CANCER CHEMOTHERAPY WITH PURINE AND PYRIMIDINE ANALOGUES[1]

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Introduction

Two articles pertaining to cancer chemotherapy have previously appeared in this series. Karnofsky & Clarkson reviewed the cellular effects of anticancer drugs (1) and Oliverio & Zubrod discussed the clinical pharmacology of selected antitumor drugs (2). The field of cancer chemotherapy is too enormous to review *in toto* in the limited space allotted here. Consequently, the present review will be restricted to purine and pyrimidine analogues that are useful or potentially so in cancer chemotherapy. It is also impossible to review comprehensively the literature that pertains to this field. Thus, the emphasis will be on publications (not including abstracts) that appeared in 1965 and the first half of 1966 and which I found of interest. Research with these analogues in systems other than mammalian ones will be arbitrarily excluded. Superficial mention will be made of clinical efficacy, but the emphasis will primarily be on results in experimental systems and on biochemical mechanisms of action.

A very important concept, enunciated by Skipper (3), is that of the necessity for 100 per cent kill of tumor cells in order to achieve cures. This concept arose from studies of the effects of chemotherapy on the kinetics of cell behavior with the L-1210 leukemia in mice, in which the transplantation of a single cell ultimately caused death from the disease. It was found that the percentage kill of leukemic cells by a given dose of a given drug is reasonably constant, which led to theoretical considerations of dose regimes that produced with several compounds significant numbers of mouse cures. Several clinical investigations in acute childhood leukemias have recently been devised in an attempt to achieve, by chemotherapy with new dose schedules and by combinations of drugs, total eradication of the leukemic cells in the patient; the preliminary results appear to be encouraging.

Recently, an evaluation of the screening results obtained in the Cancer Chemotherapy National Service Center's program has been carried out by Goldin et al. (4) who concluded that the two tumors most likely to predict clinical activity in human patients are the L-1210 leukemia in mice and the Walker 256 carcinosarcoma in rats. A valuable compendium of the toxicities of a large number of clinically useful drugs in various species including man has been prepared by Freireich et al. (5). Since the tragic inevitability of the emergence of resistance constitutes one of the major limitations to clinical cancer chemotherapy, the comprehensive review by Hutchison (6) on cross-

[1] The survey of the literature pertaining to this review was concluded in June 1966.

resistance and collateral sensitivity represents an important contribution that points to common mechanisms of action and suggests possible avenues of increased sensitivity to a second drug after resistance develops to the first.

Purine Analogues

8-Azaguanine (AzG).—The first nucleic acid antimetabolite to show definite tumor-inhibitory activity was 8-azaguanine, which Kidder et al. (7) found to inhibit the growth of *Tetrahymena* and also of adenocarcinoma E 0771 and spontaneous mammary carcinomas in mice. The earlier work with this compound has been thoroughly reviewed by Mandel (8). This analogue closely resembles guanine (II, Fig. 1), but has an isosteric replacement of the carbon at position 8 by a nitrogen atom (III, Fig. 1). There was a marked variation of the inhibitory effect of AzG among various experimental tumors, and it was shown by Hirschberg et al. (9) that the inhibition varied inversely with the activity in the tumor of the enzyme guanase, which deaminated the drug to the inactive 8-azaxanthine.

The first demonstration of the incorporation of AzG into nucleic acids was that of Lasnitzki et al. (10) who isolated 8-azaguanylic acid from alkaline hydrolysates of RNA obtained from *Bacillus coli, Staphylococcus Aureus*, and mouse liver, spleen, and sarcoma 37 after administration of unlabeled AzG. They could detect no incorporation of the analogue into DNA. However, using C^{14}-labeled AzG, Mandel et al. (11) demonstrated its incorporation into RNA, and to a much lesser extent into DNA, of mouse liver and sarcoma 37. Whereas the liver incorporated *in vivo* more guanine than AzG into nucleic acids, the converse was true for the tumor (11). Brockman et al. (12) studied the metabolism of AzG in neoplasms that were sensitive and resistant to its action and found a good correlation between the extent of conversion into 8-azaguanylic acid and incorporation into RNA with the effectiveness of the drug against the tumor; there was no indication of permeability differences between the sensitive and resistant tumors.

In subsequent years, evidence has accumulated from microbial systems to indicate that one of the primary effects of AzG is an inhibition of protein synthesis (cf. 8). This has recently been studied in HeLa cells by Zimmerman & Greenberg (13). They found that this analogue caused a preferential inhibition of protein synthesis at an early stage and that RNA and DNA biosynthesis was inhibited later and to a much lesser extent. Using a system of polysomes, they found that protein synthesis was blocked without inhibition of messenger RNA synthesis. However, the synthesis of cytoplasmic ribosomes was inhibited, and the AzG was incorporated into the ribosomal and messenger RNA's. Since AzG produced no effect on protein synthesis in rabbit reticulocytes, which have a very stable messenger RNA, it was concluded that protein synthesis was inhibited as a consequence of the incorporation of the analogue into messenger RNA (13). A somewhat similar study was carried out by Karon et al. in spinner cultures of KB cells, who also ob-

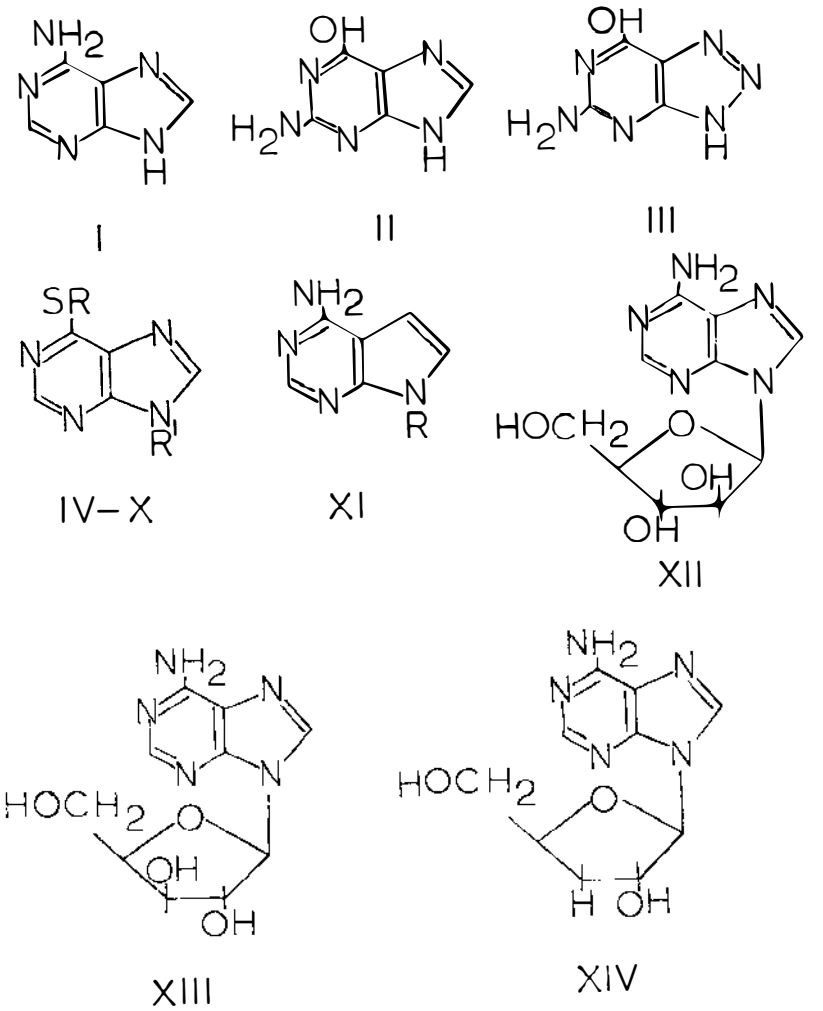

FIG. 1. Purines and purine analogues: I = Adenine; II = Guanine; III = 8-Azaguanine; IV = 6-Mercaptopurine, R = H, R′ = H; V = Methylmercaptopurine, R = methyl, R′ = H; VI = 6-Mercaptopurine ribonucleoside, R = H, R′ = ribose; VII = 6-Methylmercaptopurine ribonucleoside, R = methyl, R′ = ribose; VIII = Imuran, R = 1-methyl-4-nitro-5-imidazolyl, R′ = H; IX = Arabinosyl-6-mercaptopurine, R = H, R′ = arabinose; X = Xylosyl-6-mercaptopurine, R = H, R′ = xylose; XI = Tubercidin, R = ribose; XII = Arabinosyl-adenine; XIII = Xylosyl-adenine; XIV = Cordycepin.

served a marked inhibition of protein synthesis, but a preferential incorporation of AzG into the soluble RNA of these cells (14). Thus, one of the major biochemical effects of 8-azaguanine is to inhibit protein synthesis as a result of being incorporated into RNA to give some sort of fraudulent template, either at the messenger or soluble RNA level. Whether this effect is related to the tumor-inhibitory properties of the drug is not clear. Although AzG has not been found useful in clinical cancer chemotherapy when given systemically, it has been reported by Hall et al. (15) to produce temporary objective responses in 30 per cent of patients with head and neck cancer by continuous intra-arterial infusion.

6-Mercaptopurine (6-MP).—This compound (IV, Fig. 1) was first synthesized by Elion et al. (16) in 1952, and in 1954 a monograph appeared about its use (17). Clarke et al. (18) reported that 6-MP inhibited the growth and transplantability of sarcoma 180. Considerable activity was found in a number of tumors in mice and rats, and it is one of the important drugs for the treatment of children with acute leukemia (cf. 19). In studies with 6-MP-S^{35} in mice, Elion et al. (20) found that it was excreted in the urine primarily as thiouric acid and to a lesser extent as inorganic sulfate; there were also indications of incorporation of radioactivity into nucleic acids, which has very recently been shown to occur as thioguanylic acid (20a). A great deal of work has been done on the mechanism of action of this drug, particularly as related to the problem of resistance.

It has been clearly demonstrated by Brockman (cf. 21) that 6-MP reacts with 5-phosphoribosyl-1-pyrophosphate (PRPP) to give 6-MP ribonucleotide, which is the active tumor-inhibitory compound. This reaction is catalyzed by the enzyme that carries out a similar conversion of hypoxanthine and guanine to their corresponding nucleotides. In a number of experimental tumors that have been made resistant to 6-MP, this pyrophosphorylase is either missing or present in very small amounts; hence 6-MP is not converted into the active nucleotide form (cf. 21). However, Davidson (22) determined the nucleotide pyrophosphorylase activity of the leukemic leukocytes of a number of patients, and found no significant difference in the activity of this enzyme in the specimens from those that were susceptible and resistant to 6-MP. Hence there must be another site of action of 6-MP, not involving its ribonucleotide, since Davidson had also shown (cf. 22) that in sensitive and resistant L-1210 leukemia cells there was no difference in permeability to the drug. An interesting compound that circumvents the resistance to 6-MP resulting from the loss of pyrophosphorylase activity was synthesized and tested by Montgomery et al. (23). They found that the dinucleoside monophosphate of 6-MP, bis(thioinosine)-5′,5″-phosphate inhibited the growth of such resistant cells in culture, and presumably entered the cells (ordinary mononucleotides cannot) and was cleaved to 6-MP ribonucleotide, which then acted in an inhibitory fashion. The generality of this promising finding has apparently not been further explored.

A second site of inhibition of 6-MP has been studied by Brockman &

Chumley (24) who found that it inhibited, by a negative feedback also exhibited by natural purines, the formation of phosphoribosylamine, the first step in purine biosynthesis. This inhibition was produced equally well by 6-MP and its ribonucleotide, but occurred only in cells that were capable of forming the nucleotide (24). Further studies on these feedback mechanisms have been done by Henderson & Khoo (25).

Atkinson & Murray (26), working with Ehrlich ascites cells, found that 6-MP is a competitive inhibitor of guanylic acid pyrophosphorylase in both sensitive and resistant cells; hence, the mechanism of resistance does not involve this type of inhibition.

Salser & Balis have directed their attention toward another site of inhibition produced by 6-MP ribonucleotide, namely the enzymatic conversion of inosinic acid (IMP) to adenylic acid (AMP) (27). They found no significant difference in the ability of normal liver and sarcoma 180 (a very sensitive tumor) to form 6-MP ribonucleotide, nor in its ability to inhibit the IMP conversion to AMP. In surveying the conversion of IMP to AMP in several tissues, they found that tumors hada lesser capacity to carry out this reaction than liver, and suggested that this reduced capacity might explain the greater sensitivity of tumors to 6-MP as compared with normal tissues (27).

Thus, it can be concluded that there are at least three loci of the inhibitory action of 6-MP and its ribonucleotide, and considerably more research must be done to determine which, if any, of these is of primary importance in human cancer chemotherapy.

Another very interesting biological activity of 6-MP, the inhibition of the immune response, was first demonstrated by Schwartz & Andre (28), and it was found (29) that a derivative, azathioprine (imuran) (VIII, Fig. 1), is even more effective. The subject of the chemical suppression of immune response has been reviewed by Hitchings & Elion (30).

Other derivatives of 6-mercaptopurine.—An important derivative of 6-MP is its ribonucleoside (VI, Fig. 1), which has antitumor activity about equal to that of 6-MP, and which does not inhibit 6-MP-resistant tumors. Of greater interest is the report by Bennett et al. (31) that 6-methylmercaptopurine ribonucleoside (VII, Fig. 1) inhibits 6-MP-resistant tumors that lack the IMP pyrophosphorylase. This compound is converted into the corresponding nucleotide in cells that do not convert 6-MP, and it inhibits phosphoribosylamine formation in 6-MP-sensitive and -resistant cells. Giner-Sorolla & Bendich (32) have prepared a series of compounds that are homologues of 6-MP, of which 6-acetylthiomethylpurine has slight tumor-inhibitory activity.

6-Mercaptopurine-3-N-oxide has been synthesized and tested by Brown et al. (33) who found that it had activity against various tumors equivalent to 6-MP but at ten times the dose. It is worth noting, however, that Brown et al. (34) found that the N-oxides of adenine, guanine, and hypoxanthine are highly carcinogenic. Montgomery & Hewson (35) have synthesized the 1-, 3-, and 7-deaza-6-MP's, in which the ring nitrogens are substituted

isosterically by carbons, but they had little biological activity. They also prepared 1- and 3-deaza-6-methylthiopurine ribonucleosides, which were also not very toxic to cells in culture (36, 37).

A study of arabinosyl-6-MP (IX, Fig. 1) has been carried out by Kimball et al. (38); the compound was synthesized by Reist et al. (39). Arabinosyl-6-MP increased the survival of mice with 6-MP-resistant tumors that lacked IMP pyrophosphorylase and gave a synergistic effect in combination with azaserine (38). It was not cleaved enzymatically to 6-MP, nor was it appreciably converted to the nucleotide, suggesting that the nucleoside is the active form of this compound. It has no effect on the incorporation of glycine into nucleic acid purines, but does inhibit the incorporation of orotic acid into nucleic acid cytosine (38). Sato et al. (40) have found that xylosyl-6-MP (X, Fig. 1) is an active tumor-inhibitory compound that is cross-resistant to thioguanine. It is not cleaved to 6-MP, nor is it converted appreciably into nucleotide form. It inhibits the incorporation of guanosine into nucleic acids *in vivo* at a dose which does not influence the incorporation of glycine into nucleic acids. In a cell-free system, xylosyl-6-MP does not inhibit the conversion of guanine into guanylic acid (40).

Tubercidin.—This compound was isolated as an antibiotic by Anzai et al. (41), and its structure was shown to be 7-deaza-adenosine (42, 43) (XI, Fig. 1); the antibiotic toyocamycin was found to be 5-cyanotubercidin (43). Tubercidin is highly cytotoxic to cells in culture (44), and this activity is not reversed by the known purines, pyrimidines, or their nucleosides (45). It is also active against some of a spectrum of tumors (45). A survey of its biochemical properties in mouse fibroblasts in culture was carried out by Acs et al. (46) who found that it inhibited DNA, RNA, and protein synthesis. Tubercidin is incorporated as such into RNA, its triphosphate is a substrate for RNA polymerase, and it is incorporated into DNA as the 2′-deoxy derivative. These investigators believe that the lethal event is the incorporation into DNA (46), which is also suggested by the fact that it inhibits the growth of vaccinia, a DNA virus. A striking property of tubercidin is its rapid uptake by erythrocytes; when 100 μg/ml is added to whole human blood, 95 per cent can be recovered from the lysed erythrocytes in the form of the mono-, di-, and triphosphates (47). This uptake is also observed in cancer patients, and there are preliminary suggestions of clinical activity against some pancreatic tumors (48).

Arabinosyl-adenine (AraA).—This compound (XII, Fig. 1) was first synthesized by Lee et al. (49) in 1960. Hubert-Habart & Cohen (50) found that AraA killed *Escherichia coli*, which was correlated with the inhibition of DNA synthesis. However, this killing effect was more rapid than with other compounds that inhibit DNA biosynthesis, which suggested that AraA had a secondary effect on RNA, perhaps as a result of attachment to the terminus of soluble RNA (50).

AraA has been studied extensively in mammalian systems by Le Page and his colleagues. They reported that AraA inhibited the growth of ascites

tumors TA3 and 6C3HED, but not L-1210 leukemia and solid tumors, and it inhibited the incorporation of adenine, guanine, orotic acid, and glycine into DNA and, to a lesser extent, into RNA *in vivo* (51). It was found that AraA was readily converted into its triphosphate, which was incorporated into RNA, but not into DNA, and it was cleaved to adenine in the L-1210 leukemia, but not by the sensitive tumors (51). AraA is rapidly cleared from the blood in mice and excreted in the urine as arabinosyl hypoxanthine, and no cleavage to adenine could be detected in the blood or normal tissues although deamination was extensive (52). The compound was incorporated into acid-soluble nucleotides and RNA in liver. It inhibited DNA to a greater extent and for longer periods in sensitive than in resistant tumors, but AraG was less effective in these respects (52). AraA inhibits the incorporation of adenine and uracil into DNA in ascites cells *in vitro*, which is reversed by adenosine but not by deoxyadenosine. It is incorporated into nuclear and soluble RNA, and produces variable effects on amino acid incorporation into protein *in vivo* (53). Finally, it has been shown (54) that arabinosyl-ATP noncompetitively inhibits the incorporation of thymidine triphosphate (TTP) into DNA in extracts of TA3 ascites cells, using denatured DNA as the primer, suggesting a direct interaction with the DNA polymerase, possibly at an allosteric site.

Xylosyl-adenine (XylA).—This nucleoside analogue (XIII, Fig. 1) was synthesized by Chang & Lythgoe (55), and has been studied by Ellis & Le Page. They reported (56) that XylA significantly increased the survival of mice bearing the TA3 and Ehrlich ascites tumors and inhibited the incorporation of adenine and glycine into RNA, but not DNA, and was not cleaved. It also acted as a feedback inhibitor of purine synthesis by preventing FGAR formation, but this was probably of minor importance in tumor inhibition (56). XylA was rapidly converted to the triphosphate which inhibited PRPP formation from ribose-5-phosphate and ATP, and this was probably responsible for its biochemical effects and tumor inhibition (56, 57). In distribution studies in mice, XylA was found (58) to be rapidly excreted in the urine as xylosyl hypoxanthine, and was phosphorylated to the triphosphate level in normal and tumor tissues. The metabolism of a number of purine nucleoside analogues has been reviewed by Le Page (59).

Cordycepin and derivatives.—Cordycepin was isolated as an antibiotic and was assigned the structure of a nucleoside of adenine with a branched chain sugar, cordycepose (60). However, Kaczka et al. (61) isolated from a microorganism a compound that they identified as 3′-deoxyadenosine, which turned out to be identical with cordycepin (62) and with a sample prepared synthetically by Lee et al. (63); the structure is shown in Figure 1, XIV.

Klenow (64) found that cordycepin was readily converted to the mono-, di-, and triphosphates in Ehrlich ascites cells (and inhibited their growth), and that the incorporation of P^{32} into nucleic acids was inhibited. Overgaard-Hansen (65) found that cordycepin triphosphate inhibited the formation of PRPP from ATP and ribose-5-phosphate. In a more recent study, Shigeura

& Gordon (66) demonstrated that cordycepin inhibited the incorporation of formate, glycine, adenine, and guanine, but not orotic acid, into nucleic acids of Ehrlich ascites cells incubated *in vitro*. They also found that cordycepin triphosphate inhibited the DNA-dependent synthesis of polyadenylic acid and also polyuridylic acid-dependent polyphenylalanine synthesis in cell-free systems, and concluded that this triphosphate competed with ATP in various polymerization reactions (66).

Because of these interesting biological properties, a series of purine 3′-deoxynucleosides was synthesized by Walton et al. (67) and independently by Murray & Prokop (68), and were tested as inhibitors of the growth of KB cells in culture by Gitterman et al. (69). It was observed that 6-methylaminopurine-3′-deoxyribonucleoside was a more potent inhibitor of KB cells than cordycepin, but produced a more delayed toxicity, and both compounds inhibited the incorporation of uridine into RNA (69). 2,6-Diaminopurine-3′-deoxyribonucleoside was toxic to chick embryo fibroblasts, but not to KB cells in culture (69). Shigeura et al. (70) studied the metabolism of these compounds in Ehrlich ascites cells and found that whereas cordycepin was converted to the triphosphate, 6-methylaminopurine-3′-deoxyribonucleoside was transformed only into the monophosphate. Removal of the 6-amino function prevented phosphorylation of these compounds (70), and the extent of phosphorylation was correlated with the inhibition of PRPP formation, the monophosphates being less active than the triphosphates; the compounds that were not phosphorylated were inactive (70). They found that N-6-alkylated nucleosides were not converted to the triphosphates, and extended the studies of the inhibition of DNA-dependent RNA polymerase to show that 3′-amino-3′-deoxyadenosine triphosphate was also an effective inhibitor (70).

Other purine nucleoside analogues.—Recently a number of other compounds have been synthesized, but no biological data are yet available. Among the more interesting ones are 2′-methoxyadenosine (71), 2′,3′-dideoxy- and 2′,3′,5′-trideoxyadenosines (72), 2′,3′-dehydroadenosine (73), nucleosides of 3-deazaadenosine (a carbon isostere of adenosine) (74), 7-arabinosyl adenosine and hypoxanthine (in which the sugars are attached at the other nitrogen in the imidazole ring of the purine) (75), some 5′-deoxy-xylo purines (some of which inhibit adenosine deaminase) (76), some 3′-aminohexose derivatives of adenosine with the manno, galacto, and gluco configurations (77), and some glucopyranosides of various purines and pyrimidines (78).

Pyrimidine Analogues

6-Azapyrimidines.—6-Azauracil (AzU) (IV, Fig. 2), a nitrogen isostere of uracil (I, Fig. 2), was first prepared in 1947 (79). Its biological properties in bacterial systems were reported in 1956 simultaneously by groups in Prague (80) and in New Haven (81); since that time both groups have continued to make independent, and occasionally collaborative, contributions. AzU was

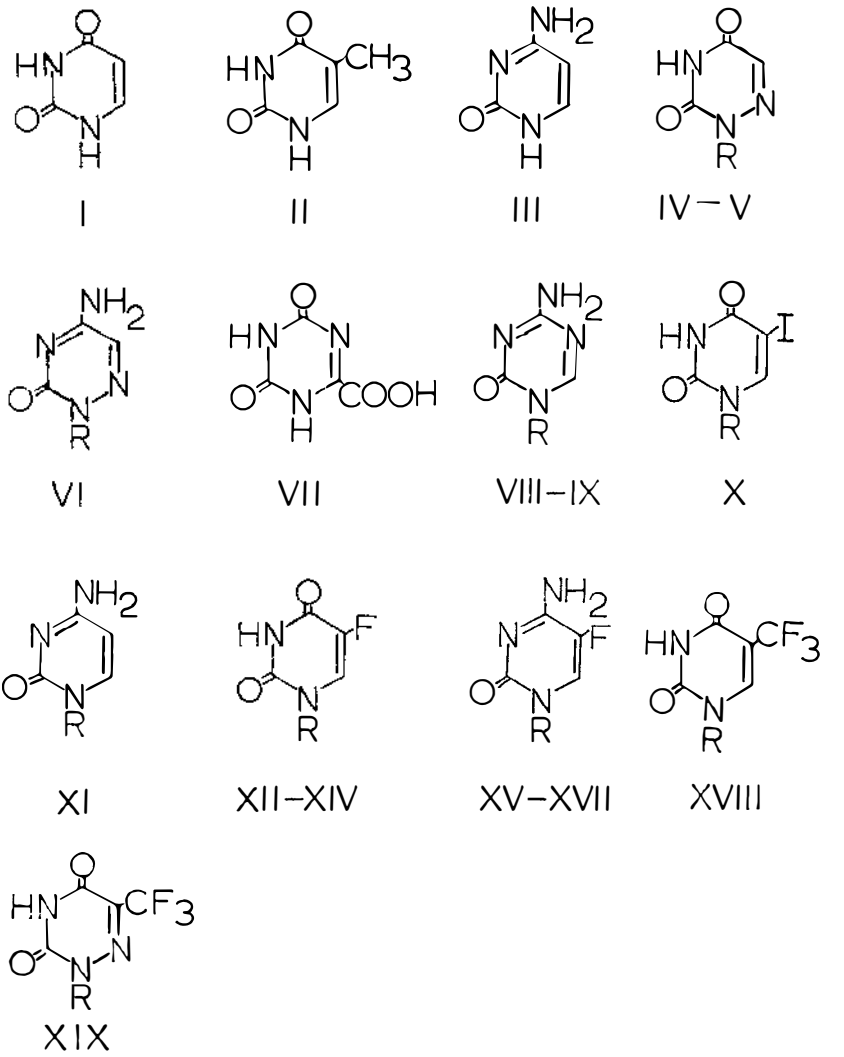

FIG. 2. Pyrimidines and pyrimidine analogues: I = Uracil; II = Thymine; III = Cytosine; IV = 6-Azauracil, R = H; V = 6-Azauridine, R = ribose; VI = 6-Azacytidine, R = ribose; VII = 5-Azaorotic acid; VIII = 5-Azacytidine, R = ribose; IX = 5-Aza-2′-deoxycytidine, R = deoxyribose: X = 5-Iodo-2′-deoxyuridine, R = deoxyribose; XI = Cytosine arabinoside, R = arabinose; XII = 5-Fluorouracil, R = H; XIII = 5-Fluorouridine, R = ribose; XIV = 5-Fluoro-2′-deoxyuridine, R = deoxyribose; XV = 5-Fluorocytosine, R = H; XVI = 5-Fluorocytidine, R = ribose; XVII = 5-Fluoro-2′-deoxycytidine, R = deoxyribose; XVIII = 5-Trifluoromethyl-2′-deoxyuridine, R = deoxyribose; XIX = 5-Trifluoromethyl-6-aza-2′-deoxyuridine, R = deoxyribose.

shown to have tumor-inhibitory activity by Sablik & Sorm (82) and by Jaffe et al. (83). Elion et al. (84) found that its activity against adenocarcinoma 755 was greatly potentiated by urethan. In a study of the pharmacology of AzU, Welch et al. (85) found that it produced considerable central nervous system toxicity.

A definite improvement in therapeutic activity came with the synthesis of 6-azauridine (AzUR) (V, Fig. 2) by a fermentation procedure (86) and later by chemical synthesis (87). This compound has considerably greater tumor-inhibitory activity than AzU in a number of systems (83, 88) and is almost free from central nervous system toxicity; in fact it is a remarkably nontoxic compound, producing virtually no side effects in human beings when given intravenously (89). Under these conditions, as reported by Handschumacher et al. (89) and discussed by Calabresi (90), a number of partial remissions have been produced in patients with acute leukemia, and the Czech experience has been similar (91). A very interesting development has been the report by Zaruba et al. (92) that AzUR is the only drug known to be effective against mycosis fungoides in man.

An improvement in therapeutic capability has resulted from the use of the triacetyl derivative of AzUR by Handschumacher et al. (93), which is absorbed from the gastrointestinal tract without the toxicity that was obtained with oral AzUR. The triacetyl compound produces a significant and sustained blood level of AzUR and is excreted in the urine solely as AzUR. This drug has produced very promising results in the treatment of patients with psoriasis and without toxicity, as reported by Turner & Calabresi (94). Another potential application of AzUR is suggested by the experiments of Sanders et al. (95) who demonstrated the termination of pregnancy in mice with its use without toxicity to the mother or unfertilized eggs. Mice that survived subabortive doses of AzUR in the early stages of gestation were normal and had normal reproductive capacities.

A number of studies on the mechanism of action of these azapyrimidines have been carried out. In the earliest of these it was found that in *E. coli* treated with AzU there was an accumulation of orotic acid and orotidylic acid (96, 97), in various mouse tumors an increased amount of orotidine was detected (98), and there was also an enhanced excretion of orotidine in the urine of mice treated with AzU (99). The conversion of AzU into the ribonucleoside and ribonucleoside monophosphate was observed in bacteria (86, 96). In tumors that were made resistant to AzU there was no conversion to the nucleotide (100). The main site of action of these compounds was ascertained as involving the block by azauridine-5′-monophosphate (AzUMP) of the enzyme orotidylate decarboxylase, which catalyzed the conversion of orotidylic acid to uridylic acid, a key step in *de novo* pyrimidine biosynthesis (101, 102). It was also demonstrated that azauridine diphosphate inhibited the enzyme polynucleotide phosphorylase, but was not incorporated into polynucleotide form (103). Similarly, azauridine triphosphate inhibited DNA-dependent RNA polymerase, and there was no evidence of

the analogue being incorporated into the RNA (104). There was no detectable incorporation of azauridine into the nucleic acids of a number of mammalian tissues (105). However, Wells et al. (106) reported that although AzUR given intraventricularly to cats inhibited the incorporation of orotic acid into brain nucleic acids as might be expected, it was also incorporated into RNA; the sole report of such incorporation. In any event, it is clear that the inhibition of orotidylate decarboxylase by AzUMP is the primary locus of the biochemical activity of the 6-azauracil series. Some aspects of this problem have been reviewed by Handschumacher (107).

6-Azacytidine (AzCR) (VI, Fig. 2) was first synthesized by Sorm et al. (108), and was shown to have antitumor activity by Sorm & Vesely (109). Handschumacher et al. (110) found that the nucleoside was converted into the monophosphate in Ehrlich ascites cells and liver *in vivo*; it was not deaminated *in vitro*, but one third of the dose given to mice was excreted as AzUR. They demonstrated that 6-azacytidine-5′-monophosphate (AzCMP) inhibited orotidylate decarboxylase to about one tenth the extent of AzUMP, and caused an accumulation of orotidine in the urine of mice (110).

Skoda & Sorm (111) have reported that whereas AzUR-diphosphate does not serve as a substrate for polynucleotide phosphorylase, AzCR-diphosphate does. They prepared a copolymer of poly-C and poly-AzCR, and showed that the melting temperature was reduced as compared with poly-C. This leads to the possibility of determining the coding properties of AzCR (111). Kara & Sorm (112) have found that AzCR-diphosphate at low concentrations activated deoxycytidylate deaminase from Ehrlich ascites cells, but was itself deaminated at higher temperatures.

The Czech group has carried out careful studies on the comparative pharmacology of AzUR and AzCR. They found that the acute toxicities of the two compounds were low and comparable (113), with the main changes in the spleen and lymph nodes, and that the central nervous system toxicity of AzUR was about four times less than AzU, but was increased 100-fold when injected intraventricularly. They attributed this toxicity to the inhibition of orotidylate decarboxylase (114). They found that AzCR was deaminated to AzUR to a different extent in various organs of various species, that about one third of the urinary excretion was accounted for by AzUR, and they suggested that AzUR was the active form of AzCR (115). The CNS effects of AzCR were less than those of AzUR, suggesting again that AzUR was the active pharmacological agent (116). They also demonstrated that AzCR successfully interrupted pregnancy in mice, but in contrast to the observations of Sanders et al. (95) with AzUR, they found that AzCR does produce some inheritable fetal abnormalities (117).

5-Azaorotic acid (*AzOA*).—This compound (VII, Fig. 2), which is a symmetrical triazine, has been found by Handschumacher (118) to inhibit the metabolism of orotic acid in extracts of tumor cells by inhibition of the enzyme orotidylate pyrophosphorylase, the step immediately preceding orotidylate decarboxylase in *de novo* pyrimidine nucleotide biosynthesis.

Hence it was possible to study AzOA and AzUR in combination in sequential blockades (107), but no synergism was observed. In an extensive preclinical and clinical pharmacological study, Granat et al. (119) showed that AzOA inhibited orotidylate pyrophosphorylase *in vitro* in various rat tissues. *In vivo*, the compound was retained longest in the liver, and in dogs the BUN rose precipitously before death. In human patients, AzOA inhibited the decarboxylation of COOH-labeled orotic acid and the latter compound was excreted in the urine; labeled AzOA was excreted mostly unchanged, but some N-formylbiuret was also excreted in the urine (119). The compound is being considered as a treatment for liver and kidney tumors in man.

5-Azacytidine (5-AzCR).—The synthesis (120) and research with this compound (VIII, Fig. 2) have been carried out entirely by the Czech group under the direction of Sorm. 5-AzCR was found to have a very high activity at inhibiting the growth of a leukemia in AK mice (121), producing some apparent cures (122). It inhibits the growth of *E. coli*, which is prevented only by the combination of uridine, cytidine, and thymidine (123), and is highly mutagenic, producing reversions of proline and tyrosine auxotrophic mutants to prototrophy to a much greater extent than IUdR (5-iodo-2′-deoxyuridine) (124), which suggests that it is incorporated into DNA. A combined spectroscopic study and molecular orbital calculations demonstrate that 5-AzCR has a free amino group and is capable of base-pairing with guanine, as does cytosine (125).

5-AzCR is chemically unstable: under mild acidic conditions it is converted into 5-azacytosine (5-AzC), 5-azauracil and D-ribose; at neutral or basic pH it is transformed into ribofuranosyl-3-guanylurea, guanidine, and D-ribose, which led to the interesting suggestion that the biological and mutagenic activity results from the incorporation of 5-AzCR into DNA and then decomposes while it is in the polynucleotide (126). This mechanism, if correct, appears to be unique among nucleic acid analogues.

The incorporation of labeled 5-AzCR into DNA was found in *Vicia faba*, and after hydrolysis of the DNA the above decomposition products were detected (127). In mice, it was demonstrated that the analogue was also incorporated into liver and tumor soluble and ribosomal RNA, resulting in a lower T_m of both; there was less incorporation into the RNA of a resistant tumor, and degradation products, including N-formylbiuret, were detected in the urine (128). Ehrlich ascites cells were found to phosphorylate 5-AzCR to the mono-, di-, and triphosphate levels and to incorporate it *in vivo* into RNA but not into DNA (129). The pharmacology of 5-AzCR and 5-azadeoxycytidine was studied in mice and found to be similar with respect to blood, urine, and tissue levels; however, the former, but not the latter, compound caused an increased urinary excretion of orotic acid and orotidine (130). In an autoradiographic investigation with the AK mouse leukemia, it was found that 5-AzCR inhibited RNA biosynthesis in myeloid cells, and that DNA was inhibited only in the mature myeloid elements of the bone marrow, the primary effect being in myeloid cells and other lymphocytes

(131). It was observed that resistance of 5-AzCR was achieved rapidly in the AK mouse leukemia and was cross-resistant to 5-fluorouracil. In the resistant tumors there was less inhibition of the incorporation of orotic acid into the nucleic acids, less uridine kinase, and less uridine phosphorylase activity than in the sensitive cells (132).

5-Iodo-2'-deoxyuridine (IUdR).—The synthesis of this nucleoside (X, Fig. 2) was first reported by Prusoff (133) in 1959, and it was found by Welch & Prusoff (134) to inhibit a number of transplanted tumors in mice. An intensive study of its toxicity and pharmacology by Prusoff et al. (135) demonstrated that its lethal toxicity could be reversed by thymidine, that the I^{131}-labeled compound was cleaved to iodouracil and then to uracil and inorganic iodide, and that there was iodination of non-nucleic acid compounds. An initial clinical study by Calabresi et al. (136) showed that a blood level could be produced, that stomatitis, alopecia, and leukopenia were the toxic symptoms, and that some "modest" tumor regressions could be produced. Calabresi (137) showed that intra-arterially administered thymidine could protect against the toxicity of systemically injected IUdR, and Mark & Calabresi (138) treated patients with head and neck tumors by intra-arterial infusions of IUdR, protecting the bone marrow by instillations of thymidine into the hypogastric artery, and obtained tumor regressions. Other clinical results in cancer chemotherapy have been reviewed by Calabresi (90).

Kaufman (139) has demonstrated that IUdR can cure herpes simplex keratitis when applied directly to the rabbit eye, and has reported similar effects ophthalmologically in man (140). Calabresi et al. (141) injected IUdR systemically into rabbits and thus protected them against vaccination, and Huebner et al. (142) were similarly able to protect newborn hamsters against oncogenesis with adenovirus 12. Thus, IUdR is systemically effective against some DNA viruses.

In one of the initial biochemical investigations with this compound, Prusoff (143) found that it inhibited the incorporation of formate and orotic acid into DNA in Ehrlich ascites cells *in vitro*, and suggested that the true inhibitor was IUdR-monophosphate. The incorporation of IUdR into the DNA of mammalian cells in culture was reported independently by Mathias et al. (144) and Eidinoff et al. (145) in 1959, and extended by Hampton & Eidinoff (146). Kriss et al. (147) measured the incorporation of IUdR into the DNA of various mouse tissues *in vivo*, and reported that the incorporation was enhanced by prior blocking of *de novo* thymidylate synthesis by FUdR (5-fluoro-2'-deoxyuridine) (see below). A quantitative comparison of the incorporation of thymidine and IUdR into tissues of tumor-bearing mice was carried out by Fox & Prusoff (148), who found that thymidine was preferentially utilized, and they discussed the relative merits of the two compounds as markers for DNA turnover. One of the consequences of the incorporation of this analogue into DNA is to confer an increased radiosensitivity to cells, as demonstrated by Erikson & Szybalski (149); this has not yet been

exploited in the clinic with conspicuous success, doubtless because of the low incorportion into DNA *in vivo*. Woodman (150) has made the intriguing observation that IUdR, or its monophosphate, reacted with the polycation polyethyleneimine and exhibited an increased uptake into DNA *in vivo*. Langen & Etzold (151) showed that administration of deoxyglucosyl thymine, which inhibits nucleoside phosphorylase cleavage of some nucleosides, caused an increased incorporation of IUdR into DNA *in vivo*. A doubling of P815Y leukemia cells in the presence of IUdR is followed by cessation of DNA synthesis, although thymidine incorporation continues, as reported by Morris & Cramer (152).

The inhibitory effects, other than the incorporation into DNA, have been studied by Delamore & Prusoff (153), who showed that IUdR inhibited thymidine kinase, the monophosphate inhibited thymidylate kinase, and there were suggestions that the triphosphate inhibited DNA polymerase.

The incorporation of IUdR into the DNA of vaccinia virus was shown by Prusoff et al. (154), and the same group has studied the inhibition of the herpes virus-induced thymidine and thymidylate kinases (155). The complex pharmacology and biochemistry of this interesting thymidine analogue (IUdR) have been reviewed by Prusoff (156).

A related compound, 5-iodo-2′-deoxycytidine (ICdR), was synthesized by Chang & Welch (157), and an extensive clinical pharmacological study by Calabresi et al. (158) demonstrated that ICdR behaved very similarly to IUdR in man as a consequence of its rapid and extensive deamination to IUdR.

Cytosine arabinoside (AraC).—The synthesis of this analogue (XI, Fig. 2) was reported by Walwick et al. (159) in a preliminary note, and by Hunter (160) in a patent. AraC was found to be a potent tumor-inhibitory compound at nontoxic levels by Evans et al. (161), and this has been confirmed in a tumor spectrum by Wodinsky & Kensler (162). Dixon & Adamson (163) found considerable activity in several mouse leukemias, resistance was easily produced and showed no cross-resistance with other pyrimidine analogues, and the compound was rapidly excreted as such in the urine. Kline et al. (164) determined that in multiple doses, AraC was more effective than amethopterin at increasing the survival of mice with L-1210 leukemia. It was found by Underwood (165) that AraC is as effective as IUdR against herpes simplex keratitis in the rabbit eye.

In clinical studies, Talley & Vaitkevicius (166) found that AraC produced megaloblastic changes in the marrows of all patients studied and minor objective responses in four patients with lymphosarcoma. Carey & Ellison (167) gave the drug by continuous intravenous infusion to patients with acute myelocytic leukemia and obtained some partial remissions. Howard et al. (168) reported good partial remissions in five of ten children suffering from acute leukemia, and the drug is currently under intensive clinical evaluation.

Papac et al. (169) found that AraC inhibited marrow repopulation by

donor cells in lethally irradiated mice, and the inhibition was reversed by deoxycytidine. Kihlman et al. (170) showed that it produced chromosomal abnormalities in human leukocytes in culture, and Buskirk et al. (171) found that AraC completely inhibited the formation of antibodies in mice to BCG vaccine.

A series of studies of the mechanism of action of AraC in cell cultures have been carried out. Chu & Fischer (172), using L5178Y leukemic cells, found that the inhibition of growth produced by AraC was completely prevented by deoxycytidine and that it inhibited the conversion of uridine to deoxycytidylate, but not to deoxyuridylate or thymidylate, by these cells; they interpreted these results as indicating that AraC inhibited the reduction of cytidine diphosphate (CDP) to deoxycytidine diphosphate (dCDP). Kim & Eidinoff (173) reported that AraC inhibited the growth and DNA synthesis of HeLa cells and produced an "unbalanced growth" due to a deficiency of deoxycytidine, and Karon et al. (174) found the same inhibition in KB cells, with the addition that RNA biosynthesis was unaffected and that there was marked cell enlargement. Silagi (175), working with L cells, found the same phenomena, but in addition reported the incorporation of AraC into DNA and to a much lesser extent into RNA in these cells. She also demonstrated that the analogue inhibited vaccinia (a DNA) virus production in these cells, but not reovirus (containing double-stranded RNA) (175). Chu & Fischer (176) obtained an AraC-resistant line of their leukemic cells and found that they had an impaired ability to convert the nucleoside into phosphorylated form, and that deoxycytidine inhibited the enzymatic phosphorylation of AraC, which also supported their thesis that the main site of AraC action involved inhibition of the reduction of cytidine diphosphate to deoxycytidine diphosphate. They also observed the incorporation of AraC into the DNA and RNA of these cells (176).

A considerable amount of work on the biochemistry of various arabinosides in bacteria has been carried out by Cohen. He has extended this to a study of AraC in various enzyme systems (177) with chemically prepared mono-, di-, and triphosphates. They found that whereas AraC-diphosphate (AraCDP) was not a substrate for purified polynucleotide phosphorylase it inhibited the polymerization of adenosine diphosphate (ADP) and cytidine diphosphate (CDP) (177), but AraC-triphosphate (AraCTP) had no effect on the incorporation of CTP into sRNA, nor was it incorporated to any significant extent. In studies of DNA polymerase, AraCTP had no effect on the incorporation of dCTP into DNA, nor could its incorporation into DNA be detected (177). The exclusion of the incorporation of AraC into DNA and RNA by these bacterial enzyme systems is in disagreement with the incorporations reported (175, 176) in mammalian cells.

5-Fluoropyrimidines.—These compounds exert profound effects in a variety of microbiological and mammalian systems, which I have recently reviewed thoroughly (178). Only the studies in mammalian systems will be briefly summarized here. The specific rationale for the synthesis of 5-fluoroura-

cil (FU) (XII, Fig. 2) has been thoroughly described (178). The synthesis of 5-fluorouracil and some of its biological properties were announced in 1957 (179, 180), and it was demonstrated to exert powerful tumor-inhibitory activity in a number of transplanted tumors in rats and mice (181). 5-Fluorouridine (FUR) (XIII, Fig. 2) was more toxic and less tumor-inhibitory than FU, whereas 5-fluoro-2′-deoxyuridine (FUdR) (XIV, Fig. 2) was less toxic and more effective than FU (182). FU produces a significant incidence of objective responses in patients suffering from advanced solid tumors, particularly in breast and gastrointestinal cancers (183, 184), and prolongs the life of patients with breast carcinomas, but at the expense of some bone marrow and gastrointestinal toxicity (178). Prolonged intravenous infusion of FU has been reported by several investigators to produce less systemic toxicity without loss of therapeutic effect (cf. 185). In a randomized study of patients with primary inoperable lung carcinomas, the combination of FU plus radiotherapy prolonged life considerably more than radiotherapy alone; 3 of the 13 patients in the combined group of this report are currently alive and symptom-free more than five years after the single course of treatment (186). Continuous intra-arterial infusions of FU into patients with carcinomas of the head and neck produced massive tumor regressions in the majority of the cases (187). It has been reported by Curreri & Ansfield (188) that FUdR is superior to FU in the treatment of gastrointestinal cancers, but this finding has been disputed (189).

The metabolic degradation of FU proceeds to dihydro-FU, α-fluoro-β-ureidopropionic acid, urea, carbon dioxide, and α-fluoro-β-alanine, which occurs in most normal tissues but not in two mouse tumors, which fact is probably responsible for the selectivity of action against tumors (190, 191). The first step in the degradation of FUdR is its enzymatic cleavage to FU (192). The blood levels, excretion, and metabolic degradation of FU and FUdR in patients have been studied by means of tracer (193), microbiological (194), and cell culture (195) techniques, with relatively good agreement. The tissue distribution of FU and FUdR in patients with specimens taken at surgery was studied, and it was found that carcinomas of the colon converted more drug to the active nucleotide form than did the rapidly dividing normal intestinal mucosa (196).

In studies on the mechanism of action of these compounds, it was found, as expected, that FU is incorporated into RNA by mouse tissues and tumors *in vivo*, but not into DNA (197). In suspension of Ehrlich ascites cells, FUdR, FUR, and FU, in decreasing order, inhibited the incorporation of formate into the methyl group of DNA thymine (198), which resulted from the inhibition by the nucleotide 5-fluoro-2′-deoxyuridine-5′-monophosphate (FUdRP), of the enzyme thymidylate synthetase that converts deoxyuridylate into thymidylate, as first shown by Cohen et al. (199) in a bacterial system. With the enzyme from Ehrlich ascites cells it was shown that FUdRP is a powerful and competitive inhibitor, whether or nor preincubation is carried out (200, 201). In cells in culture, FU also inhibits DNA synthesis without

impairing RNA and protein synthesis to produce enlarged cells that undergo a "thymineless death" (202, 203); this effect is prevented by thymidine. Bresnick (204) found that FU and FUdR produced a feedback inhibition of aspartate transcarbamylase, and Anderson & Brockman (205) reported that 5-fluorouridine triphosphate (FUTP) exerted feedback inhibition of uridine kinase. Pitot & Peraino (206), among others, have found that FU and fluoroorotic acid block enzyme induction in rat liver *in vivo*.

In studies of the resistance of tumors to the fluorinated pyrimidines, several mechanisms have been elucidated, all indicating that the inhibition of thymidylate synthetase is more important in tumor inhibition than the incorporation into RNA. These mechanisms of resistance include: an altered thymidylate synthetase that is not inhibited by FUdRP (207), decreased uridine kinase activity (208), and decreased uridine pyrophosphorylase activity (209). Other biological effects of these compounds are: FU counteracts the testosterone stimulation of seminal vesicles in castrate rats (210), is teratogenic in rats (211), is an insect chemisterilant (212), and produces chromosome breakage (213). Related compounds that have been synthesized, but which have little biological activity are: AraFU (214), lyxo-FU (215), and 5-fluoro-2′-fluoro-2′-deoxyuridine (216).

5-Fluorocytosine (FC) (XV, Fig. 2) (180), although inactive against tumors, is inhibitory to *Candida albicans in vitro* and *in vivo* (217), and its metabolism has been studied in mice and man; it is not degraded, and is excreted in the urine as such (218). 5-Fluoro-2′-deoxycytidine (FCdR) (XVII, Fig. 2) was synthesized by Wempen et al. (219), and found by Burchenal et al. (220) to be as active as FUdR against some mouse leukemias, and FCdR is rapidly deaminated to FUdR (178). Duschinsky et al. (221) demonstrated that the N-*p*-toluyl-FCdR is very active against some mouse leukemias, AraFC was made by Fox et al. (222), and found by Burchenal et al. (223) to be very active against some mouse leukemias.

5-Trifluoromethyl-2′-deoxyuridine ("trifluorothymidine," F_3TdR).—This compound (XVIII, Fig. 2) was synthesized in this laboratory (224), and found to have a better therapeutic index than FUdR against adenocarcinoma 755 in mice (225). F_3TdR is also more effective than IUdR against herpes simplex keratitis in the rabbit eye, and is active against an IUdR-resistant line of the virus (226). The compound is mutagenic to and is incorporated into DNA in bacteriophage T4 (227), is incorporated into DNA in mammalian cells and radiosensitizes them (228), and inhibits the incorporation of formate into DNA thymine (229). The nucleotide, F_3TdRP, also inhibits thymidylate synthetase, but after preincubation inhibits it noncompetitively (201) and possibly irreversibly. *In vivo*, F_3TdR is catabolized only to the free base and to a small extent to 5-carboxyuracil and is incorporated into tissue and tumor DNA to only a very slight extent (230); it is about to undergo clinical evaluation.

Other pyrimidine analogues.—5-Trifluoromethyl-6-aza-2′-deoxyuridine (XIX, Fig. 2) has been synthesized independently in three laboratories (231–

233), but appears to be devoid of significant biological activity (233). The following interesting compounds have been synthesized, but no biological information on them is yet available: 2′,3′-unsaturated derivatives of uracil and thymine (234), 3′-aminohexapyranosyl uracil (235), arabinohexapyranosyl and deoxyribohexapyranosyl uracils (236), uridine and arabinosyl uridine 5′-carboxylic acids and uridine-5-carboxaldehyde and various hydrazone derivatives (237), 2′-O-methyl uridine and cytidine (238), and α- and β-anomers of arabinosyl and lyxosyl uracil and thymine (239).

Conclusions

It is evident from this brief and superficial review that a number of clinically useful and biochemically interesting compounds have resulted from a concerted interdisciplinary effort in the field of purine and pyrimidine analogues. Most of the obvious modifications of the structures of the naturally occurring compounds have already been made, and the production of future novel analogues may require an increased sophistication in biochemistry and molecular design. In spite of the limited successes of some of these compounds, none of them cures cancer, but with increased knowledge of the biochemical mode of action and pharmacological disposition, the therapeutic effectiveness of the existing compounds is certain to improve. The two major stumbling blocks to successful cancer chemotherapy remain: a lack of real selectivity against tumor cells and the certainty of the emergence of resistance. It will require greater insight into the biochemistry of malignancy and more subtlety in molecular design before these obstacles can be overcome.

LITERATURE CITED

1. Karnofsky, D. A., and Clarkson, B. D., *Ann. Rev. Pharmacol.*, **3,** 357–428 (1963)
2. Oliverio, V. T., and Zubrod, C. G., *Ann. Rev. Pharmacol.*, **5,** 335–56 (1965)
3. Skipper, H. E., *Cancer Res.*, **25,** 1544–50 (1965)
4. Goldin, A., Serpick, A. A., and Mantel, N., *Cancer Chemotherapy Rept.*, **50,** 173–218 (1966)
5. Freireich, E. J., Gehan, E. A., Rall, D. P., Schmidt, L. H., and Skipper, H. E., *Cancer Chemotherapy Rept.*, **50,** 219–44 (1966)
6. Hutchison, D. J., *Cancer Res.*, **25,** 1581–95 (1965)
7. Kidder, G. W., Dewey, V. C., and Parks, R. E., Jr., *Science*, **109,** 511–14 (1949)
8. Mandel, H. G., *Pharmacol. Rev.*, **11,** 743–838 (1959)
9. Hirschberg, E., Kream, J., and Gellhorn, A., *Cancer Res.*, **12,** 524–28 (1952)
10. Lasnitzki, I., Matthews, R. E. F., and Smith, J. D., *Nature*, **173,** 346–49 (1954)
11. Mandel, H. G., Carlo, P.-E., and Smith, P. K., *J. Biol. Chem.*, **206,** 181–89 (1954)
12. Brockman, R. W., Bennett, L. L., Jr., Simpson, M. S., Wilson, A. R., Thomson, J. R., and Skipper, H. E., *Cancer Res.*, **19,** 856–69 (1959)
13. Zimmerman, E. F., and Greenberg, S. A., *Mol. Pharmacol.*, **1,** 113–25 (1965)
14. Karon, M., Weissman, S., Meyer, C., and Henry, P., *Cancer Res.*, **25,** 185–92 (1965)
15. Hall, T. C., Krant, M. J., Lloyd, J. B., Patterson, W. B., Ishibara, A., Potee, K. G., Lovina, T. O., and Mullen, J. M., *Cancer*, **15,** 1156–64 (1962)
16. Elion, G. B., Burgi, E., and Hitchings, G. H., *J. Am. Chem. Soc.*, **74,** 411–14 (1952)
17. Miner, R. W., Ed., *Ann. N. Y. Acad. Sci.*, **66,** 183–508 (1954)
18. Clarke, D. A., Philips, F. S., Sternberg, S. S., and Stock, C. C., *Cancer Res.*, **13,** 593–604 (1953)
19. Burchenal, J. H., *Bull. N. Y. Acad. Med.*, **30,** 429–47 (1954)
20. Elion, G. B., Bieber, S., and Hitchings, G. H., *Ann. N. Y. Acad. Sci.*, **66,** 297–303 (1954)

20a. Scannell, J. P., and Hitchings, G. H., *Proc. Soc. Exptl. Biol. Med.*, **122,** 627–29 (1966)

21. Brockman, R. W., *Cancer Res.*, **25,** 1596–1605 (1965)
22. Davidson, J. D., *Cancer Res.*, **25,** 1606–7 (1965)
23. Montgomery, J. A., Dixon, G. T., Dulmadge, E. A., Thomas, H. J., Brockman, R. W., and Skipper, H. E., *Nature*, **199,** 769–72 (1963)
24. Brockman, R. W., and Chumley, S., *Biochim. Biophys. Acta*, **95,** 365–79 (1965)
25. Henderson, J. F., and Khoo, M. K. Y., *J. Biol. Chem.*, **240,** 3104–9 (1965)
26. Atkinson, M. R., and Murray, A. W., *Biochem. J.*, **94,** 64–70, 71–74 (1965)
27. Salser, J. S., and Balis, M. E., *Cancer Res.*, **25,** 539–43, 544–51 (1965)
28. Schwartz, R., and Andre, J., in *Second International Symposium on Immunopathology*, 385–409 (Miescher, R., and Grabar, P., Eds., Benno Schwabe & Co., Basel, Switzerland, 1962)
29. Calne, R. Y., *Transplant. Bull.*, **28,** 65–81 (1961)
30. Hitchings, G. H., and Elion, G. B., *Pharmacol. Rev.*, **15,** 365–405 (1963)
31. Bennett, L. L., Jr., Brockman, R. W., Schnebli, H. P., Chumley, S., Dixon, G. J., Schabel, F. M., Jr., Dulmadge, E. A., Skipper, H. E., Montgomery, J. A., and Thomas, H. J., *Nature*, **205,** 1276–79 (1965)
32. Giner-Sorolla, A., and Bendich, A., *J. Med. Chem.*, **8,** 667–72 (1965)
33. Brown, G. B., Levin, G., Murphy, S., Sele, A., Reilly, H. C., Tarnowski, G. S., Schmid, F. A., Teller, M. N., and Stock, C. C., *J. Med. Chem.*, **8,** 190–95 (1965)
34. Brown, G. B., Sugiura, K., and Creswell, R. M., *Cancer Res.*, **25,** 986–92 (1965)
35. Montgomery, J. A., and Hewson, K., *J. Med. Chem.*, **8,** 708–10 (1965)
36. Montgomery, J. A., and Hewson, K., *ibid.*, **9,** 105–7 (1966)
37. Montgomery, J. A., and Hewson, K., *ibid.*, **9,** 354–57 (1966)
38. Kimball, A. P., Le Page, G. A., and Bowman, B., *Can. J. Biochem. Physiol.*, **42,** 1753–68 (1964)
39. Reist, E. J., Benitez, A., Goodman, L., Baker, B. R., and Lee, W. W., *J. Org. Chem.* **27,** 3274–81 (1962)

40. Sato, K., Le Page, G. A., and Kimball, A. P., *Cancer Res.*, **26,** 741–47 (1966)
41. Anzai, K., Nakamura, F., and Suzuki, S., *J. Antibiotics (Tokyo)*, **10,** 201–4 (1957)
42. Suzuki, S., and Marumo, S., *J. Antibiotics (Tokyo)*, **14,** 34–38 (1961)
43. Mizuno, Y., Ikehara, M., Watanabe, K. A., Suzuki, S., and Itoh, T., *J. Org. Chem.*, **28,** 3229–30 (1963)
44. Smith, C. G., Lummis, W. L., and Grady, J. R., *Cancer Res.*, **19,** 847–52 (1959)
45. Owen, S. P., and Smith, C. G., *Cancer Chemotherapy Rept.*, **36,** 19–22 (1964)
46. Acs, G., Reich, E., and Mori, M., *Proc. Natl. Acad. Sci. U.S.*, **52,** 493–501 (1964)
47. Smith, C. G., Reineke, L. M., and Harpootlian, H., *Proc. Am. Assoc. Cancer Res.*, **7,** 66 (1966)
48. Johnson, R. O. (Private communication)
49. Lee, W. W., Benitez, A., Goodman, L., and Baker, B. R., *J. Am. Chem. Soc.*, **82,** 2618–19 (1960)
50. Hubert-Habart, H., and Cohen, S. S., *Biochim. Biophys. Acta*, **59,** 468–71 (1962)
51. Brink, J. J., and Le Page, G. A., *Cancer Res.*, **24,** 312–18 (1964)
52. Brink, J. J., and Le Page, G. A., *ibid.*, 1042–49
53. Brink, J. J., and Le Page, G. A., *Can. J. Biochem. Physiol.*, **43,** 1–15 (1965)
54. York, J. L., and Le Page, G. A., *Can. J. Biochem. Physiol.*, **44,** 19–26 (1966)
55. Chang, P., and Lythgoe, B., *J. Chem. Soc.*, 1992–93 (1950)
56. Ellis, D. B., and Le Page, G. A., *Mol. Pharmacol.*, **1,** 231–38 (1965)
57. Ellis, D. B., and Le Page, G. A., *Can. J. Biochem. Physiol.*, **43,** 617–19 (1965)
58. Ellis, D. B., and Le Page, G. A., *Cancer Res.*, **26,** 893–97 (1966)
59. Le Page, G. A., and Junga, I. G., *Cancer Res.*, **25,** 46–52 (1965)
60. Bentley, H. R., Cunningham, K. G., and Spring, F. S., *J. Chem. Soc.*, 2301–9 (1951)
61. Kaczka, E. A., Dulaney, E. L., Gitterman, C. O., Woodruff, H. R., and Folkers, K., *Biochem. Biophys. Res. Commun.*, **14,** 452–55 (1964)
62. Kaczka, E. A., Trenner, N. R., Arison, B., Walker, R. W., and Folkers, K., *Biochem. Biophys. Res. Commun.*, **14,** 456–57 (1964)
63. Lee, W. W., Benitez, A., Anderson, C. D., Goodman, L., and Baker, B. R., *J. Am. Chem. Soc.*, **83,** 1906–11 (1961)
64. Klenow, H., *Biochim. Biophys. Acta*, **76,** 347–53, 354–65 (1963)
65. Overgaard-Hansen, K., *Biochim. Biophys. Acta*, **80,** 504–7 (1964)
66. Shigeura, H. T., and Gordon, C. N., *J. Biol. Chem.*, **240,** 806–10 (1965)
67. Walton, E., Holly, F. W., Boxer, G. E., Huff, R. F., and Jenkens, S. R., *J. Med. Chem.*, **8,** 659–63 (1965)
68. Murray, D. H., and Prokop, J., *J. Pharm. Sci.*, **54,** 1468–73 (1965)
69. Gitterman, C. O., Burg, R. W., Boxer, G. E., Meltz, D., and Hitt, J., *J. Med. Chem.*, **8,** 664–67 (1965)
70. Shigeura, H. T., Boxer, G. E., Meloni, M. L., and Sampson, S. D., *Biochemistry*, **5,** 994–1004 (1966)
71. Broom, A. D., and Robins, R. K., *J. Am. Chem. Soc.*, **87,** 1145–46 (1965)
72. Robins, M. J., McCarthy, J. R., and Robins, R. K., *Biochemistry*, **5,** 224–31 (1966)
73. McCarthy, J. R., Robins, M. J., Townsend, L. B., and Robins, R. K., *J. Am. Chem. Soc.*, **88,** 1549–53 (1966)
74. Rousseau, R. J., Townsend, L. B., and Robins, R. K., *Biochemistry*, **5,** 756–60 (1966)
75. Thomas, H. J., and Montgomery, J. A., *J. Org. Chem.*, **31,** 1413–16 (1966)
76. Shah, R. H., Schaeffer, H. J., and Murray, D. H., *J. Pharm. Sci.*, **54,** 15–20 (1965)
77. Baranek, J., Friedman, H. A., Watanabe, K. A., and Fox, J. J., *J. Heterocyclic Chem.*, **2,** 188–91 (1965)
78. Yamaoka, H., Aso, K., and Matsuda, K., *J. Org. Chem.*, **30,** 149–52 (1965)
79. Seibert, W., *Ber. Deut. Chem. Ges.*, **80,** 494–502 (1947)
80. Sorm, F., and Skoda, J., *Collection Czech. Chem. Commun.*, **21,** 487–88 (1956)
81. Handschumacher, R. E., and Welch, A. D., *Cancer Res.*, **16,** 965–69 (1956)
82. Sablik, J., and Sorm, F., *Neoplasma*, **4,** 113–17 (1957)
83. Jaffe, J. J., Handschumacher, R. E., and Welch, A. D., *Yale J. Biol. Med.*, **30,** 168–75 (1957)
84. Elion, G. B., Bieber, S., Nathan, H.,

and Hitchings, G. H., *Cancer Res.*, **18,** 802–17 (1958)
85. Welch, A. D., Handschumacher, R. E., and Jaffe, J. J., *J. Pharmacol. Exptl. Therap.*, **129,** 262–70 (1960)
86. Skoda, J., Hess, V. F., and Sorm, F., *Experientia*, **13,** 150–51 (1957)
87. Handschumacher, R. E., *J. Biol. Chem.*, **235,** 764–68 (1960)
88. Sorm, F., and Keilova, H., *Experientia*, **14,** 215–17 (1958)
89. Handschumacher, R. E., Calabresi, P., Welch, A. D., Bono, V., Fallon, H., and Frei, E., *Cancer Chemotherapy Rept.*, **21,** 1–18 (1962)
90. Calabresi, P., *Cancer Res.*, **23,** 1260–67 (1963)
91. Waelsch, J. H., *Rev. Czech. Med.*, **3,** 170–74 (1959)
92. Zaruba, F., Kuta, A., and Elis, J., *Lancet*, **7275,** 255–58 (1963)
93. Handschumacher, R. E., Creasey, W. A., Fink, M. E., Calabresi, P., and Welch, A. D., *Cancer Chemotherapy Rept.*, **16,** 267–69 (1962)
94. Turner, B. W., and Calabresi, P., *J. Invest. Dermatol.*, **43,** 551–59 (1961)
95. Sanders, M. A., Wiesner, B. P., and Yudkin, J., *Nature*, **189,** 1015–16 (1961)
96. Handschumacher, R. E., *Nature*, **182,** 1090–91 (1958)
97. Skoda, J., and Sorm, F., *Collection Czech. Chem. Commun.*, **24,** 1331–37 (1959)
98. Pasternak, C. A., and Handschumacher, R. E., *J. Biol. Chem.*, **234,** 2292–97 (1959)
99. Habermann, V., and Sorm, F., *Collection Czech. Chem. Commun.*, **23,** 2201–6 (1958)
100. Handschumacher, R. E., *Biochim. Biophys. Acta*, **23,** 428–30 (1957)
101. Handschumacher, R. E., *J. Biol. Chem.* **235,** 2917–19 (1960)
102. Bruemmer, N. C., Holland, J. F., and Sheehe, P. R., *Cancer Res.*, **22,** 113–19 (1962)
103. Smrt, J., Beranek, J., and Sorm, F., *Collection Czech. Chem. Commun.*, **25,** 130–37 (1960)
104. Goldberg, I. H., and Rabinowitz, M., *Biochim. Biophys. Acta*, **72,** 116–19 (1963)
105. Schindler, R., and Welch, A. D., *Biochem. Pharmacol.*, **1,** 132–36 (1958)
106. Wells, W., Gaines, D., and Koenig, H., *J. Neurochem.*, **10,** 709–23 (1963)
107. Handschumacher, R. E., *Cancer Res.*, **25,** 1541–43 (1965)
108. Sorm, F., Smrt, J., and Cerneckij, V., *Experientia*, **17,** 64–65 (1961)
109. Sorm, F., and Vesely, J., *Experientia*, **17,** 355 (1961)
110. Handschumacher, R. E., Skoda, J., and Sorm, F., *Collection Czech. Chem. Commun.*, **28,** 2983–90 (1963)
111. Skoda, J., and Sorm, F., *Biochim. Biophys. Acta*, **91,** 352–54 (1964)
112. Kara, J., and Sorm, F., *Biochim. Biophys. Acta*, **80,** 154–57 (1964)
113. Jiricka, Z., Smetana, R., Janku, I., Elis, J., and Novotny, J., *Biochem. Pharmacol.*, **14,** 1517–23 (1965)
114. Janku, I., Krsiak, M., Volicer, L., Capek, R., Smetana, R., and Novotny, J., *Biochem. Pharmacol.*, **14,** 1525–36 (1965)
115. Novotny, J., Smetana, R., and Raskova, H., *Biochem. Pharmacol.*, **14,** 1537–44 (1965)
116. Janku, I., Krsiak, M., Novotny, J., Volicer, L., and Capek, R., *Biochem. Pharmacol.*, **14,** 1545–48 (1965)
117. Cerey, K., Elis, J., and Raskova, H., *Biochem. Pharmacol.*, **14,** 1549–56 (1965)
118. Handschumacher, R. E., *Cancer Res.*, **23,** 634–39 (1963)
119. Granat, P., Creasey, W. A., Calabresi, P., and Handschumacher, R. E., *Clin. Pharmacol. Therap.*, **6,** 436–47 (1965)
120. Piskala, A., and Sorm, F., *Collection Czech. Chem. Commun.*, **29,** 2060–76 (1964)
121. Sorm, F., Piskala, A., Cihak, A., and Vesely, J., *Experientia*, **20,** 202–3 (1964)
122. Sorm, F., and Veseley, J., *Neoplasma*, **11,** 123–30 (1964)
123. Cihak, A., and Sorm, F., *Collection Czech. Chem. Commun.*, **30,** 2091–2102 (1965)
124. Fucik, V., Zadrazil, S., Sormova, Z., and Sorm, F., *Collection Czech. Chem. Commun.*, **30,** 2883–86 (1965)
125. Pithova, P., Piskala, A., Pitha, J., and Sorm, F., *Collection Czech. Chem. Commun.*, **30,** 1626–34 (1965)
126. Pithova, P., Piskala, A., Pitha, J., and Sorm, F., *ibid.*, 2801–11
127. Pithova, P., Fucik, V., Zadrazil, S., Sormova, Z., and Sorm, F., *Collection Czech. Chem. Commun.*, **30,** 2879–82 (1965)
128. Cihak, A., Veseley, J., and Sorm, F., *Biochim. Biophys. Acta*, **108,** 516–18 (1965)

129. Jurovcik, M., Raska, K., Sormova, Z., and Sorm, F., *Collection Czech. Chem. Commun.*, **30**, 3370–76 (1965)
130. Riska, K., Jurovcik, M., Sormova, Z., and Sorm, F., *Collection Czech. Chem. Commun.*, **30**, 3001–6 (1965)
131. Veseley, J., and Sorm, F., *Neoplasma*, **12**, 3–9 (1965)
132. Veseley, J., Seifert, J., Cihak, A., and Sorm, F., *Intern. J. Cancer*, **1**, 31–40 (1966)
133. Prusoff, W. H., *Biochim. Biophys. Acta*, **32**, 295–96 (1959)
134. Welch, A. D., and Prusoff, W. H., *Cancer Chemotherapy Rept.*, **6**, 29–36 (1960)
135. Prusoff, W. H., Jaffe, J. J., and Gunther, H., *Biochem. Pharmacol.*, **3**, 110–21 (1960)
136. Calabresi, P., Cardozo, S. S., Finch, S. C., Kligerman, M. M., von Essen, C. F., Chu, M. K., and Welch, A. D., *Cancer Res.*, **21**, 550–59 (1961)
137. Calabresi, P., *J. Clin. Invest.*, **41**, 1484–91 (1962)
138. Mark, J. B. D., and Calabresi, P., *Cancer Chemotherapy Rept.*, **16**, 545–51 (1962)
139. Kaufman, H. E., *Proc. Soc. Exptl. Biol. Med.*, **109**, 251–52 (1962)
140. Kaufman, H. E., Nesburn, A. B., and Maloney, E. D., *Arch. Ophthalmol.*, **67**, 583–91 (1962)
141. Calabresi, P., McCollum, R. W., and Welch, A. D., *Nature*, **197**, 767–69 (1963)
142. Huebner, R. J., Lane, W. T., Welch, A. D., Calabresi, P., McCollum, R. W., and Prusoff, W. H., *Science*, **142**, 488–90 (1963)
143. Prusoff, W. H., *Cancer Res.*, **20**, 92–95 (1960)
144. Mathias, A. P., Fischer, G. A., and Prusoff, W. H., *Biochim. Biophys. Acta*, **36**, 560–61 (1959)
145. Eidinoff, M. L., Cheong, L., and Rich, M. A., *Science*, **129**, 1550–52 (1959)
146. Hampton, E. G., and Eidinoff, M. L., *Cancer Res.*, **21**, 345–52 (1961)
147. Kriss, J. P., Tung, L., and Bond, S., *Cancer Res.*, **22**, 1257–64 (1962)
148. Fox, B. W., and Prusoff, W. H., *Cancer Res.*, **25**, 234–40 (1965)
149. Erikson, R. L., and Szybalski, W., *Cancer Res.*, **23**, 122–30 (1963)
150. Woodman, R. J., *Nature*, **209**, 1362–63 (1966)
151. Langen, P., and Etzold, G., *Mol. Pharmacol.*, **2**, 89–92 (1966)
152. Morris, N. R., and Cramer, J. W., *Mol. Pharmacol.*, **2**, 1–9 (1966)
153. Delamore, I. W., and Prusoff, W. H., *Biochem. Pharmacol.*, **11**, 101–12 (1962)
154. Prusoff, W. H., Bakhle, Y. S., and McCrea, J. F., *Nature*, **199**, 1310–11 (1963)
155. Prusoff, W. H., Bakhle, Y. S., and Sekely, L., *Ann. N. Y. Acad. Sci.*, **130**, 135–50 (1965)
156. Prusoff, W. H., *Cancer Res.*, **23**, 1246–59 (1963)
157. Chang, P. K., and Welch, A. D., *Biochem. Pharmacol.*, **8**, 327–28 (1961)
158. Calabresi, P., Creasey, W. A., Prusoff, W. H., and Welch, A. D., *Cancer Res.*, **23**, 583–92 (1963)
159. Walwick, E. R., Roberts, W. K., and Dekker, C. A., *Proc. Chem. Soc.*, 84 (1959)
160. Hunter, J. H., *U. S. Patent 3,183,226* (May 11, 1965)
161. Evans, J. S., Musser, E. A., Mengel, G. D., Forsblad, K. R., and Hunter, J. H., *Proc. Soc. Exptl. Biol. Med.*, **106**, 350–53 (1961)
162. Wodinsky, I., and Kensler, C. J., *Cancer Chemotherapy Rept.*, **47**, 65–68 (1965)
163. Dixon, R. L., and Adamson, R. H., *Cancer Chemotherapy Rept.*, **48**, 11–16 (1965)
164. Kline, I., Venditti, J. M., Tyrer, D. D., and Goldin, A., *Cancer Res.*, **26**, 853–59 (1966)
165. Underwood, G. E., *Proc. Soc. Exptl. Biol. Med.*, **111**, 660–64 (1962)
166. Talley, R. W., and Vaitkevicius, V. K., *Blood*, **21**, 352–62 (1963)
167. Carey, R. W., and Ellison, R. R., *Clin. Res.*, **13**, 337 (1965)
168. Howard, J. P., Cevik, N., and Murphy, M. L., *Cancer Chemotherapy Rept.*, **50**, 287–91 (1966)
169. Papac, R. J., Calabresi, P., Hollingsworth, J. W., and Welch, A. D., *Cancer Res.*, **25**, 1459–62 (1965)
170. Kihlman, R. A., Nichols, W. W., and Levan, A., *Hereditas*, **50**, 139–43 (1963)
171. Buskirk, H. H., Crim, J. A., Petering, H. G., Merrit, K., and Johnson, A. G., *J. Natl. Cancer Inst.*, **34**, 747–58 (1965)
172. Chu, M. Y., and Fischer, G. A., *Biochem. Pharmacol.*, **11**, 423–30 (1962)
173. Kim, J. N., and Eidinoff, M. L., *Cancer Res.*, **25**, 698–702 (1965)
174. Karon, M., Henry, P., Weissman, S., and Meyer, C., *Cancer Res.*, **26**, 166–71 (1966)

175. Silagi, S., *Cancer Res.*, **25,** 1446–53 (1965)
176. Chu, M. Y., and Fischer, G. A., *Biochem. Pharmacol.*, **14,** 333–41 (1965)
177. Cardeilhac, P. T., and Cohen, S. S., *Cancer Res.*, **24,** 1595–1603 (1964)
178. Heidelberger, C., *Progr. Nucleic Acid Res.*, **4,** 1–50 (1965)
179. Heidelberger, C., Chaudhuri, N. K., Danneberg, P. B., Mooren, D., Griesbach, L., Duschinsky, R., Schnitzer, R. J., Pleven, E., and Scheiner, J., *Nature*, **179,** 663–66 (1957)
180. Duschinsky, R., Pleven, E., and Heidelberger, C., *J. Am. Chem. Soc.*, **79,** 4559–60 (1957)
181. Heidelberger, C., Griesbach, L., Montag, B. J., Mooren, D., Cruz, O., Schnitzer, R. J., and Grunberg, E., *Cancer Res.*, **18,** 305–17 (1958)
182. Heidelberger, C., Griesbach, L., Cruz, O., Schnitzer, R. J., and Grunberg, E., *Proc. Soc. Exptl. Biol. Med.*, **97,** 470–75 (1958)
183. Heidelberger, C., and Ansfield, F. J., *Cancer Res.*, **23,** 1226–43 (1963)
184. Rochlin, D. B., Shiner, J., Langdon, E., and Ottoman, R., *Ann. Surg.*, **156,** 105–13 (1962)
185. Cressy, N. L., and Schell, H. W., *Am. J. Med. Sci.*, **249,** 52–55 (1965)
186. Gollin, F. F., Ansfield, F. J., Curreri, A. R., Heidelberger, C., and Vermund, H., *Cancer*, **15,** 1209–17 (1962)
187. Johnson, R. O., Kisken, W. A., and Curreri, A. R., *Surg. Gynecol. Obstet.*, **120,** 530–36 (1965)
188. Curreri, A. R., and Ansfield, F. J., *Cancer Chemotherapy Rept.*, **16,** 387–88 (1962)
189. Nivinny, H. B., *Proc. Am. Assoc. Cancer Res.*, **5,** 47 (1964)
190. Chaudhuri, N. K., Mukherjee, K. L., and Heidelberger, C., *Biochem. Pharmacol.*, **1,** 328–41 (1958)
191. Mukherjee, K. L., and Heidelberger, C., *J. Biol. Chem.*, **235,** 433–37 (1960)
192. Birnie, G. D., Kröger, H., and Heidelberger, C., *Biochemistry*, **2,** 566–72 (1963)
193. Mukherjee, K. L., Boohar, J., Wentland, D., Ansfield, F. J., and Heidelberger, C., *Cancer Res.*, **23,** 49–66 (1963)
194. Clarkson, B., O'Connor, A., Winston, L. R., and Hutchison, D. J., *Clin. Pharmacol. Therap.*, **5,** 581–610 (1964)
195. Smith, C. G., Grady, J. E., and Kupiecki, F. P., *Cancer Res.*, **25,** 241–45 (1965)
196. Mukherjee, K. L., Curreri, A. R., Javid, M., and Heidelberger, C., *Cancer Res.*, **23,** 67–77 (1963)
197. Chaudhuri, N. K., Montag, B. J., and Heidelberger, C., *Cancer Res.*, **18,** 318–28 (1958)
198. Bosch, L., Harbers, E., and Heidelberger, C., *Cancer Res.*, **18,** 335–43 (1958)
199. Cohen, S. S., Flaks, J. G., Barner, H. D., Loeb, M. R., and Lichtenstein, J., *Proc. Natl. Acad. Sci. U. S.*, **44,** 1004–12 (1958)
200. Hartmann, K.-U., and Heidelberger, C., *J. Biol. Chem.*, **236,** 3006–13 (1961)
201. Reyes, P., and Heidelberger, C., *Mol. Pharmacol.*, **1,** 14–30 (1965)
202. Cheong, L., Rich, M. A., and Eidinoff, M. L., *Cancer Res.*, **20,** 1602–7 (1960)
203. Paul, J., and Hagiwara, A., *Biochim. Biophys. Acta*, **61,** 243–49 (1962)
204. Bresnick, E., *Cancer Res.*, **22,** 1246–51 (1962)
205. Anderson, E. P., and Brockman, R. W., *Biochim. Biophys. Acta*, **91,** 380–86 (1964)
206. Pitot, H. C., and Peraino, C., *J. Biol. Chem.*, **239,** 1783–88 (1964)
207. Heidelberger, C., Kaldor, G., Mukherjee, K. L., and Danneberg, P. B., *Cancer Res.*, **20,** 903–9 (1960)
208. Reichard, P., Sköld, O., Klein, G., Revesz, L., and Magnusson, P. H., *Cancer Res.*, **22,** 235–43 (1962)
209. Kasbekar, D. K., and Greenberg, D. M., *Cancer Res.*, **23,** 818–24 (1963)
210. Cantarow, A., and Zagerman, A. J., *Proc. Soc. Exptl. Biol. Med.*, **115,** 1052–54 (1964)
211. De Ri, F., and Mascitelli-Coriandoli, E., *Tumori*, **48,** 411–20 (1962)
212. La Brecque, G. C., Adcock, P. H., and Smith, C. H., *J. Econ. Entomol.*, **53,** 802–5 (1961)
213. Taylor, J. H., Haut, W. F., and Tung, J., *Proc. Natl. Acad. Sci. U. S.*, **48,** 190–98 (1962)
214. Yung, N. C., Burchenal, J. H., Fecher, R., Duschinsky, R., and Fox, J. J., *J. Am. Chem. Soc.*, **83,** 4060–65 (1961)
215. Fox, J. J., and Miller, N. C., *J. Org. Chem.*, **28,** 936–41 (1963)
216. Codington, J. F., Doerr, I. L., and Fox, J. J., *J. Org. Chem.*, **29,** 558–64 (1964)

217. Grunberg, E., Titsworth, E., and Bennett, M.E., *Antimicrobial Agents Ann.*, 566–68 (1963)
218. Koechlin, B. A., Rubio, F., Palmer, S., Gabriel, T., and Duschinsky, R., *Biochem. Pharmacol.*, **15**, 435–46 (1966)
219. Wempen, I., Duschinsky, R., Kaplan, L., and Fox, J. J., *J. Am. Chem. Soc.*, **83**, 4755–66 (1961)
220. Burchenal, J. H., Holmberg, E. A. D., Fox, J. J., Hemphill, S. C., and Reppert, J. A., *Cancer Res.*, **19**, 494–500 (1959)
221. Duschinsky, R., Gabriel, T., Hoffer, M., Berger, J., Titsworth, E., Grunberg, E., Burchenal, J. H., and Fox, J. J., *J. Med. Chem.*, **9**, 566–72 (1966)
222. Fox, J. J., Miller, N. C., and Wempen, I., *J. Med. Chem.*, **9**, 101–5 (1966)
223. Burchenal, J. H., Adams, H. H., Newell, N. S., and Fox, J. J., *Cancer Res.*, **26**, 370–73 (1966)
224. Heidelberger, C., Parsons, D. G., and Remy, D. C., *J. Med. Chem.*, **7**, 1–5 (1964)
225. Heidelberger, C., and Anderson, S. W., *Cancer Res.*, **24**, 1979–85 (1964)
226. Kaufman, H. E., and Heidelberger, C., *Science*, **145**, 585–86 (1964)
227. Gottschling, H., and Heidelberger, C., *J. Mol. Biol.*, **7**, 541–60 (1963)
228. Szybalski, W., Cohn, N. K., and Heidelberger, C., *Federation Proc.*, **22**, 532 (1963)
229. Heidelberger, C., Boohar, J., and Birnie, G. D., *Biochim. Biophys. Acta*, **91**, 636–38 (1964)
230. Heidelberger, C., Boohar, J., and Kampschroer, B., *Cancer Res.*, **25**, 377–81 (1965)
231. Shen, T. Y., Ruyle, W. V., and Bugianesi, R. L., *J. Heterocyclic Chem.*, **2**, 495–96 (1965)
232. Mertes, M. P., Saheb, S. E., and Miller, D., *J. Heterocyclic Chem.*, **2**, 493–94 (1965)
233. Dipple, A., and Heidelberger, C., *J. Med. Chem.*, **9**, 715–18 (1966)
234. Horwitz, J. P., Chua, J., de la Rooge, M. A., Noel, M., and Klundt, I. L., *J. Org. Chem.*, **31**, 205–11 (1966)
235. Watanabe, K. A., and Fox, J. J., *J. Org. Chem.*, **31**, 211–17 (1966)
236. Zorbach, W. W., Munson, H. R., and Bhat, K. V., *J. Org. Chem.*, **30**, 3955–57 (1965)
237. Imai, K., and Honjo, M., *Chem. Pharm. Bull.* (*Tokyo*), **13**, 7–16 (1965)
238. Furukawa, Y., Kobayashi, K., Kanai, Y., and Honjo, M., *Chem. Pharm. Bull.* (*Tokyo*), **13**, 1273–78 (1965)
239. Nishimura, T., and Shimizu, B., *Chem. Pharm. Bull.* (*Tokyo*), **13**, 803–10 (1965)

CONTENTS