CLINICAL PHARMACOLOGY OF THE EFFECTIVE ANTITUMOR DRUGS[1,2]

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Pharmacology covers a wide variety of drug phenomena such as animal screening, toxicology, therapeutic trials, pharmacological disposition by the host, and mechanism of action. The modern department of pharmacology tends to concentrate on the last item, since it often involves interesting techniques and concepts of biochemistry. Those who must advise physicians on the best way to use drugs need to collect and interpret other data about drugs—absorption, binding, excretion, distribution, metabolism, and relation of drug-dosage schedule to effectiveness and toxicity. Further, they must interpret and correlate the effects of drugs in the animals used for experimental therapy and toxicology; the same types of data must be examined in the experimental model. The pharmacological disposition of antitumor drugs in man or animal has not been emphasized. This is reflected in the fact that there has been only one earlier review (1). Although the present review may serve largely to call attention to the gaps in our knowledge, the authors have chosen to review the disposition of antitumor drugs, limiting the discussion largely to (*a*) the findings available from clinical studies, or, where these are lacking, to a few relevant animal data; and (*b*) those drugs that produce complete or nearly complete regression in at least one form of clinical cancer.

Alkylating Agents

Since the discovery of the antineoplastic effect of nitrogen mustard in Hodgkin's disease in 1943, a large number of alkylating agents have been synthesized. However, only six alkylating agents are, at present, in widespread use. These are shown in Table I, noted with the disease in which they are unequivocally effective. Since the dosage of each of these drugs must be decided on an individual basis, a full description of clinical usage cannot be explained in a table; the route and dosage are shown to give the pharmacologist some idea of how the drugs are used. Since there are clinical pharmacologic data for only two of the six agents, we have decided to include data in man for alkylating agents other than the six most commonly used and to mention a few observations in the dog and rat for compounds for which clinical observations are lacking (Table II).

[1] The survey of literature pertaining to this review was concluded in July, 1964.

[2] The following abbreviations are used: CSF (cerebrospinal fluid); FU (fluorouracil); FUdR (5-fluoro-2′-deoxyuridine); HN_2 (mechlorethamine); I.V. (intravenous); MeGAG (methylglyoxal-*bis*-guanylhydrazone); MEPA (morpholine diethylene phosphoramide); 6-MP (6-mercaptopurine); MTX (methotrexate); TEM (Triethylene melamine); and TEPA (triethylene phosphoramide).

TABLE I

CLINICAL USEFULNESS OF SIX COMMONLY USED ALKYLATING AGENTS

Drug	Disease	Route	Oral dose
Mechlorethamine (nitrogen mustard, HN_2, Mustargen)	Hodgkin's disease Lymphosarcoma Wilm's tumor	I.V.	0.4 mg per kg single dose
Chlorambucil (Leukeran)	Chronic lymphatic leukemia Hodgkin's disease Lymphosarcoma Carcinoma of ovary	Oral	0.1 to 0.2 mg per kg per day
Triethylene thiophosphoramide (TSPA, Thiotepa)	Chronic lymphatic leukemia Hodgkin's disease Lymphosarcoma Carcinoma of ovary	I.V.	0.4 mg per kg weekly or biweekly
L-sarcolysin (Melphalan, Alkeran)	Myeloma	Oral	6 to 10 mg per day (initially) 2 to 4 mg per day (maintenance)
Cyclophosphamide (Endoxan, Cytoxan)	Myeloma Acute lymphatic leukemia Wilm's tumor	I.V. Oral	3.5 to 5.0 mg per kg per day × 10 or 15 mg per kg once weekly 50 to 300 mg per day
Busulfan (Myleran)	Chronic myelocytic leukemia	Oral	2 to 8 mg per day

Absorption.—Most of the six agents are absorbed from the gastrointestinal tract. Mechlorethamine (nitrogen mustard, HN_2) is, perhaps, the exception. Krakoff & Karnofsky (2) report that HN_2, given to seven patients, produced neither bone marrow depression nor therapeutic effect in spite of doses up to 1.3 mg per kg. The failure of patients to absorb mechlorethamine is curious, since normal volunteers absorb HN_3 after oral administration (3). Absorption of the five other alkylating agents is probably incomplete and erratic, although no clear judgment can be made except in the case of thiotepa. A comparison (Table III) of the total amounts of drug and labeled end products, excreted in the urine after oral or intravenous administration, shows no significant differences for busulfan, (4), TEM (4), or thiotepa (5) in man, and for thiotepa (6) in the dog. Mellett, Hodgson & Woods (5) have shown, in three patients receiving oral C^{14}-thiotepa, that 84, 24, and 37 percent of the label were recovered in the urine. The plasma levels were correspondingly variable. Similar data on tritiated cyclophosphamide in the

TABLE II

TOXICITY OF ALKYLATING AGENTS WHEN USED AT THERAPEUTICALLY EFFECTIVE DOSAGES

Drug	Nausea and vomiting	Granulocytopenia	Thrombocytopenia	Other
Mechlorethamine	+++	+++	++++	Local vesication and tissue necrosis
Chlorambucil	0	++	++++	
Thiotepa	+	+++	++++	
Sarcolysin	+	+++	++++	Mucosal ulceration
Cyclophosphamide	++	+++	+	Alopecia 10–25%; mucosal ulceration; sterile cystitis
Busulfan	0	++++	+++	Depression of ovarian function; skin pigmentation

dog (7) suggest quite incomplete absorption. This is confirmed by the recovery from the stools of 31 to 63 percent of the label. It is unfortunate that more comparisons on the oral vs. parenteral effects of the six alkylating agents are not available. Even though these agents are used in a highly individualized fashion, it is apparent that erratic absorption occurs, and this may result in sharp excursions in antitumor effect or toxicity.

Distribution and excretion.—Because of the reactivity of most of the alkylating agents, events move so quickly that distribution and excretion are difficult to study. As seen in Table III, 5 min after injection in man, the plasma concentrations of busulfan-C^{14} and TEM-C^{14} (4) have fallen more than 90 percent. C^{14}-N-(3-oxapentamethylene)-N′,N″-diethylene thiophosphoramide (OPSPA), another compound that has been studied in man, remains in the plasma for at least 24 hr (8). In this period, about 40 percent of the dose appears in the urine, but only a small fraction appears as the parent compound. C^{14}-Thiotepa remains in the plasma of patients for several hours (5), with 75 to 85 percent of the labeled materials appearing in the urine. When a specific fluorimetric method was used for thiotepa (6), in a study in dogs, only 10 to 15 percent could be recovered in the urine. Cyclophosphamide (7) has similar characteristics in the dog, although larger amounts are found in the urine. It also appears in the bile, is excreted in the feces, and accumulates in the liver, spleen, and kidney of the dog. HN_2 studied in dogs by a fluorimetric method had a disappearance time of 4 min (9).

Ruddon & Mellett (10) recently reported on the distribution of C^{14}-labeled thiotepa in rats, both normal and tumor bearing (Walker-256 carcinosarcoma). The drug and its metabolites were uniformly distributed in all tissues studied, including tumor. However, at 1 hr after injection, there was accumulation of a metabolite in the tissues of the stomach, small intestine,

TABLE III

PHARMACOLOGICAL DISPOSITION OF SEVERAL ALKYLATING AGENTS

Drug	% of dose excreted in urine during first 24 hr		Time for plasma value to fall 90–95%	% of drug excreted in urine as		Nature of urinary metabolite	Reference
	I.V.	Oral		Parent compound	Metabolite		
			Man				
Busulfan							
C^{14}	10	12	3 min	none	75	Two unknown metabolites	(3)
S^{35}	40	53	5 min	none	95	Methane sulfonic acid	(3)
TEPA							
P^{32}		15		none	88–93	Inorganic phosphates	(3)
TEM							
C^{14}	20	30	5 min	none	8	Urea and creatinine	(3)
					92	Twelve unknown metabolites	
OPSPA-C^{14}[a]	40		24 hr	2.6	25	MEPA	(9
					1.8	Morpholine	
Thiotepa-C^{14}	63	24–84	1–4 hr	0.1–1.0 (oral) 0.2 (I.V.)	0.3	TEPA in 2 of 4 patients	(6)
			Dog				
Mechlorethamine[b]			4 min	0.01			10
Thiotepa[b]	8.3–13.3		3 hr	0.3–0.7	8–	TEPA	7
Thiotepa-C^{14}	75–8		1–2 hr	0.2–0.3 (I.V.)			6
Cyclophosphamide-H^3	43	15–23[c]	6 hr	4–6 (oral) 11–14 (I.V.)	30	Unknown	8

[a] C^{14}-N(3-oxapentamethylene)-N′,N″-diethylene thiophosporamide)

[b] Fluorimetric method

[c] Total radioactivity in stools = 31–63% of dose
Fecal recovery of unchanged cyclophosphamide = 17–31% of dose

and colon. More label was found in nuclear fractions of both tumor and normal cells than in the cytoplasmal constituents. In autoradiographs, the activity was associated with chromosomal material. Similar studies in the dog with TEPA, using a fluorimetric method, showed selectively high concentrations in the bone marrow 15 min after intravenous administration (6).

Metabolism.—These agents remain as parent compounds in the fluids and the patient for relatively short periods. Of the five compounds studied in man, none of the parent compounds could be found in the urine after administration of busulfan (4), TEPA (4), thiotepa (5) or TEM (4). Nadkarni, Trams & Smith (4) have studied busulfan, TEPA, and TEM. When busulfan-C^{14} was given, 75 percent of the dose was recovered in the urine as unknown metabolites. With busulfan-S^{35}, 95 percent was recovered as methane sulfonic acid. About 90 percent of TEPA-P^{32} was recovered as inorganic phosphates. After a dose of TEM-C^{14}, patients excreted 8 percent as urea and creatinine, with 92 percent of the label in 12 unidentified metabolites. Thiotepa (5), as such was not found in the urine, but there were small amounts of

TEPA. C^{14}-N-(3-oxapentamethylene)-N′,N″-diethylene thiophosphoramide again differed somewhat from these agents in that 2.6 percent of a dose administered as the C^{14} compound was recovered in the urine (11) unaltered. An additional 25 percent of the label was found in MEPA, an active antitumor agent, and 1.8 percent was found as morpholine. In addition, there were unidentified metabolites. In the dog, thiotepa studied by a fluorimetric method somewhat resembled C^{14}-N-(3-oxypentamethyl)-N′,N″-diethylene thiophosphoramide in that parent compound was found in the urine in small amounts and 10 percent of the label was found in the metabolite TEPA (6). Cyclophosphamide gave similar results, although the metabolites were not identified (7).

There are then two types of alkylating agents. One group (HN_2, busulfan, and TEM) remain as such in the plasma for a matter of a few minutes; the radioactive labels appear in the urine in a variety of degradation products. A second group C^{14}-N-(3-oxapentamethylene)-N′,N″-diethylene thiophosphoramide, thiotepa, and cyclophosphamide) have a much longer half life; parent compound can be found in the urine in small amounts, and probably the number of metabolites is relatively small. These studies should be extended and the different patterns of pharmacological disposition related to their modes of clinical effectiveness and toxicity. Cyclophosphamide is of importance in this regard, since it, alone, brings about remission in acute leukemia and has slight effect on platelets. Because of its interesting pharmacology, OPSPA should be studied in a wider variety of diseases, especially acute leukemia and myeloma.

Toxicity.—The serious toxicity of the alkylating agents resides mainly in their effects upon the bone marrow; this property is common to all six of the agents. There are other less threatening side effects that are not seen for all of the agents. In Table II, there is a quasiquantitative summary of these toxicities (12).

In a general way, the toxicities of the alkylating agents resemble those of other antitumor drugs with selective action upon rapidly dividing cells, such as those of the bone marrow, hair root, and gastrointestinal tract. There are, however, some interesting differences. Two side effects are seen with one agent each—alopecia with cyclophosphamide (13), and skin pigmentation with busulfan (14). Another difference is that ulceration of mucosa of the mouth and gastrointestinal tract, while common with antimetabolites, rarely occurs with alkylating agents, perhaps only with sarcolysin (L-phenylalanine mustard) (15). Finally, while hematopoietic effects of alkylating agents are usually equally expressed upon granulocytes and platelets, cyclophosphamide is an exception in that there is a relative sparing of the platelets (13). While this results in a safer drug, its use unfortunately leads to morale-depleting alopecia.

6-Mercaptopurine

6-Mercaptopurine (6-MP), was synthesized and developed by Hitchings & Elion (16) and their colleagues and was shown to be active against animal

tumors. Burchenal et al. (17) found that it was active in both types of acute leukemia. It has subsequently been shown to be active in choriocarcinoma (18) and chronic granulocytic leukemia (19) and to suppress the immune responses in man (20). 6-MP diminishes purine synthesis *de novo* by inhibition of phosphoribosylamine formation (21), but there is uncertainty concerning the precise relation of this inhibition to its antitumor or immune suppressive effects.

Absorption.—6-MP is readily absorbed from the gastrointestinal tract, and it is usually given by mouth. Balance studies have not been published, and a precise quantitative estimate of absorption cannot be made. Elion et al. (22) and Hamilton & Elion (23) report that 30 to 35 percent of an oral dose and 60 percent of an intravenous dose of 6-MP-S^{35} was recovered in the urine in 24 hr. This is a minimal figure, since only two of the metabolites were studied. Absorption of 6-MP in man after an oral dose must clearly be more than 50 percent. Toxicity comparisons of oral and intravenous 6-MP have not been made in the same individual with the same total dose and schedule. Intravenous administration gives rise to no greater apparent toxicity than similar amounts given orally, when both are given as a single daily dose (24). However, the toxicity of 6-MP was much greater when it was given by continuous infusion for 6 to 10 days.

Distribution.—The half time of 6-MP in the blood has been calculated at 90 min (23, 25). Three minutes after injection, the concentration in whole blood is only 80 percent that of plasma. The concentrations approximate each other at 5 hr, and, thereafter, concentration is higher in whole blood (23). The calculated volume of distribution of 6-MP-S^{35} at 1 hr, 60 percent (23), suggested that it was distributed into total body water rather than into a volume corresponding to the extracellular fluid. The probability that 6-MP is incorporated into human white cells lends further support for intracellular distribution. Small amounts of 6-MP can be found in the cerebrospinal fluid after intravenous administration (23). For drugs that are rapidly catabolized, the extent to which the parent substance enters the CSF can be calculated only when a constant concentration is maintained until a steady-state is approached; this has not been studied in patients. In the dog under steady-state conditions, the amount of 6-MP in the CSF is 80 percent that of plasma (26). 6-MP given orally does not prevent or control meningeal leukemia. This suggests that administration of the usual clinical doses of 6-MP orally once a day does not maintain a plasma-CSF gradient of active drug long enough to achieve effective concentrations in the CSF.

Excretion.—Although it has been shown that 6-MP is rapidly metabolized, it has been possible to study the renal excretion of the parent compound. Loo, Michael & Rall (25) state that no more than 50 percent of a large intravenous dose can be recovered from the urine as 6-MP. Elion et al. (22) could recover from the urine only 4.8 and 7.2 percent of an oral dose of 6-MP. Most of the 6-MP is excreted in 15 hr (23, 25). In two studies, clearance of 6-MP has been found to approximate glomerular filtration. Loo, Michael & Rall (25) state that it was 5 ml per min per kg in the dog, while

Hamilton & Elion (23) calculated that in a 75-kilo adult the clearance was 125 to 132 ml per min. At 24 hr, 60 percent of the administered radioactivity could be recovered in the urine. There was, then, a gradual decline until day 17. Small amounts of radioactivity were irregularly present up to 45 days. Net excretion into the gastrointestinal tract is negligible after an intravenous dose (23).

Metabolism.—The metabolites of 6-MP appearing in the urine have been shown to be 6-thiouric acid (6-TU), sulfates, and unknown moieties. Loo et al. (27), using unlabeled 6-MP, confirmed this finding, stating that at least two other metabolites appeared which could not be identified. Hamilton & Elion (23) gave an intravenous dose of 6-MP-S^{35} and found that, in the 1 hr urine collection, 39 percent of the radioactivity was in 6-MP, 36 percent in 6-thiouric acid, 13 percent unknown, and a trace was in sulfate. At 5 hr the proportion of 6-MP and 6-TU remained the same, the unknown increased to 21 percent, and sulfate to 21 percent. In the 24 to 48 hr collection, the 6-MP and 6-TU were down to 8 percent, while sulfate increased to 75 percent. In another study after oral administration of unlabeled 6-MP in two patients, Elion et al. (22) found in the 24 hr urine collection 4.8 and 7.2 percent, respectively, as 6-MP, and 25.6 and 26.5 percent as 6-TU. Loo et al. (27) only state that in a study of 20 leukemic patients, receiving 50 to 200 mg of 6-MP per day, about 15 percent of the daily dose was regularly excreted as 6-TU. Oxidation of 6-MP is, thus, rapid and extensive. Presumably this is achieved in man by means of xanthine oxidase.

Toxicity.—The major toxic effect of 6-MP is bone marrow depression, all three elements being affected (17). 6-MP can usually be employed for acute leukemia without serious depression of blood elements. Depression of platelets and red cells by 6-MP is less of a problem in patients with chronic granulocytic leukemia. Adults and children rarely show nausea and vomiting while taking 6-MP. Mucosal ulceration of the mouth is occasionally seen but occurs less often than with methotrexate. Rashes can occur. Up to one third of patients on 6-MP develop jaundice; this is often disease, rather than drug, related, but there is no question that 6-MP causes mild jaundice (28). Liver biopsies usually show moderate bile stasis. As with other active antileukemic agents, hyperuricemia with hyperuricosuria and renal impairment may occur from 6-MP (19).

Fluorinated Pyrimidines

The observation by Rutman, Cantarow & Paschkis (29) of the greater utilization of uracil by rat hepatomas, as compared to normal rat liver, led to the design and synthesis of two antimetabolites, fluorouracil (FU) and 5-fluoro-2′-deoxyuridine (FUdR), by Heidelberger and associates (30, 31). Following the demonstration of the activity of FU and the slightly less toxic but more effective FUdR against the growth of a variety of transplanted rodent tumors (32, 33, 34), initial clinical trials of FU (35, 36) produced palliative effects in patients with cancers of the gastrointestinal tract, breast, and female genital tract but not with leukemias or lymphomas (36, 37, 38).

Objective responses were also reported (39, 40) with FUdR. Heidelberger & Ansfield (41) as well as Zubrod (42) have recently reviewed the literature on clinical responses to FU and FUdR in human solid tumors. The biochemical mechanism of action of the fluorinated pyrimidines also has been recently reviewed by Heidelberger (43).

Absorption.—FU-2-C^{14} and, to a lesser extent, FUdR-2-C^{14} were rapidly absorbed after oral or intramuscular administration (44, 45). This was evidenced by the appearance of radioactivity in the plasma as early as 10 min after administration. Erratic absorption was obtained by the intraperitoneal dose route. Using a newly developed microbiological assay system which is specific for FU and FUdR, Clarkson et al. (46) observed that orally administered FUdR reached only one tenth the peak of plasma concentration of FU given by the same route.

Distribution.—The initial studies by Chaudhuri, Montag & Heidelberger (47) of the distribution of radioactivity in various tissues and body fluids of cancer patients receiving FU-2-C^{14} led to the first demonstration in man of the selective localization of FU or its metabolite(s) in tumor tissue. The specific activity of a specimen of an anaplastic lung carcinoma was higher than those of the adjacent skin, muscle, fat, and a liver biopsy from a patient who had been given a single intravenous injection of radioactive FU. This finding was subsequently confirmed in the same laboratory (44, 48), but, in addition, the specific activity of normal intestinal mucosa and malignant-tumor tissue was higher than liver and surrounding tissues. In contrast, benign lesions (fibroadenomas) had a lower specific activity than liver. Thus, in these tissue distribution studies, differences in the degree of uptake of radioactivity between normal and cancerous tissues generally correlated with differences in the degree of cell proliferation (turnover) and vascularity.

Radioactivity in plasma, following a single intravenous injection of FU-2-C^{14} (44, 45) or FUdR-2-C^{14} (45), was highest during the first 2 hr and decreased linearly to 24 hr. One fourth of the radioactivity in the blood during the first hour was unchanged drug which was calculated to be equally distributed throughout body water in patients who received FU-2-C^{14} intravenously (44). No significant difference was observed in the plasma concentration of FU or FUdR between patients with normal and impaired renal function (46). Mukherjee et al. (45) observed a slow entry of radioactivity into the CSF, which gradually increased to the concentration of radioactivity in the plasma following intravenous doses of FU-2-C^{14} or FUdR-2-C^{14}. Thereafter, a similar pattern of the rate of disappearance of radioactivity was established between plasma and CSF. In contrast, Clarkson et al. (46), using the microbiological assay, found that the highest concentration of FU in the CSF was only one fiftieth or less of the initial plasma concentration. In any event, it appeared that fluorinated pyrimidines do not freely diffuse across the blood-brain barrier in sufficient concentration to be of practical value in the treatment of neoplasms associated with the central nervous system.

Excretion.—Approximately 20 to 90 percent of the C^{14} of radioactive FU was excreted as respiratory CO_2 during the 12 hr following a single intraven-

ous injection in man (44, 45, 47). More rapid appearance and consistently larger amounts of the dose in respiratory CO_2 were observed after oral administration, while excretion following administration by the intramuscular or intraperitoneal routes was generally lower and more variable (45). Following intravenous injection of FU-2-C^{14}, between 10 and 20 percent of the radioactive dose was excreted in the urine during the initial 24 hr. A smaller proportion of the radioactive dose was excreted following oral administration of the drug. However, a significantly greater proportion of unchanged FU-2-C^{14} persisted in the urines of patients who received the drug intravenously (45). Insignificant amounts of radioactivity were detectable in stool samples (44).

Radioactive FUdR was rapidly converted into respiratory CO_2 following oral or intravenous administration in man, but more of the drug was degraded after the oral route (70 to 80 percent) than after the intravenous route (50 to 60 percent) during the initial 24 hr (45). The enhanced degradation following the oral administration was reflected in urine excretion, where no unchanged FUdR was recoverable. Small but detectable amounts of parent compound occurred in the urine following intravenous, intramuscular, or intraperitoneal injection. It thus would be expected that FUdR would be least active by the oral route.

Since it was observed clinically (49) that continuous intravenous infusion resulted in a twofold decrease in toxicity of FU and a twentyfold increase in toxicity of FUdR, the excretion of the drugs was studied following administration by this technique. Both Mukherjee et al. (45) and Clarkson et al. (46) found that FU was degraded more completely after continuous intravenous infusion with reduced excretion in the urine and increased excretion of radioactivity in respiratory CO_2. It was also noted (45) that by continuous intravenous administration a greater proportion of FUdR was excreted as respiratory CO_2 than by single intravenous injection. This coincided with the reduced urinary excretion of FUdR (46). Generally then, the employment of slow, continuous, intravenous administration leads to a more extensive degradation of FU or FUdR with consequent alteration of the toxicity and therapeutic effectiveness of these compounds in man.

Metabolism.—It is now firmly established that the fluorinated pyrimidines are metabolized in man by anabolic and catabolic pathways. The catabolic route of FU, which is analogous to the degradative pathway taken by uracil, gives rise to dihydrofluorouracil, α-fluoro-β-ureido-propionic acid α-fluoro-β-alanine, and urea or carbon dioxide, and ammonia (44, 50). The liver is the primary site of degradation of the fluorinated pyrimidines (44), and the products formed are devoid of significant toxicity or antitumor activity. The anabolic pathway, in which FU and FUdR are converted into nucleotides, is considered to be of primary importance in effecting carcinostatic activity (48).

Urine samples from patients who received an intravenous injection of FU-2-C^{14} contained unchanged drug, urea, and α-fluoro-ureido-propionic acid, the proportion of which varied with time of collection. Urea, α-fluoro-

ureido-propionic acid, FU (as a degradation product), and unchanged FUdR were identified in the urine of patients intravenously receiving radioactive FUdR.

Fractionation of various tumors and tissues to determine content of degradation products and nucleotides revealed that, in addition to unchanged drug, the concentration of urea and α-fluoro-ureido-propionic acid of both tumor and corresponding normal tissue was the same, while the nucleotide content of several carcinomas of the colon was approximately equivalent to the adjacent intestinal mucosa of patients injected intravenously with FU-2-C^{14} (48). However, following intravenous administration of FUdR-2-C^{14}, the proportion of labeled nucleotide in the carcinoma of the colon was higher than the normal intestinal mucosa. While there were considerable variations in the uptake of radioactivity in tumors of different organs, it was postulated that the more cellular the tumor, the higher the uptake. This might be expected because of the relation of FU to nucleic acids and the correlation of nucleic acid concentration with cellularity. In the case of the intestinal mucosa with its high cell-turnover rate, the uptake was also high. However, it is significant, at least in the case of FUdR, that the capacity of the carcinoma of the colon for converting this fluorinated pyrimidine into nucleotide was greater than that of the corresponding intestinal mucosa. Conversely, an astrocytoma, which does not respond clinically to FU or FUdR, had a low capacity for converting fluorinated pyrimidines into nucleotides, despite its high cellularity and vascularity (48).

Toxicity and dosage.—The two major toxic effects of fluorinated pyrimidines in man are gastrointestinal damage and bone marrow suppression. Manifestation of toxicity, in which there is also damage to normal proliferating tissue as a result of inhibition of DNA synthesis, seems prerequisite to an objective antitumor response.

Toxic reactions observed in patients treated with FU and FUdR include diarrhea, stomatitis, nausea, vomiting, alopecia, dermatitis, pharyngitis, esophagitis, epistaxis, and leukopenia (40). Diarrhea occurred most commonly and was often the initial sign of toxicity with FUdR, while stomatitis more often indicated onset of toxicity with FU. Alopecia was rare with FUdR, while dermatitis was observed more frequently with FU. Leukopenia was manifested more often with FUdR than with FU (40).

The Wisconsin dosage schedule (41) has been most widely adopted in the clinic for the single or repeated intravenous injection of FU or FUdR. With FU, 15 mg per kg per day was given for 5 days followed by 7.5 mg per kg every other day until the appearance of toxicity. The most effective intravenous dose for FUdR was twice that of FU, 30 mg per kg per day followed by 15 mg per kg every other day for 11 such doses or until toxicity appeared. With both drugs, a new course of therapy is usually given after an interval of approximately 4 wk.

The toxicity in man from single, daily, intravenous injections of FU or FUdR given on an equal molar basis was not appreciably different. However, Sullivan et al. (49) observed that when fluorinated pyrimidines are given by

slow intravenous infusion, the toxicity of FU is decreased at least two times and, conversely, that of FUdR is increased approximately 20 times. The type of toxicity observed with continuous intravenous infusion was similar to that seen after a single intravenous injection.

The decreased toxicity of FU, given by continuous infusion as compared to single intravenous injections, was also observed by Lemon (51), Staley et al. (52), and Reitemeier & Moertel (53). In the study by Staley et al. (52), diarrhea was the most common toxic reaction, while severe leukopenia was avoided. However, only minimal objective responses (12 percent) were seen in patients with various advanced neoplasma. Similar results were obtained by Ansfield, Schroeder & Curreri (54) for FUdR.

Patients with metastatic liver disease (55) and tumors of the head, neck, and extremities (56, 57, 58) have shown variable objective responses to continuous intra-arterial infusions of FU or FUdR.

On occasion, FU has been given by mouth (52) with reduced toxicity but with less antitumor effect. This results from more extensive degradation of the drug, thus making oral dosage less desirable.

While these pharmacological findings have undoubtedly contributed significantly to the intelligent clinical use of fluorinated pyrimidines, additional data are required to correlate the variations in the capacity of tumors in different patients to convert these drugs into lethal nucleotides with variations in clinical responses to chemotherapy. Efforts to decrease the toxicity of FU or FUdR by varying the dose and route of administration have most often led to a decreased antitumor response. Further investigation, into several proposed explanations for the altered toxicity and therapeutic effects of these compounds following continuous intravenous infusion (46, 49, 51, 59), is desirable for unveiling the responsible biochemical mechanism(s). Thus far, the best clinical results have been achieved by employing these drugs, as in the case of the folic and antagonists, at doses near or at the toxic level. Unfortunately, the margin is narrow between effective antitumor doses and mortality.

Folic Acid Antagonists

Aminopterin, methotrexate (MTX), and the halogenated 4-amino analogs, particularly dichloromethotrexate, have shown consistently marked antitumor effects in experimental rodent tumor systems (60, 61, 62). Clinically, the most significant achievements have been made with MTX, especially in the chemotherapy of choriocarcinoma in women (63, 64, 65). Aminopterin and MTX have been effectively employed in the therapy of systemic (66, 67, 68, 69) and meningeal (69, 70, 71) acute lymphocytic leukemia. Occasional responses have also been elicited by MTX in a variety of other human neoplasms (12). Dichloromethotrexate, the least tried of the clinically useful folic acid antagonists, produced therapeutic effects comparable to MTX in patients with lymphosarcoma and Hodgkin's disease (72), and acute lymphocytic leukemia (73). The mechanism of action by which the folic acid antagonists exert their carcinostatic activity is generally accepted to be the

inhibition of folic or dihydrofolic reductase which catalyzes the conversion of folic acid to tetrahydrofolic acid. The latter provides one carbon unit in the *de novo* synthesis of purines, pyrimidines, and nucleic acids. The reductase enzyme has a greater affinity for the antagonist than for folic acid. For detailed discussions of the mechanism of action of folic acid antagonists, the reader is referred to recent reviews by Werkheiser (74), Bertino (75), and Mead (76).

Until recently, studies of the physiological disposition of folic acid antagonists have been impeded by the inherent impurity and instability of the available drugs and the lack of methods which were adequately sensitive, precise, and specific for the determination of minute quantities of these compounds or their possible metabolites in biological tissues and fluids. The most recent and reliable data on the fate of folic acid antagonists in man and animals have come from studies in which radioactive labeled compounds, i.e., methotr

combination with cellulose ion-exchange column techniques (77, 78) and the modified Schöniger oxygen-flask combustion method for biological tissues and fluids (79, 80). The ensuing discussion of the metabolic fate of these agents, namely MTX and DCM, will be drawn largely from recent investigations reported by Johns et al. (77) and several published reports and unpublished data from the laboratory of one of the authors (Oliverio) of this review. The results of related studies, carried out earlier by other investigators, have been reviewed by Mandel (1), Holland (81), and Delmonte & Jukes (82).

Absorption.—Methotrexate was readily absorbed from the gastrointestinal tract following oral administration. This was evidenced by its rapid appearance (within one half hour) in the plasma (75, 83, 84, 85). The extent of absorption was governed by the size of the administered dose. Thus, 0.5 mg per kg or less of MTX taken by mouth resulted in more complete absorption (with plasma levels equivalent to intravenous administration) than ingestion of 2 to 10 mg per kg of the drug (75, 84, 85). In addition, delayed absorption and lower plasma concentrations were obtained in nonfasting patients (84), while prolonged plasma levels were observed in the presence of renal insufficiency (83, 84). The rates of disappearance from the plasma ($T\frac{1}{2} = 1.5$ hr) were similar between high and low oral or intravenous doses of MTX (85). Plasma protein binding of the drug amounted to approximately 50 percent (84, 85).

Unlike its nonhalogenated predecessor, dichloromethotrexate was less incompletely absorbed from the gastrointestinal tract of man (86, 87) and animals (78, 87). Peak plasma concentrations were achieved 2 to 4 hr after oral dichloromethotrexate, and these were about one fourth of the plasma concentrations 2 to 4 hr following comparable doses of intravenous or intramuscular dichloromethotrexate (87, 88). The plasma protein binding of the drug (90 percent) differed substantially from that of MTX (85).

Distribution.—Following a single intravenous injection of MTX, the rate of disappearance of the drug from the plasma was rapid with a half life

ranging from 0.5 to 1.5 hr (85, 89). The plasma concentrations extrapolated to a volume of distribution of about 20 to 30 percent of body weight, suggesting that, in man, relatively little drug enters intracellluar spaces (84). However, that some MTX penetrates cells and is bound to folic acid reductase was evidenced by its detection in mouse liver and kidney tissues for a period up to 8 mo after administration of the drug (90). Both liver and kidney tissues contain the highest concentrations of folic acid coenzymes and folic acid reductase (75). Werkheiser (91) noted that, in contrast to the more slowly dividing liver and kidney tissues, the highly proliferating intestinal mucosa of mice released aminopterin more rapidly. Condit (92) observed that human liver and kidney tissue, obtained at autopsy, showed MTX for as long as 116 days following administration of the drug. Studies in rodents with MTX-H^3 demonstrated that the drug distributed in all tissues, although liver, kidney, and small intestines with contents accounted for the bulk of tissue radioactivity 1 hr following parenteral administration of the drug (85). Tissue distribution studies in man with radioactive MTX have not been described.

Following a single intramuscular injection of MTX in man, very little drug entered the cerebrospinal fluid (93). Similar results were obtained in dogs with MTX and aminopterin. After 4 hr infusions, a steady-state CSF: plasma-MTX concentration ratio of less than 0.004 was approached.

Initial studies with dichloromethotrexate-Cl^{36} by Oliverio & Davidson (78) demonstrated that the pattern of distribution of radioactivity in the tissues and organs of rodents was similar to MTX-H^3 distribution following parenteral dosage. After a single intravenous dose of dichloromethotrexate-Cl^{36} in man, the radioactivity disappeared rapidly from the plasma up to 3 hr and, thereafter, declined more slowly and exponentially to 24 hr, exhibiting a half time of 8 hr for the latter phase of disappearance (88). This is in marked contrast to the more rapid rate of disappearance of MTX-H^3 from human plasma. Generally, as with other folic acid antagonists, dichloromethotrexate was excluded from the cerebrospinal fluid after systemic administration (78).

Excretion.—MTX was excreted by the urinary and fecal routes in man and animals (80, 84). The bulk of fecal MTX was of biliary origin (80). One day following a single intravenous dose of 0.1 to 10 mg per kg of MTX-H^3 in man, about three fourths of the radioactive dose was excreted in the urine as unaltered drug, and less than 10 percent was recovered in stool samples (80). Slightly lower urinary excretion values in man were observed for the first 24 hr in studies by Johns et al. (77). In addition, some of the radioactivity excreted in the urine of patients after the first 48 hr was attributed to two conversion products of MTX. Although these metabolically altered products accounted for a small fraction of the total radioactivity excreted, it was proposed (77) that these were derived from MTX bound to tissues, and slowly released at the time of cell death. On the other hand, 2 wk after the initial radioactive dose in man, radioactive MTX, presumably bound to intact living cells, was displaced by flushing doses of unlabeled MTX and was excreted in the urine unaltered. The fact that tritium was recovered other than

in the form of MTX suggested that it probably had little or no affinity for dihydrofolic reductase, since it might have been expected to be bound to excess enzyme in neighboring intact living cells.

The recovery of radioactivity from the urine and stools of man following either ingestion or intravenous administration of 0.1 mg per kg of MTX-H^3 was comparable. Doses of 10 mg per kg, however, resulted in the recovery of considerably more radioactivity in the stools following oral than following intravenous administration (85).

Clearance studies have indicated that MTX is both secreted by renal tubules and filtered by the glomeruli (84, 94).

The excretion of DCM in man was found to differ significantly from that of MTX, most likely as a result of its metabolic alteration (78, 86). Following intravenous administration of 5 mg per kg of DCM-Cl^{36}, about 30 percent of the dose, representing the unchanged compound and its metabolite, was excreted in the urine in 24 hr and 40 percent in 72 hr. Approximately one half the radioactive dose was excreted in the stools via the bile. Analysis of urine collected for the first 24 hr showed that one third of the radioactivity was present in the form of a metabolite. Following oral administration in man, about 80 percent of the dose was excreted in the stool, with less than 10 percent in the form of the metabolite. The greater fecal excretion, together with the low level of metabolite observed following oral dosage, suggested that a considerable portion of the dose was not absorbed from the gastrointestinal tract. Because of the extensive fecal excretion and metabolic inactivation of DCM in contrast to the nearly quantitative urinary excretion of unchanged MTX, it was suggested that DCM might be employed in patients with renal insufficiency (86).

Metabolism.—Although an abundance of evidence supports the contention that MTX is not metabolized to any extent in man and animals, the recent investigations of Johns et al. (77) indicate that MTX bound to dihydrofolic acid reductase in tissues may be catabolically altered over an extended period of time and slowly excreted into the urine at the time of cell death. However, recent investigations in the laboratory of one of the review authors (Oliverio) have indicated that altered products of MTX may be due to bacterial contamination of urine samples or perhaps bacterial degradation of parent drug in the gastrointestinal tract, followed by reabsorption and excretion into the urine of its catabolic products (85). In any event, these unidentified breakdown products only account for a minute fraction of the total administered dose, and they do not appear until most of the dose has been excreted.

Unlike MTX, dichloromethotrexate is rapidly and partially converted in rodent and man (but not dog) into a 7-OH derivative (78a) following oral or parenteral dosage (78, 86). The metabolite has been shown by Misra et al. (95) to be a very weak inhibitor of dihydrofolic acid reductase. Thus, metabolism may explain toleration of larger doses of dichloromethotrexate than MTX, but it does not account for the superior antileukemic effect of dichloro-

methotrexate in mice (61). Unfortunately, this advantage has not been experienced clinically.

Toxicity and dosage.—The toxicological effects of the folic acid antagonists in man result from damage primarily to normal rapidly proliferating tissues—the bone marrow, gastrointestinal mucosa, hair follicles, gonads, and fetal tissue. Thus, with bone marrow damage, anemia, granulocytopenia, and thrombocytopenia are the chief factors limiting the dosage of these agents. Increased susceptibility to infection, bleeding, diarrhea, moderate to complete alopecia, and ulceration of oral and intestinal tract mucosa are also prominent. Depression of spermatogenesis and a variety of skin rashes, usually in the form of a perifolliculitis, vesiculation, and desquamation, are seen (96). Abortion or teratogenic effects may prevail during the early stages of pregnancy in patients on folic acid antagonist therapy (97, 98, 99). Many of these toxic symptoms are similar to those identified with folic acid nutritional deficiencies. Werkheiser (91) has suggested that prolonged inhibition by the antifolics of the high dihydrofolic reductase activity in rapidly dividing tissues, such as the intestinal mucosa, bone marrow, and certain neoplasms, is causal in the toxic effects of this class of antimetabolites.

Since methotrexate is almost completely absorbed at low dose levels when given orally, it has been administered by this route to children with acute lymphocytic leukemia. Thus, objective responses have been effected on an oral dose schedule of 2.5 to 5 mg (0.1 mg per kg) daily, usually for 6 to 8 wk until onset of an objective response or for shorter periods in cases of early development of toxicity. The dose can be increased or decreased depending on the size of the individual. The intermittent, oral dose schedule of two to three times weekly was less effective than the daily schedule in prolonging remissions. The rate of survival and incidence of remission appeared to depend on the total dose rather than on dose schedule (68, 100). Complete remission rates of 21 percent have been obtained in children with acute lymphocytic leukemia who were on the daily oral dose regimen of MTX (101); lower remission rates were experienced in adults. Recently, however, it has been demonstrated in a combined study involving several institutions (101) that MTX given intravenously every four days results in remission rates of 70 percent in children with acute lymphocytic leukemia. This schedule permitted the use of larger doses of 30 mg per m^2 of body surface area twice weekly (total dose of 60 mg per m^2 per wk), as compared to the daily oral dose schedule of approximately 3 mg per m^2 per day (total dose of 21 mg per m^2 per wk). Furthermore, the intermittent parenteral schedule was more effective in prolonging remissions than the daily oral dose regimen.

In the treatment of choriocarcinoma, daily intramuscular doses of 10 to 30 mg of MTX given in 5 day courses has been the most commonly adopted dose schedule by Hertz and associates (64, 65). Occasionally, oral or intravenous routes of administration have been employed. Elsewhere (102) lower levels of MTX consisting of 0.75 to 1.5 mg per kg per day given orally in courses of 4 or 5 days have been used for treatment of choriocarcinoma.

Hertz & associates have obtained 47 percent complete remission rates in choriocarcinoma patients treated with MTX alone (65). There were indications that MTX led to hepatic damage in choriocarcinoma patients, as evidenced by increases in plasma alkaline phosphatase and serum glutamic oxaloacetic transaminase. It was recommended (96) that the patients with pre-existing hepatic disease be excluded from treatment with MTX "except under most desperate circumstances."

One of the most common manifestations of systemic acute leukemia has been the proliferation of leukemic cells in the subarachnoid space of the brain, resulting in obstruction to spinal fluid flow and increased intracranial pressure. This has led to nausea, vomiting, headache, blindness, deafness, and nerve palsies (69). Thus, even while patients are in systemic remission, one third of these may develop acute lymphocytic meningeal leukemia. Both MTX and aminopterin have been useful in the treatment of meningeal leukemia when administered intrathecally. Dosage for MTX has been 0.2 mg per kg given on alternate days for three to six doses or 0.8 mg per kg for four doses (81). Rieselbach et al. (70, 71) reported a complete remission rate of 100 percent in patients treated with intrathecal aminopterin in doses ranging from 0.5 to 3 mg per m^2 of body surface at intervals of 6 to 7 days up to 9 mo. Minimal systemic toxicity was produced at 2.5 mg per m^2. At all dose levels, there was significant platelet and white cell depression. No acute or chronic neurotoxicity was noted in any patient. Henderson et al. (103), in an attempt to obtain higher, more uniform exposure of the meninges and brain surface to folic acid antagonists, have perfused the cerebrospinal fluid space of several patients with 10 to 25 μg per ml solutions of MTX over repeated 4 to 5 hr periods. This resulted in minimal toxicity and absorption of MTX into the brain, apparently by passive diffusion.

The clinical experience (59, 104) with continuous intravascular infusions of MTX has been too limited to permit an evaluation.

Patients with metastatic carcinoma given intramuscular or oral dichloromethotrexate at 1 to 2 mg per kg daily for 2 wk developed bone marrow and gastrointestinal toxicity which was qualitatively similar to that seen with MTX (72). No significant difference was noted in tolerated dose and qualitative toxicity between intramuscular and oral routes of administration of dichloromethotrexate. Somewhat lower doses (0.25 to 0.5 mg per kg daily up to 42 days) of dichloromethotrexate were tolerated in patients with acute lymphocytic leukemia. It was observed from these combined clinical studies (72) that the tolerated dose of dichloromethotrexate is five times that of MTX. At equitoxic doses, the antitumor effects of oral dichloromethotrexate, oral MTX, and intramuscular dichloromethotrexate were comparable in patients with lymphosarcoma and Hodgkin's disease. Dichloromethotrexate was not superior to MTX in patients with lymphomas nor was the parenteral route superior to the oral route of administration. A greater than twofold difference in regression rate was elicited with a twofold difference in dose with either MTX or dichloromethotrexate.

METHYLGLYOXAL-*BIS*-GUANYLHYDRAZONE

In 1958, Freedlander & French (105) first described the marked antitumor activity of methylglyoxal-*bis*-guanylhydrazone (MeGAG) in leukemia-L1210- and adenocarcinoma-755-bearing rodents. This finding has been confirmed and extended to other transplanted tumors (106, 107). Although initial clinical trials with an oral regimen of MeGAG showed the drug to be inactive, subsequent studies by various investigators at the National Cancer Institute (108–111) and Roswell Park Memorial Institute (112) proved its uniquely high activity against acute myelocytic leukemia and showed its occasional effectiveness against malignant lymphoma and myeloma, despite concomitant profound toxicity. Several possible mechanisms of action of MeGAG have been discussed in a recent review by Mihich (113).

Physiological disposition.—The results of pharmacological studies of MeGAG-C^{14} in normal and tumor-bearing animals have been published (114, 115). Current studies in man with MeGAG-C^{14} have shown that following a single intravenous infusion (20 min), the radioactivity rapidly disappeared from the plasma and approximately 60 percent of the dose was excreted unchanged in the urine over an extended period of 3 wk (88). Stool samples obtained during this period contained less than 20 percent of the dose radioactivity. In contrast, following a single oral dose, only 10 percent of the radioactivity was recovered in the urine while 68 percent was present in stools. The poor absorption of the drug from the gastrointestinal tract probably accounts for the clinical ineffectiveness of the drug when given by the oral route (116). No radioactivity was detectable in expired carbon dioxide. This parallels the results of animal studies (114) and supports the observation that MeGAG is not measurably degraded in man or animals.

Toxicity and dosage.—The toxicological effects of MeGAG in several animal species have been described by Mihich et al. (117) and Tidball & Rall (118). The side effects observed, some of which are peculiar to certain species, included gastrointestinal toxicity, delayed and fatal hypoglycemia, hepatic and renal damage, bone marrow depression, and pneumonia. These effects, with the exception of hepatic and renal tubular damage, have also prevailed in humans undergoing MeGAG treatment. Certain additional toxic effects, however, have been unique to man. These include esophagitis, ulcerative pharyngitis, laryngitis, stomatitis, genital mucosa swelling, conjunctivitis, erythemia, edema, desquamating dermatitis, and pain of the palms, soles, and abdomen (108, 110–112, 116). The development of leukopenia and thrombocytopenia, which are seen mostly at high dose levels, have not been dose-limiting toxic effects in most patients (110, 112).

MeGAG is usually administered as the acetate or hydrochloride salt by intravenous infusion (0.5 to 1 hr) in 5 percent glucose water. The most effective dose level for acute myclocytic leukemia patients has been approximately 150 mg per m^2 of body surface daily, until onset of toxicity or definite therapeutic effect (108, 111, 112). The effective dose range, however, is quite

narrow with slight alterations markedly affecting the toxic or therapeutic response. The initial toxicity usually elicited is associated with gastrointestinal effects (nausea, vomiting, diarrhea). The median duration of remissions has been 4 mo. while the complete remission rate (45 percent) and median increase in survival from 2.5 to 6.5 months are encouraging when compared to acute myclocytic leukemia patients treated with 6-MP (111). Maintenance of remissions has not been practicable with MeGAG, since it is ineffective orally and must be administered parenterally. Patients are usually maintained on 6-MP or occasionally receive twice-weekly intramuscular injections of MeGAG (111).

Discussion.—Clinical experience has shown MeGAG to be unique in its chemotherapeutic management of acute myelocytic leukemia, but as Mihich (113) has so aptly observed, "the enthusiasm elicited by the activity of the drug . . . is somewhat tempered by the frequent occurrence of dramatic toxicity." Although lack of cumulative toxicity has been observed in animals which received repeated parenteral doses of MeGAG (113), toxicological effects observed clinically have been attributed to cumulative effects of repeated daily doses (111). The latter is possibly explained by the unusually protracted urinary excretion of MeGAG in man (88). On this basis, attempts were made to modify the course of toxicity in humans by initial treatment

TABLE IV

CLINICAL USEFULNESS OF MISCELLANEOUS AGENTS

Drug	Disease	Route	Dose	Pharmacology (References)
Actinomycin D	Wilm's tumor Hodgkin's disease Choriocarcinoma Embryonal rhabdomyosarcoma	I.V.	15 μg per day	(119)
Demecolcin (Colcemide)	Chronic granulocytic leukemia	Oral	3–10 mg per day	(120)
MIH (N-isopropyl-α-(2-methylhydrazino)-*p*-toluamide)	Hodgkin's disease	Oral I.V.	200–500 mg per day	(121–124)
Op'-DDD (2,2-*bis*(4-cholorophenyl, 2,chlorophenyl)-1,1-dichloroethane)	Adrenal cortical carcinoma	Oral	7–10 gm per day	(125)
Vinblastine (VLB, Velban)	Hodgkin's disease Lymphosarcoma	I.V.	0.01–0.10 mg per kg per week	(126–128)
Vincristine (VCR, Oncovin)	Acute lymphocytic leukemia Hodgkin's disease Lymphosarcoma Wilm's tumor Carcinoma of breast and ovary	I.V.	0.01–0.10 mg per kg per week	(126, 127, 129)

with a "high loading dose" followed by lower-than-usual maintenance doses (111). Unfortunately, the results were unsuccessful and this regimen was discontinued. However, because of the very few cases evaluated, further exploration of a proper loading dose is warranted before discarding this approach to broadening the therapeutic index of MeGAG in man.

Miscellaneous Agents

Several active drugs have not been covered in detail because there has been only fragmentary data on their pharmacological disposition. These agents are listed in Table IV, together with the diseases in which they have been most effective and pertinent references to their pharmacology, when available. Another active group of antitumor agents, the steroid hormones, has not been included in this review because of limitations of space.

Concluding Remarks

The reviewers have attempted to summarize some of the more recent advances in the knowledge of the fate in man of a number of clinically useful cancer chemotherapeutic agents. It is evident that the type of biological response elicited by an antitumor agent is governed not only by its intrinsic activity, but also by factors of (*a*) drug penetration or uptake into body fluids, (*b*) tissue localization, (*c*) anabolic or catabolic alterations, and (*d*) rate of elimination from the site of reaction. The study of these factors, together with observed toxicological effects and their relationship to dose levels and routes of administration, provides the clinician with useful information for the intelligent and effective use of these agents in man.

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