin and phleomycin discussed above. Studies with purified DNA have disclosed no effect of bleomycin when used alone. However, in the presence either of hydrogen peroxide or of a variety of sulfhydryl-containing compounds the antibiotic lowered the thermal melting point of DNA (109, 112, 113) and produced single strand breaks in the polynucleotide chain (113). Prior exposure of DNA to a mercaptan sensitized the DNA to

later bleomycin exposure; the converse sequence was ineffective (112). The copper chelate of bleomycin was not active against purified DNA even in the presence of sulfhydryl compounds (112). Single-strand scissions have also been demonstrated in DNA extracted from HeLa cells and *E. coli* following treatment of the intact cells with bleomycin (113).

Other Chemotherapeutic Agents

Alkylating agents.—The clinical pharmacology of cytotoxic alkylating agents was discussed in this series in 1965 (114); they have subsequently received a more general review by Ochoa & Hirschberg (115). Recent laboratory investigations have considered drug effects upon progression of cells through the cleavage cycle and biochemical differences between sensitive and resistant cell populations. Mechlorethamine (HN2), inhibited progression from the DNA synthetic or "S" period into "G_2", the post synthetic period, in cells in culture (116). In a similar test system 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), inhibited progression through all phases of the cell cycle saving late G_2 and mitosis (117). A comparable result, i.e., prolongation of S, was observed in the ascites form of L-1210 leukemia following therapy with BCNU or 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) (118, 119). Studies on the binding of radioactive nitrogen mustard HN2-^{14}C by L5178Y lymphoblasts in vitro have disclosed significantly reduced binding in resistant cells (120). These findings were interpreted in terms of decreased membrane permeability. Further observations (121) suggested the presence of a transport carrier for HN2; in resistant cells an elevated K_m and diminished V_{max} was observed. Comparable studies have been carried out in vivo on sensitive and resistant lines of Lettre-Ehrlich ascites. Although the results were in accord on those on the L5178Y leukemic line, the resistant cells showed only a two-fold reduction in alkylation and cross-linking in spite of being 10- to 50-fold less sensitive to the cytotoxic effects of alkylating agents (122). In view of this discrepancy it was proposed that other factors were operant, such as an increased repair of alkylated DNA in the resistant cells (122). Repair of cross-linked DNA has been demonstrated in *E. coli* (123) and in mammalian cells (124). In the former study, resistant lines of *E. coli* possessed a significantly greater capacity for repair than did the sensitive parent strain.

Whisson & Connors (125–127) have investigated the unusual sensitivity of a murine plasma cell tumor to the therapeutic effects of N,N-bis(2-chloroethyl)-aniline (aniline mustard). Structure-activity relationships indicated the requirement of a free para position for therapeutic activity. Since p-hydroxylation is a known catabolic sequence for N, N diethylaniline in mammals, Whisson & Connors postulated hepatic p-hydroxylation of aniline mustard, (producing thereby p-hydroxyaniline mustard, a very toxic alkylating agent), followed by intrahepatic conjugation with sulfate or glucuronic acid. Hydrolysis of the p-hydroxyaniline mustard conjugate in tissue would regenerate the toxic intermediate and cause local tissue damage. This might be accomplished by at least two known enzymes β-glucuronidase and

aryl sulfatase. Further analysis disclosed that sensitive tumors had markedly increased levels of β-glucuronidase, but that aryl sulfatase levels in sensitive and resistant tumors were comparable.

Nitrosourea derivatives known to be active against intracerebral inoculated L-1210 leukemia (128), have now received extensive clinical trial. BCNU possesses significant therapeutic activity in Hodgkin's disease (129); it is also being used extensively in combination with other agents in the treatment of leukemia although its therapeutic utility as a single agent in this disorder has not been firmly established. The limiting toxicity with BCNU in clinical studies has been myelosuppression, with a characteristically delayed onset and persistent duration (129). The physiologic disposition of BCNU is complex. At physiologic pH BCNU is relatively unstable in aqueous solution, a loss of 70% occurring over a 5-hour period (130). Degradation of the compound in vivo is even more rapid; intact BCNU-^{14}C could not be detected in plasma samples taken 5 minutes following parenteral drug administration in man. Similarly urine and CSF samples collected as early as 15 minutes following drug dosage in monkeys failed to reveal intact BCNU. In contrast, disappearance from plasma and urine of ^{14}C derived from labeled BCNU is very slow. The data suggest that the antitumor effects and myelosuppression are caused by a catabolite of BCNU rather than by the intact compound.

Cross-resistance between nitrosoureas and established alkylating agents has been demonstrated in a hamster plasma-cytoma and in microorganisms. Further, nitrosoureas and alkylating agents produce similar biochemical changes in transplanted tumors (131–133) and in mouse liver (131). These effects included decreased incorporation of ribonucleotides into nucleic acids (131) and production of elevated levels of nicotinamide adenine dinucleotide glycohydrylase (NADase) (132, 133). In a cell-free system BCNU inhibited DNA nucleotidyltransferase by direct attack on the enzyme rather than upon the DNA primer; however, both primer and enzyme extracted from inhibited cells were capable of normal reactivity in vitro (134). Green and coworkers have demonstrated an increase in tumor cell NADase and a decrease in NAD following treatment of a variety of transplanted tumors with either HN2 or BCNU (132, 133). These observations support the concept that nitrosoureas function as alkylating agents in biologic systems. In addition they suggest that induction of NADase may be a significant cytotoxic consequence of cellular exposure to alkylating agents.

In chemical tests of alkylation, several nitrosourea derivatives complex with 4-(p-nitrobenzyl)pyridine; however, the nature of the intermediate that alkylates this receptor is unknown (135).

L-Asparaginase.—The historical background, animal experimentation, and early clinical trials of asparaginase in the treatment of cancer have been reviewed in this series by Cooney & Handschumacher (3) and by Capizzi et al (136). The basic concepts outlined in those two reviews are still current (137); now however, detailed reports have been published on clini-

cal results in adults (138) and children (139). Plasma levels obtained during these therapeutic trials and the clinical toxicity observed have been reported separately (140, 141). The limitations of L-asparaginase as a single agent in the treatment of human cancer seem now well established. It will produce complete remission of brief duration in roughly two-thirds of patients with lymphoblastic leukemia; responses with a lower frequency are also seen in lymphosarcoma and myeloblastic leukemia. Future therapeutic approaches with this agent will involve combining it with other effective agents in the treatment of sensitive tumors (142–144). In addition, an effort is being made to broaden the agent's spectrum of activity by coupling it with glutamine antagonists in the hope that this will produce a moderate asparagine deficiency in spite of adequate cellular quantities of asparagine synthetase. This strategy has had some success in asparaginase-insensitive rodent tumors when the enzyme was given in combination with 6-diazo-5-oxo-L-norleucine (DON) or DL-methionine-DL-sulfoxamine (145). Azaserine, another glutamine antagonist, failed to potentiate the effects of L-asparaginase. Preliminary clinical trials using asparaginase in combination with arabinosylcytosine (146) or as part of a multiple drug combination in acute leukemia (147) have been reported.

In line with current practice (148) with respect to new cytotoxic agents, L-asparaginase has been tested for immunosuppressive capacities in a variety of test systems. In intact animals asparaginase is immunosuppressive with respect to homograft rejection (149), generation of antibody-forming clones, and development of circulating antibody to particulate or soluble antigens (150–153). The apparent inconsistency between these observations and the uniform rise in immunoglobulins seen in patients receiving asparaginase (140) may indicate that the immunosuppression is greatly dependent upon precise timing of antigenic stimulus and enzyme administration. Asparaginase is also inhibitory to the blastogenic response induced in cultures of human lymphocytes; attempts to reverse this inhibition by addition of exogenous asparagine, or glutamine, or both, have given conflicting results (154, 155).

Plant Extracts.—Since the detailed discussion by Sartorelli & Creasey on the mechanism of action of vinca alkaloids and other chemicals producing mitotic arrest (2), a new agent, 4′-demethyl-epiodophyllotoxin thenylidene glucoside (VM-26) has appeared and been entered into clinical trial. According to the observation of Stähelin (156), in addition to production of mitotic-arrest, VM-26 inhibited entry of cells into mitosis and led to relatively rapid cell lysis. It also potentiated the antileukemic effects of L-asparaginase in mice (142). Furthermore, it produced significant inhibition of thymidine uptake into DNA in short-term experiments at relatively low drug concentrations. In the initial clinical assessment, VM-26 has shown definite therapeutic activity in patients with Hodgkin's Disease; limiting toxic manifestations appeared to be myelosuppression (157).

Folic acid antagonists.—Investigative interest in this area has centered upon increasingly distant structural analogs of folate such as quinazoline derivatives (158, 159), and 2,4-diaminopyrimidines (160). Experimental focus in these studies has been on the delineation of favorable inhibitor-enzyme relationships in extensively purified systems followed by testing in intact cells, in animals and finally in man.

Albrecht & Hutchinson have presented detailed observations on methasquin, a new quinazoline antifolate (158). They observed that the interaction between methasquin and folate reductase was slightly firmer than that between the same enzyme and methotrexate and was to a greater degree independent of experimental conditions. In studies on sublines of the L-1210 leukemia, the quinazoline antifolates proved cross-resistant with methotrexate; however, methasquin has a somewhat higher therapeutic index than did the earlier folic acid antagonist (159).

An extensive series of studies on 2,4-diaminopyrimidines has been summarized recently by Hitchings (160). These studies account at least in part for the differing toxicities of the diaminopyrimidines to various bacterial species on the basis of the inhibition kinetics demonstrated with the inhibitors and the isolated folic reductase enzymes from these bacteria. In further investigations along this line Baker has synthesized a large number of "active-site-directed irreversible inhibitors" with considerable attention being given to inhibitors of folic reductase (161). The potential power of this approach is amply demonstrated by the recent observations of Baker & Meyer that a 5-(p-aminophenoxypropyl)-2,4-diamino-6-methyl-pyrimidine derivative bearing a terminal sulfonyl fluoride was a powerful irreversible inhibitor of folate reductase isolated from L-1210 cells but not from mouse liver, spleen, or intestine (162). That this compound failed to inhibit the growth of L-1210 cells in vitro presumably indicates that they are impermeable to the inhibitor.

Thiosemicarbazones.—Investigation has continued on the series of thiosemicarbazones with antileukemic activity, originally reported by Brockman (163) and reviewed recently by Sartorelli & Creasey (2). Brockman has reported on the correlation between structure, inhibition of ribonucleotide reductase, and inhibition of the antiviral activity of these compounds, particularly the 5-hydroxypicolinaldehyde derivative (5-HP) against Herpes simplex and cytomegalovirus (164). The antileukemic effect of this compound in mice, and lack of cross resistance to conventional antileukemic agents such as methotrexate, mercaptopurine, arabinosylcytosine, vincristine, and L-asparaginase, has been reported (143). The ability of 5-HP to potentiate the antileukemic effects of asparaginase has also been stressed (142–144). This compound is now in clinical trial.

Topical chemotherapy.—The therapeutic effects of topically applied antigens and of topically applied cytotoxic agents with particular emphasis on

5-fluorouracil have recently been summarized elsewhere (165–167). Apparently comparable results can be achieved in premalignant keratoses, superficial basal cell carcinomas, and epidermoid cell carcinomas in situ either by inducing a localized hypersensitivity reaction with dinitrochlorobenzine or by a cytotoxic effect of fluorouracil. In a series of 700 patients, fluorouracil produced resolution of the neoplastic or preneoplastic lesion in more than 95% of appropriately selected patients. Local side effects in both instances were tolerable; systemic side toxicity was not observed. The reason for this apparently highly selective effect on these epidermal lesions is not clear, though a breakdown in local barriers to permeability is a reasonable supposition.

Chemotherapeutic Applications of Cell Proliferation Kinetics

In the last half decade the closely interrelated concepts of log-kill, surviving fraction, cell proliferation kinetics, and cycle-dependent and cycle-independent cytotoxic drugs have come to dominate much of the clinical and laboratory endeavor in cancer chemotherapy. Although this work has not lacked for reviewers elsewhere (168), its increasingly significant role in cancer chemotherapeutic practice mandates a discussion in this series as well.

By detailed analysis of the relationship between the number of leukemia L-1210 cells inoculated into mice and the duration of the animals' survival thereafter, Skipper, Schabel & Wilcox (169) derived a credible figure for the total number of leukemic cells present, within the animal, at any given time. In like manner, by examining the effect of chemotherapy upon the duration of survival in mice containing a known burden of leukemic cells they could calculate the number of tumor cells killed by a single administration or by a series of treatments. They observed that the percentage of a leukemic cell population killed with a given dose of an anticancer drug was reasonably constant regardless of the size of the leukemic population over a range of 10^0 to 10^6 cells (169). Further, a close relationship existed between the dosage level and the percent (or log) kill. Having derived a system to measure cell-kill they proposed that total eradication of all tumor cells was a reasonable chemotherapeutic goal. Subsequently, they and others have tested a number of drugs and therapeutic strategies toward this end. In this work the concepts of cycle-dependent and independent cytotoxicity and an increasing knowledge of proliferation kinetics have contributed greatly.

Drug-induced cell injury is dependent upon numerous factors; among them are the extent of drug entry into the cell, the nature of the metabolic (or structural) lesion produced, the cell's capacity to circumvent this lesion by an alternate pathway, replacement, or repair, and the time permitted for recovery or repair before, by reason of some cellular event, the drug-induced lesion becomes lethal. It should be noted that we are currently considering lethality only in terms of proliferative capacity. According to current theory, if an injured cell is to produce viable progeny, repair must be accomplished before the proliferative sequence actually commences. There-

fore, the time available for repair is greatest in nondividing cells and shortest in cells proliferating at the time of drug exposure. Based on in vitro (170, 171) and in vivo (172, 173) exposure of drug-sensitive populations, many of the currently employed cytotoxic agents have been characterized as producing cell-cycle-dependent and cell-cycle-independent cell killing. Drugs in the former group damage only proliferating cells actually in the cell cycle, i.e. mitosis— G_1— S — G_2 — mitosis-etc., at the time of drug exposure. This can be manifested in vivo either by a plateau in cell kill with increasing drug dose (vinblastine, methotrexate, arabinosylcytosine) or by positive correlation between the slope of an exponential dose-effect curve and the rate of cell proliferation (fluorouracil, actinomycin, cyclophosphamide) (173–175). Cell-cycle-independent agents produce long-term injury to the proliferative capacity of cells in a resting or "G_0" phase as well as damaging cells actually "in cycle." HN2 and BCNU are cited most commonly as exemplifying this type of agent (170, 173). Current therapeutic strategy attempts to fit the drug to the kinetics of the tumor in question. In some animal models at least, this has proven to be an approach of impressive merit.

Below a cell burden of 10^6 cells/mouse the transplanted leukemia L-1210 consists entirely of cells in cycle (i.e. there are no cells in G_0) which have a generation time of 12 hours; of this about 10 hours is spent in the S phase (176, 177). Cures of mice bearing this leukemia can be obtained by appropriate timing of cytotoxic levels of arabinosylcytosine, (an S phase specific agent), to include at least part of the S phase of all cells in the asynchronous population (177, 178). In contrast, in the advanced murine leukemias (L-1210 or AK), in murine solid tumors, and in clinically evident human neoplasms, significant numbers of the tumor cell population are in G_0 (179–184). In these animal models, even with "optimal" spacing, cycle-dependent (or at least S-phase-dependent) chemotherapy fails to "cure" or produce prolonged survival (179–182); a similar lack of success has classically attended clinical efforts. According to current treatment strategy in such disease situations, an initial course of cycle-independent therapy should be given in the hope that the drug-induced reduction in neoplastic cell numbers will be followed by a recruitment of the remaining cells into the proliferation cycle (185). With a higher growth fraction, the population may then become more susceptible to cycle-dependent cytotoxic agents. This strategy has provided cures in advanced murine leukemias L-1210 and AK (179–181), as well as in the murine adenocarcinoma 755 (182) and the hamster plasmacytoma 1 (186).

The clinical concomitant of this work has included an increasingly detailed examination of tumor cell kinetics (183, 184), manipulation of dosage schedules of cycle-specific agents in the light of the newly acquired kinetic data, and therapeutic employment of a variety of cycle-dependent and independent drugs in combination. The most direct clinical extension of the mouse leukemia studies has been a comparison of the therapeutic utility of arabinosylcytosine by acute daily injection and by continuous infusion in

five-day courses (187–188). Both schedules produce remissions in acute leukemia, but there have been no published studies as yet to show a demonstrable superiority of one over the other.

Current practice in the management of acute leukemia calls for remission induction followed by "consolidation" therapy with drug combinations; this is a direct application of the concept of "maximal kill." The median survival time in acute lymphoblastic leukemia has markedly improved in the past five years; during this period kinetic considerations and drug combinations have played an ever increasing role in therapy. The case for a cause and effect relationship between the two has recently been summarized by Henderson & Samaha (189). Similar efforts are being made in the treatment of lymphomas (190) and solid tumors (191, 192). In the absence of curative single agents this approach will doubtless be extended in the coming decade.

Cycles abound in the affairs of man; they occur in scientific enthusiasms as well as in cell proliferation and in mitochondria. For the moment the excitement of fresh discovery has left the clinical pharmacologists, and more recently the molecular pharmacologists; it now dwells with the kineticists. However the newly, and perhaps temporarily, drab disciplines have much yet to contribute in the kineticists home territory. The effect in animals of several simultaneously or sequentially administered cytotoxic agents upon their individual tissue distributions and metabolisms has gone unexamined. Similarly, drug combinations have been studied in vitro primarily for enhanced cytotoxicity rather than their effects on specific biochemical lesions. Since combination chemotherapy is fast becoming a clinical rule rather than an exception these points should receive intense pharmacologic study in the next few years.

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