Cancer Chemother Pharmacol (1987) 19: 91–102

Cancer Chemotherapy and Pharmacology

Review

N-Methyl antitumour agents

A distinct class of anticancer drugs?

David Newell[1], Andreas Gescher[2], Stephen Harland[1], David Ross[3], and Christine Rutty[1]

[1] Department of Biochemical Pharmacology, Institute of Cancer Research, Belmont, Sutton, Surrey, United Kingdom
[2] Pharmaceutical Sciences Institute, Aston University, Birmingham, United Kingdom
[3] Department of Pharmaceutical Chemistry, School of Pharmacy, University of Colorado, Boulder, Colorado, USA

Summary. This article reviews the structure-activity characteristics, mode of action, pharmacokinetics and clinical utility of a group of chemically dissimilar antitumour agents which have as a common structural feature the *N*-methyl moiety. The importance of this feature is shown by the fact that molecules without a substituent on the nitrogen or compounds with *N*-alkyl groups other than methyl are usually inactive in experimental systems. This observation is supported by structure-activity studies with *N*-alkyl derivatives of *s*-triazines, triazenes, formamides, hydrazines and nitrosoureas. Representatives of these structural types which have found clinical application are, respectively, hexamethylmelamine, dacarbazine, *N*-methylformamide, procarbazine and streptozotocin. Mode of action studies have shown that dacarbazine, procarbazine and streptozotocin can give rise to species capable of methylating nucleic acid. This may be the lesion which produces antitumour activity. The mechanism of action of *N*-methylmelamines and *N*-methylformamide remains unclear. There is good evidence that, with the exception of *N*-methylnitrosoureas, host metabolism is prerequisite for activity with these agents.

Although not pronounced, the clinical activity of *N*-methyl antitumour agents is useful, particularly as activity is not associated with severe haematological toxicity. Furthermore, responses may be observed in patients resistant to bifunctional alkylating agents. It is concluded that the drugs reviewed herein show a degree of coincidence in terms of their biological properties which may warrant a common classification. The term *N*-methyl antitumour agent is proposed.

Introduction

The subdivision of anticancer drugs into various classes of agents forms the basis for the ordered study of cancer chemotherapy. Of the various classes, alkylating agents comprise the first used and probably the most widely studied type of compound. In early work it was recognised that the biological activity of alkylating agents was markedly increased by the presence of two or more alkylating functions in the molecule [131]. A range of polyfunctional compounds have subsequently found widespread clinical use, namely, nitrogen mustards, aziridines, methane sulphonates and epoxides. More recently, it has been shown that the apparently monofunctional chloroethylnitrosoureas are indirectly bifunctional, being capable of secondary alkylation following the initial reaction of the chloroethyl carbonium ion [88]. However, there remains a group of compounds, sometimes referred to as non-classical alkylating agents [176], where there is no evidence for bifunctionality either in their structure or in their mechanism of action.

Despite the disparate range of chemical structures which can be placed in this grouping, i.e. *s*-triazines, triazenes, formamides, hydrazines and methylnitrosoureas, a feature common to the more potent representatives of these structural types is the *N*-methyl moiety. Thus, clinically used examples of these groups are, respectively, hexamethylmelamine, dacarbazine, *N*-methylformamide, procarbazine and streptozotocin. The purpose of this paper is to review the structure-activity relationships, mode of action, pharmacokinetics and clinical properties of the antitumour agents which posses the *N*-methyl moiety. On the basis of these data conclusions are drawn as to the similarities, or otherwise, between compounds, particularly with regard to the importance of the *N*-methyl group for activity and its relevance to the mechanism of action.

Structure-activity studies, metabolism, pharmacokinetics and mechanism of action

Methylmelamines

Hexamethylmelamine (HMM; Fig. 1, I) is an *s*-triazine derivative which has shown limited activity against a number of experimental rodent tumours [66, 134]. The antitumour activity of HMM is inherently associated with the presence of *N*-methyl moieties; substitution with other alkyl groups, such as ethyl, or with hydrogen leads to compounds which are devoid of antitumour activity [94, 134]. Furthermore, activity is related not only to the absolute presence of *N*-methyl groups, but also to the extent to which the compounds undergo oxidative *N*-demethylation [134]. Thus, within the homologous series, mono-, di- and trimethylmelamine (Fig. 1, V) are poorly demethylated in vitro and devoid of antitumour activity in vivo [134]. In contrast, tetramethylmelamine (Fig. 1, IV) and in particular pentamethylmelamine (PMM; Fig. 1, III) are well demethylated in vitro and active in vivo [134]. This latter observation, coupled with the greater aqueous solubility of PMM, led to

Offprint requests to: David Newell

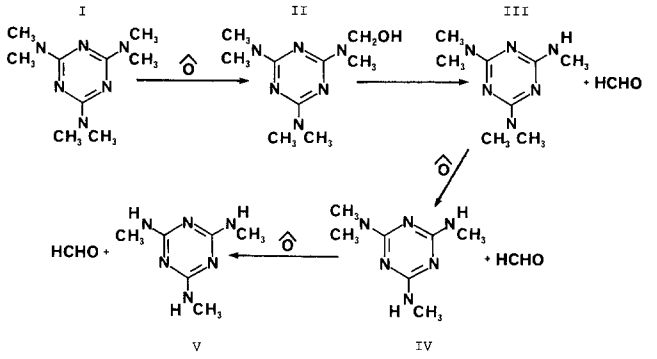


Fig. 1. Methylmelamine metabolism: *I*, hexamethylmelamine; *II*, *N*-hydroxymethylpentamethylmelamine; *III*, pentamethylmelamine; *IV*, tetramethylmelamine; *V*, trimethylmelamine

the selection of PMM as an alternative to HMM suitable for intravenous use (see below).

HMM and PMM are rapidly and extensively metabolised in mice, rats and humans. Thus, the majority of the drug administered is excreted in the urine in the form of demethylated metabolites, i.e. N^2, N^4, N^6-trimethylmelamine (Fig. 1, V), N^2,N^4-dimethylmelamine, monomethylmelamine and melamine [182, 183], with very little parent drug being present [4, 25, 135]. HMM is metabolised in vitro, in the presence of various hepatic and intestinal preparations, via oxidative *N*-demethylation which yields PMM after the decomposition of an unstable carbinolamine intermediate (Fig. 1) [18, 41, 59, 134]. The intermediate produced during the *N*-demethylation of HMM to PMM, *N*-hydroxymethyl PMM (Fig. 1, II), has been identified as a major in vitro metabolite [59]. Although the formation of this same metabolite in vivo could not be demonstrated, Rutty et al. [135, 137] have provided indirect evidence for the formation of formaldehyde-precursor metabolites from HMM and PMM in vivo. More recently, Borm et al. [18] have shown that glutathione may be involved in the metabolism of HMM. If, as suggested by these authors, *N*-hydroxymethyl PMM is subject to glutathione conjugation, this may explain the failure to detect *N*-hydroxymethyl PMM in vivo.

In patients, D'Incalci et al. [36] have reported considerable variability in plasma concentration and half-life for HMM following oral administration. This does not appear to be due to poor absorption of the drug, as very little drug-derived material is excreted in the faeces [183], the majority being eliminated in the urine [4, 183]. Studies in rabbits [4] and rats [87] have confirmed that there is extensive first-pass metabolism, and hence probably activation.

Despite the fact that HMM has been used clinically for some 20 years, the mechanism by which it exerts its antitumour action remains unknown. Although *N*-methylmelamines do not have direct alkylating activity, it has been suggested that intermediate metabolites, i.e. *N*-hydroxymethylmelamines or formaldehyde, may possess such activity [94, 134, 182]. Rutty and Connors [134] have shown that whilst HMM requires metabolism in order to exert a significant cytotoxic effect in vitro, *N*-hydroxymethylmelamines are highly cytotoxic per se. *N*-Hydroxymethylmelamines are also active antitumour agents in vivo [33, 66, 71, 94, 134]. Rutty and Connors concluded, therefore, that HMM and PMM exert their antitumour effect via the formation of *N*-hydroxymethyl metabolites [134]. Furthermore, in tumour cell lines which are sensitive to these agents in vivo, the observed cytotoxicity appears to be due to the *N*-hydroxymethylmelamine itself and not to the formaldehyde it releases on chemical decomposition [133]. Although there is evidence that HMM is itself weakly cytotoxic in vitro [37, 133], the concentrations and duration of exposure necessary to produce this effect are greatly in excess of those seen in vivo in man [36, 38, 39] and in experimental animals [4, 21, 87].

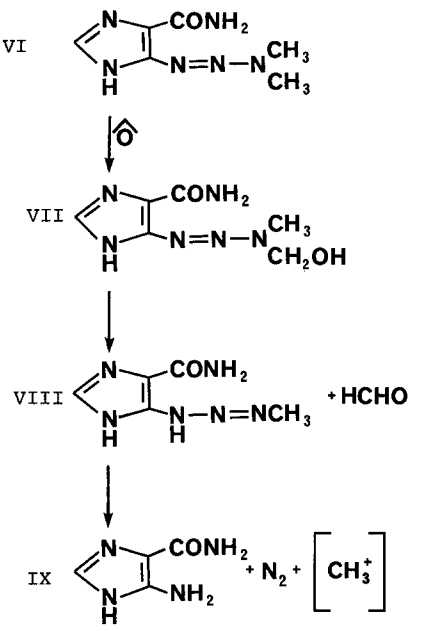


Fig. 2. Dacarbazine metabolism: *VI*, dacarbazine; *VII*, 5-(3-hydroxymethyl-3-methyltriazen-1-yl)imidazole-4-carboxamide; *VIII*, 5-(3-methyltriazen-2-yl)imidazole-4-carboxamide; *IX*, 5-aminoimidazole-4-carboxamide

HMM and PMM have been shown to inhibit both DNA and RNA synthesis in vitro [37, 136] and in vivo [111]; however, *N*-hydroxymethylmelamines are more potent and inhibit nucleic acid synthesis irreversibly [136]. Studies with ring-^{14}C HMM and PMM in vitro and in vivo suggest that following activation, HMM and PMM can bind to DNA [5, 6, 57, 111]. However, only limited DNA-protein cross-linking occurs either with activated HMM and PMM or with *N*-hydroxymethyl PMM in L1210 cells [132]. Furthermore, in the sensitive PC6 cell line no significant DNA cross-linking could be demonstrated [114]. Thus it seems unlikely that DNA cross-linking plays a major role in the molecular mechanism of action of the *N*-methylmelamines.

Dacarbazine

After 10 years of clinical experience with and experimental work on dacarbazine (DTIC; Fig. 2, VI), its mode of antineoplastic activity remains an enigma. It was synthesised in 1961 as a prodrug of 5-diazoimidazole-4-carboxamide [151], an agent which had been shown to possess antitumour activity [149, 150]. Most studies on the biological properties and structure-antitumour activity relationships of 1-aryl-3,3-dialkyltriazenes have been conducted with the 1-phenyl-substituted analogues of dacarbazine [7, 32, 60], whose chemical properties and spectrum of antineoplastic activity against rodent malignancies resemble those

of dacarbazine. In one of the first reports on the antineoplastic activity of 1-aryl-3,3-dialkyltriazenes, against the murine sarcoma 180, the structural requirement of N^3-methyl substitution in the triazene molecule was demonstrated [29]. More recently, in an investigation of the activity of 35 different 1-aryl-3,3-dialkyltriazenes against the mouse TLX/5 lymphoma, Connors et al. [32] deduced that only those aryldialkyltriazenes that can be metabolised in vivo to an arylmonomethyltriazene possess antitumour properties. However, the diethyl analogue of dacarbazine and several related compounds with alkyl moieties other than methyl have shown marginal activity against the L1210 leukaemia [70].

In addition to their activity against primary tumours, it has been shown by Giraldi, Sava and co-workers that certain 1-aryl-3,3-dimethyltriazenes can inhibit the metastatic spread of tumours in mice [63, 64, 140–142], and dacarbazine also appears to possess this property [30].

There is now convincing evidence that 1-aryl-3,3-dimethyltriazenes require metabolic activation by the host for antitumour activity. Most of the results corroborating this contention have been obtained in studies on phenyl dimethyltriazenes rather than on dacarbazine [7, 32, 60]. These triazenes undergo oxidative *N*-demethylation catalysed by cytochrome P450 to form 1-aryl-3-monomethyltriazenes, direct methylating agents. For dacarbazine this metabolic route is outlined in Fig. 2. Aminoimidazole carboxamide (AIC; Fig. 2, IX) is the major urinary metabolite of dacarbazine in man [19, 75, 76, 158, 159] and is formed from dacarbazine in mouse liver microsomes [73] and human and animal tumour tissue [58, 107]. It is difficult to envisage from the mechanistic point of view how AIC could be formed by a metabolic pathway other than one implicating 5-(3-methyl-triazen-2-yl)imidazole-4-carboxamide (MTIC; Fig. 3, VIII). Recently, Kolar et al. [90] identified 5-(3-hydroxymethyl-3-methyltriazen-1-yl)-imidazole-4-carboxamide (HMTIC; Fig. 2, VII) as a urinary metabolite of dacarbazine in rats. As this compound appeared to be more stable than MTIC in polar solvents, the authors suggested that HMTIC may act as a transport form of MTIC, the postulated ultimate antineoplastic species derived from dacarbazine. An alternative transport form of MTIC might be the hydroxymethyltriazene glucuronide of HMTIC, given the observation that the hydroxymethyltriazene glucuronide is a metabolite of 1(2,4,6-trichlorophenyl)-3,3-dimethyltriazene in rats [89].

MTIC decomposes spontaneously in aqueous media to form AIC, with the concomitant alkylation of nucleophiles [118], e.g. DNA [106]. Recently, however, evidence has been presented which casts doubt on the contention that an indiscriminantly reactive species such as MTIC can be responsible for the selective antitumour activity of dacarbazine [60, 70, 72].

The most extensive study of dacarbazine pharmacokinetics in man reported to date has shown that AIC and the parent compound can be readily detected in both plasma and urine [19], confirming earlier studies which showed that renal excretion is a major route of elimination [76, 100, 157–159]. Studies currently underway have shown that both HMTIC and MTIC are dacarbazine metabolites in mice, rats and man, although differences apparently exist between species in terms of the formation and persistence of these metabolites [138]. Finally, the observation that 7-methylguanine excretion is enhanced in patients and rats treated with dacarbazine confirms that this agent can give rise to methylating species in vivo [160].

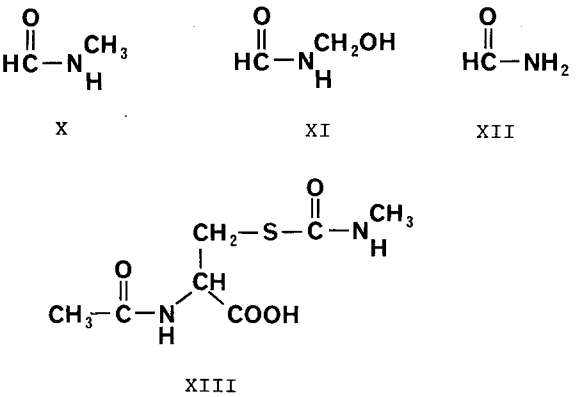


Fig. 3. *N*-methylformamide and its metabolites: *X*, *N*-methylformamide; *XI*, *N*-hydroxymethylformamide; *XII*, formamide; *XIII*, *S*-(*N*-methylcarbamoyl)*N*-acetylcysteine

N-Methylformamide

The antitumour activity of *N*-methylformamide (NMF; Fig. 3, X) against murine tumours was first described in the early 1950s. In studies of the antitumour efficacy of a series of formamide derivatives against the S180 sarcoma [28] and Ehrlich ascites tumour [55], NMF was found to be the most potent inhibitor of tumour growth. This result has been confirmed recently using two other murine tumour models, although formamide (Fig. 3, XII), did display marginal antitumour activity in these systems [61]. Interestingly, *N,N*-dimethylformamide, like *N*-ethylformamide, is only slightly active or inactive in most rodent antitumour tests [95], although both DMF and NMF similarly inhibited the growth of two human colon tumours grown in immune-deprived mice [45].

The mechanism by which NMF exerts its antitumour activity is under discussion. The work of Clarke et al. [28] suggests that NMF is not a folic acid antagonist. Further studies indicated that NMF may interfere with nucleic acid synthesis [28, 48, 113, 139, 161, 178], although it appeared to stimulate the incorporation of formate into nucleic acids in the liver [9], an organ to which it is toxic [117]. A rodent tumour which had acquired esistance to aryldimethyltriazenes and procarbazine in vivo was not resistant to NMF [61] and hence NMF apparently has a different mechanism of action. NMF and related polar solvents can induce terminal differentiation to a more mature phenotype in certain human leukaemia cell lines, and the suggestion has been tendered that this is related to their antineoplastic activity in mice [164].

Whether NMF requires bioactivation in order to exert an antineoplastic effect is difficult to establish, as it is not metabolised to an appreciable extent in vitro by liver preparations [61]. However, the fact that *N*-ethylformamide, although devoid of any in vivo antitumour activity, exhibited toxicity in vitro comparable to that of NMF [61] suggests that metabolism may be a determinant of the selective in vivo antitumour activity of NMF. Evidence that NMF may be bioactivated to reactive metabolites in vivo has emerged from studies of its effect on hepatic non-protein thiols. Of a series of formamide derivatives, only NMF caused a reduction in hepatic non-protein thiols, an

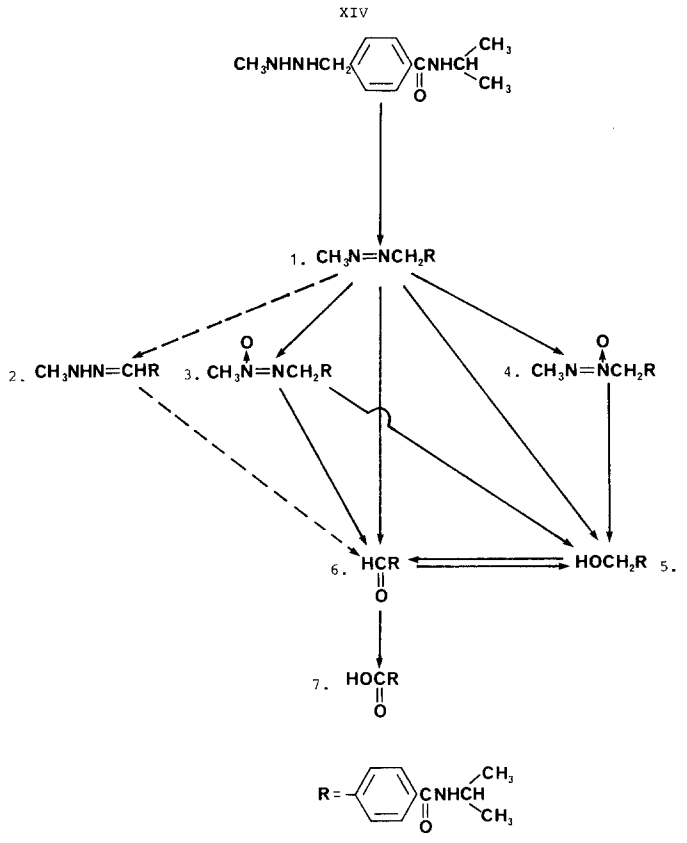


Fig. 4. Metabolism (——) and chemical decomposition (------) of procarbazine (XIV): *1.* azoprocarbazine; *2. N*-isopropyl-*p*-formylbenzamide methylhydrazone; *3.* methylazoxyprocarbazine; *4.* benzylazoxyprocarbazine; *5. N*-isopropyl-*p*-hydroxymethylbenzamide; *6. N*-isopropyl-*p*-formylbenzamide; *7. N*-isopropyl-terephthalamic acid

effect which could be partially abolished by pretreatment of animals with SKF-525A [61]. This finding implicates the formation of a reactive – presumably electrophilic – metabolite in vivo.

Metabolism studies in mice have shown that NMF is biotransformed to CO_2, which is exhaled in the breath, and to methylamine, *N*-hydroxymethylformamide (HMF; Fig. 3, XI), formamide (Fig. 3, XII) and a mercapturic acid conjugate, all of which are excreted in the urine [85]. The latter metabolite has subsequently been identified as *S*-(*N*-methylcarbamoyl)*N*-acetylcysteine (Fig. 3, XIII) and was also found in the urine of patients treated with NMF [86]. Although HMF is unlikely to contribute to the antitumour activity of NMF [34], the as yet unknown precursor of the mercapturic acid may be a cytotoxic species.

After the administration of NMF or $^{14}CH_3$-NMF to mice the plasma concentration vs time profiles did not fit a linear pharmacokinetic model, and low levels of radioactivity were measurable in the plasma for up to 8 days [20, 22].

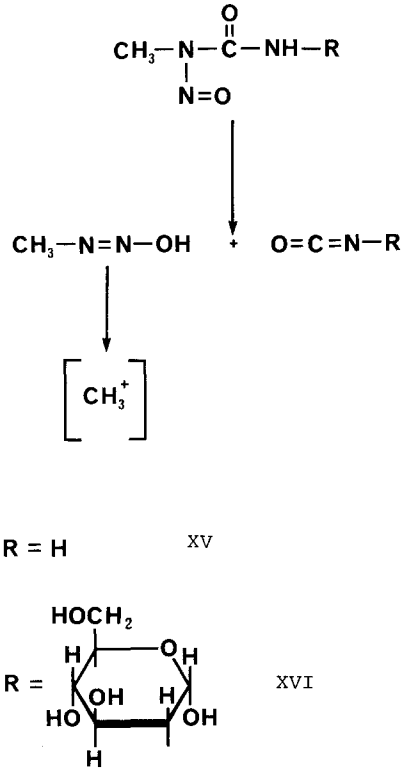
Procarbazine

Procarbazine (Fig. 4, XIV) was originally synthesised in the laboratories of Hoffman-La Roche following the observation that 1-methyl-2-benzylhydrazine displayed antitumour activity in experimental systems [13, 184]. Although the data has never been presented, it has been stated that only 1-methylhydrazines possess antitumour activity, ethyl and unsubstituted hydrazines being inactive [13, 91]. Early workers showed that procarbazine is not cytotoxic to cultured cells [69], and this in turn stimulated metabolism studies in an attempt to identify an active metabolite. As these studies have progressed, it has become apparent that the metabolism and chemical decomposition of procarbazine are particularly complicated, with some 15 different metabolites or intermediates being implicated to date [35, 153].

The chemical decomposition of procarbazine involves oxidation to azoprocarbazine and subsequent isomerisation of the double bond to form the methylhydrazone which hydrolyses to yield *N*-isopropyl-*p*-formylbenzamide (Fig. 4) [67, 175]. Although it was originally proposed that this pathway was also the major route of procarbazine metabolism [130], more recent data has shown that the methylhydrazone is not a major product of procarbazine metabolism and that azoprocarbazine is relatively stable under physiological conditions [8, 93, 110, 175]. More importantly, the in vitro oxidation of procarbazine to azoprocarbazine can be catalysed by various liver preparations [8, 93, 110, 175], a process which is probably dependent on cytochrome P450 [47]. The subsequent metabolism of azoprocarbazine can apparently follow four routes, namely, direct conversion to *N*-isopropyl-*p*-formylbenzamide or *N*-isopropyl-*p*-hydroxymethylbenzamide [35] and *N*-oxidation to produce two azoxyprocarbazine isomers (Fig. 4) [175, 179]. In vitro the formation of the methylazoxy isomer is quantitatively the most important reaction [35, 179], and in rat and human plasma this compound is the major primary azoprocarbazine metabolite [153]. Both of the azoxy metabolites can be converted in vitro by hepatic preparations to *N*-isopropyl-*p*-hydroxymethylbenzamide or, in the case of the methylazoxy isomer, the corresponding aldehyde (Fig. 4) [35, 175]. All of the above reactions are probably mediated by cytochrome P450 [35, 179] and, for the *N*-oxidation of azoprocarbazine, the isoenzymes responsible have been identified [128]. Finally, *N*-isopropyl-*p*-formylbenzamide and *N*-isopropyl-*p*-hydroxymethylbenzamide are oxidised to *N*-isopropyl-terephthalamic acid (Fig. 4) the major urinary metabolite [122, 130]. Urinary excretion in man, dogs and rats is extensive, with up to 75% of the dose excreted in the urine within 24 h [143].

Recently, it has been shown that azoprocarbazine metabolism in vitro is associated with methyl-radical-derived methane generation [129]. The methyl radical is responsible, in part, for the methylation of microsomal proteins during in vitro azoprocarbazine metabolism. In addition, electron spin resonance studies have directly demonstrated the existence of carbon-centred free radicals during procarbazine metabolism [156]. These reactive species may therefore be involved in mediating procarbazine antitumour activity; however, the exact nature of the metabolite responsible is currently unknown. In addition to procarbazine, azoprocarbazine and a mixture of the two azoxy isomers posses in vivo antitumour activity [14, 152], an observation which is consistent with the hypothesis that antitumour activity is related to the presence of an intact *N*-methyl moiety.

A number of studies using methyl-radiolabelled procarbazine have confirmed that extensive metabolism of the methyl group occurs in vivo. Thus radioactivity is expired



R = H XV

Fig. 5. The chemical decomposition of methylnitrosoureas: *XV*, methylnitrosourea; *XVI*, streptozotocin

by rats in the form of CO_2 and methane [46, 143]. In addition to elimination, there is incorporation of radioactivity into the DNA and RNA of tumour cells from mice treated with procarbazine [24, 91]. Two major routes of incorporation into nucleic acid have been identified; direct methylation of bases, involving complete transfer of the intact *N*-methyl group, and incorporation during de novo purine biosynthesis via labelled formate [91]. Although the most thorough study has involved RNA [91], DNA methylation has also been shown [24].

N-Methylnitrosoureas

Apart from the well-known *N*-chloroethylnitrosoureas (BCNU, CCNU, MeCCNU, etc.) two *N*-methylnitrosoureas have also received clinical evaluation, i.e. *N*-methylnitrosourea (MNU Fig. 5, XV) and streptozotocin (Fig. 5, XVI). Although MNU is known to western science primarily as an experimental carcinogen [78] it has been used in the USSR as an antitumour agent (see below). Streptozotocin is a fermentation product which was initially developed as an antibiotic [171]. Following the demonstration of activity in experimental systems [51], it was hoped that, because of its low bone marrow toxicity [126], streptozotocin would have greater utility than *N*-chloroethylnitrosoureas. Unfortunately, clinical trials have shown that the activity of streptozotocin is limited to certain rare tumour types (see below).

Early structure-activity studies demonstrated that nitrosourea antitumour activity resides only in the *N*-methyl and *N*-chloroethyl analogues [82]. This is in marked contrast to the carcinogenic activity of nitrosoureas, where there is an extensive literature documenting the activity of, for example, *N*-ethyl- [77] and *N*-propylnitrosoureas [168], as well as MNU [78].

No definite evidence for the metabolic activation of either MNU or streptozotocin has been reported. However, both compounds are known to undergo spontaneous chemical decomposition to yield alkylating species [126]. The decomposition pathway for MNU and streptozotocin, shown in Fig. 5, leads to the release of a methylating species which may be the ultimate cytotoxic species.

Studies on the mechanism of action of MNU have focussed mainly on the DNA lesion responsible for carcinogenesis [96]. A number of methylated bases have been isolated from nucleic acid extracts of organs from animals treated with MNU. Quantitatively, the most important methylated base is N^7-methylguanine, although evidence from a number of studies suggests that O^6-methylation of guanine is the lesion which leads to carcinogenesis via anomalous base pairing [78, 96].

More recent studies on the mechanism of action of methylating agents have focussed on the importance of DNA repair enzymes as determinants of cell sensitivity. For example, the enzyme responsible for the repair of O^6-methylguanine (O^6-methylguanine-DNA methyltransferase) is absent in cells which are particularly sensitive to the cytotoxic effects of methylating agents [154]. However, it does not necessarily follow that O^6-methylguanine is the sole cytotoxic lesion, since the extent of O^6-guanine methylation does not always correlate with cell survival [119].

N-Methylnitrosourea-induced changes in poly (ADP-ribose) polymerase, a second DNA repair enzyme, may be an additional determinant of activity in certain human tumours. Treatment of cultured cells with MNU or streptozotocin stimulates poly (ADP-ribose) polymerase, resulting in an increased utilisation, and thereby depletion, of the substrate nicotinamide adenine dinucleotide (NAD) [162]. NAD depletion is thought to be the reason why *N*-methylnitrosoureas induce diabetes in experimental animals, as pretreatment with nicotinamide prevents the onset of diabetes [144]. In experimental systems nicotinamide pretreatment does not impair the antileukaemic activity of streptozotocin, and hence, in this situation, the mechanism of action is probably alkylation alone [144]. Clinically, however, the activity of streptozotocin against islet cell carcinomas may be due in part to the diabetogenic properties of the molecule (see below).

Clinical utility

Methylmelamines

Due to its poor aqueous solubility HMM is given as an oral preparation, usually as a daily dose over several weeks. Whereas it can cause leukopaenia and thrombocytopaenia, these effects are usually mild, and the common limiting toxicity is patient intolerance due to cumulative nausea and vomiting. The other important side effects of HMM are neurological [101, 181] and may be ameliorated by pyridoxine [52].

HMM has a wide spectrum of antitumour activity, responses being seen in breast, ovarian, lung and cervical carcinomas [11, 97], lymphomas [124] and sarcomas [17]. Unfortunately, its activity is poor in patients already treated with standard drugs. Thus, of 104 patients with breast cancer resistant to conventional therapy only two responded [52, 98]. In ovarian cancer the response rate is

30%–40% in patients not previously treated [97, 177], but in disease resistant to nitrogen mustard alkylating agents it is modest: 15% [12], 16% [123], 27% [16] and 29% [81], giving an overall response rate of 19%. However, Vogl et al. [173] found a response rate of 54% to an HMM-cisplatin combination in melphalan-resistant disease. Ovarian carcinoma is the only disease where HMM is used with any frequency.

PMM, being water-soluble can be given intravenously, thereby circumventing variation in bioavailability (see above). Furthermore, it was hoped that the drug-induced nausea would be less than that seen with HMM. In fact it is considerably worse, and is the limiting toxicity. It has been difficult to produce any myelotoxicity with this drug. In a number of phase I studies, nearly 200 patients have been treated, most with unassessable disease. Only eight minor responses and one partial response have been seen [3, 26, 65, 80, 115, 170]. In a comparative trial of PMM and HMM for the treatment of bilharzial bladder cancer, the two agents were similar in terms of toxicity, response and survival [56].

Dacarbazine

Dacarbazine has unquestioned activity in Hodgkin's disease, sarcomas and melanoma. However, its most notable side effect, nausea and vomiting, has made it an unpopular drug. Dacarbazine has been used most extensively in malignant melanoma, reviewed by Comis [31]. As a single agent it consistently produces remissions in the range of 16%–31%. Its superiority over other agents in this disease is not established, however [1, 2].

In a single-agent study [54] of pretreated patients with Hodgkin's disease, 10 of 18 patients responded to dacarbazine. Hence it is now used frequently as second-line therapy in this disease in the ABVD regimen [15]. In non-Hodgkin's lymphomas, remissions are fewer and shorter [54].

A number of studies have shown the activity of dacarbazine against a variety of sarcomas [53, 102, 172, 174]. Gottlieb et al. [68] reviewed the sequential M. D. Anderson and Southwest Oncology Group studies. The addition of dacarbazine to adriamycin apparently increased the response rate for sarcomas from 31% to 42%. The survival of the patients treated in the combination studies was significantly longer than that of those in the single-agent adriamycin study.

Nearly all patients receiving dacarbazine experience nausea and vomiting [102]. When given on a daily $\times 5$ schedule it is frequently observed that these effects are worst on the first day. Leukopaenia is variable and usually less severe than thrombocytopaenia.

Dacarbazine is always given intravenously, although it has been given by mouth (100, 157] in daily doses sufficient to produce myelosuppression. Variability in absorption [100, 157] caused the intravenous route to be preferred.

N-Methylformamide

A number of clinical trials with *N*-methylformamide have been described [50, 105, 121, 167, 180]. Mild nausea is seen at higher doses, which commonly also cause elevations of liver enzyme and bilirubin levels. Hepatotoxicity or geneval malaise are dose-limiting; little activity has been seen.

Procarbazine

The clinical use of procarbazine was reviewed in 1974 by Spivack [163]. No new role has been found for the drug since then.

Single-agent activity in Hodgkin's disease was found early on [104] in both previously untreated disease and disease resistant to other agents [40, 74, 169]. The inclusion of procarbazine in four-drug combinations with a nitrogen mustard, vinca alkaloid and prednisone has proved highly successful in the management of advanced Hodgkin's disease, producing complete remission rates of over 80% for previously untreated patients [43, 84, 120]. Interestingly, the combination of cyclophosphamide, vincristine and prednisone, omiting procarbazine, gave an equivalent figure of only 31% [103].

Similarly, single-agent activity against non-Hodgkin's lymphomas [99, 169] has led to procarbazine's inclusion in a successful four-drug regimen (cyclophosphamide, vincristine, prednisone and procarbazine) [44]. Good, possibly better results have been obtained, however, with several drug combinations which exclude procarbazine [10, 147].

The use of procarbazine in lung cancer remains controversial. An early report [148] of a 67% response rate in a small number of previously untreated patients with small cell histology has led to its inclusion in a number of drug combinations for this disease. Its efficacy has recently been questioned, however, by Gersel Pedersen et al. [62], who observed no responses in a phase II single-agent study in 24 patients with disease resistant to other agents.

Although procarbazine can produce shrinkage of non-small cell lung carcinomas in about 18% of cases, the remission is brief in duration [99] and confers no survival benefit [125].

Procarbazine is usually given as a daily oral dose. Mild nausea and vomiting are frequently seen initially, but tend to subside. Myelotoxicity is dose-limiting. Peripheral neuropathy and effects on the central nervous system are occasionally seen. Flushing after alcohol is a fairly common interaction. As procarbazine is a weak monoamine oxidase inhibitor [42], the usual exaggerated pressor responses to sympathomimetics and tyramine-containing foods occur.

The intravenous route of administration has not been directly compared with the oral route. Those who have used low repeated doses i.v. have found comparable myelosuppression and equivalent efficacy [104, 112, 155]. Following intermittent high intravenous doses, central nervous system effects are dose-limiting, with prolonged somnolence and disorientation followed by restlessness and emotional lability in the recovery period. Nausea and vomiting may be severe, and peripheral neuropathy is also more pronounced [27].

N-Methylnitrosoureas

Streptozotocin has been used with some success in islet cell tumours of the pancreas [23, 116]. Whether its efficacy is related to the diabetogenic effect seen in some animal species [92, 144] is impossible to say, since there are no reports of other nitrosoureas used against this tumour. The improvement in the response rate seen when 5-fluorouracil is added to streptozotocin [108] suggests that islet cell tumours may be relatively chemosensitive. Activity against carcinoid tumours is less impressive [109, 145, 166]. Re-

sponses of short duration have been seen in Hodgkin's disease resistant to conventional chemotherapy. In one instance tumour shrinkage was seen in a patient resistant to BCNU [146].

The limiting toxicity of streptozotocin is renal, very little myelosuppression being seen at the doses used.

MNU has been used clinically in the USSR [49]. Good responses were reported in undifferentiated lung cancer and previously treated Hodgkin's disease. Almost all patients experienced severe nausea and vomiting, often accompanied by diarrhoea, within 40 min of receiving the drug. Myelotoxocity was also seen.

Conclusions and future developments

From the extensive literature concerning the clinical and experimental properties of the antitumour agents reviewed herein it is possible to draw several conclusions. Structure-activity studies have shown that in the majority of in vivo rodent antitumour screens, only compounds with an *N*-methyl moiety display significant activity. Obvious exceptions to this generalisation are the *N*-chloroethylnitrosoureas, the *N*-chloroethyltriazenes and the aziridinyl melamine triethylenemelamine. These agents are, however, distinct from their *N*-methyl analogues by virtue of their potential for bifunctional alkylation. Although inadequate absorption, metabolism or distribution may be invoked to explain the inactivity of compounds with *N*-alkyl groups other than methyl, e.g. hexaethylmelamine [21], it seems unlikely that this is sufficient as a general explanation. For example, *N*-ethylnitrosourea has potent in vivo biological activity in terms of carcinogenicity [77] yet is ineffective against an MNU-sensitive tumour [82]. Similarly, *N,N*-diethyltriazenes are carcinogenic [127] yet induce little or no tumour inhibition [29, 32, 70]. Thus, on balance, it appears that the *N*-methyl group is an important determinant of antitumour activity.

In marked contrast to the stringent requirement for activity at the *N*-alkyl position are the wide range of nitrogen-containing structures which can bear the *N*-methyl group, i.e. triazines, triazenes, formamides, hydrazines and nitrosoureas. Although it might be suggested that the requirement for an *N*-methyl moiety in these various classes of molecules implies a common mechanism of action, caution should be exercised. Whilst it is true that *N*-methyltriazenes, *N*-methylnitrosoureas and procarbazine can give rise to methylating species, there is no evidence that methylmelamines or NMF can do so. If the former compounds do in fact act by methylation of nucleic acid, then for methylmelamines and NMF the structural requirement for a methyl group is apparently coincidental. Therefore, on the basis of the available data, a common mechanism of action for all of the *N*-methyl drugs is not implicated, a view which is supported by the observed differences in their activities in murine antitumour screens [66]. However, there is still no convincing evidence that any of these compounds act via a mechanism involving bifunctional alkylation.

With the exception of nitrosoureas, all of the agents reviewed herein probably require metabolic activation, although in the case of NMF the evidence is somewhat circumstantial. These observations argue strongly for the application of clinical pharmacokinetic studies to demonstrate that adequate metabolic activation is achieved in man. It has been suggested on the basis of such studies that, in the case of methylmelamines, the direct administration of the *N*-hydroxymethyl metabolic intermediate may result in greater antitumour activity in man [137]. Preliminary clinical results with one such compound, N^2,N^4,N^6-*tris* (hydroxymethyl)-N^2,N^4,N^6-trimethyl melamine, indicate that *N*-hydroxymethylmelamines are active antitumour agents in man [83]. Similarly, a novel class of monoalkyltriazene prodrugs has recently been described which again do not require metabolic activation in order to display activity [165]. This approach is currently the most exciting area of drug development in the field of *N*-methyl antitumour agents.

Clinically, it must be admitted that *N*-methyl drugs have not had a major impact on cancer chemotherapy. However, their redeeming features are that they do not produce severe haematological toxicity and that in some instances they are not cross-resistant with nitrogen mustard alkylating agents. These properties have made them valuable components of many combination schedules, particularly in second-line therapy.

With regard to disease-specific activity in man, although there is some overlap between compounds, e.g. in lymphomas, adequate comparative single-agent studies have not been reported. Hence it is difficult to comment on the similarities, or otherwise, between the various agents. In terms of toxicity, although nausea and vomiting is the side effect most frequently observed, the most worrying long-term effect is that of carcinogenicity. For example, it is accepted that dacarbazine, procarbazine and methylnitrosoureas are carcinogenic in man [78, 79].

In summary, the antitumour agents reviewed in this article show a degree of coincidence in terms of structure-activity characteristics, mode of action and metabolic activation. Notable exceptions to this generalisation are the weak activity of certain dialkyltriazenes without an *N*-methyl group, the lack of nucleic acid methylation following HMM or NMF treatment and the chemical activation of methylnitrosoureas. Despite these discrepancies, none of the compounds appear to act via a polyfunctional mechanism, and as such they clearly differ from classical bifunctional alkylating agents. Furthermore, their in vivo properties distinguish them from the latter class of compounds. Thus, in conclusion, the description of this group of compounds as *N*-methyl antitumour agents may form the basis of a more rational classification.

Acknowledgements. The authors gratefully acknowledge the support and encouragement of past and present members of the CRC Experimental Chemotherapy Research Group, Aston University and the Department of Biochemical Pharmacology, Institute of Cancer Research. They also wish to thank Miss Ann Robinson for carefully typing this manuscript more times than they would care to remember!

References

1. Ahmann DL, Hahn RG, Bisel HF (1972) A comparative study of 1(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (NSC 79037) and imidazole carboxamide (NSC 45388) with vincristine (NSC 67574) in the palliation of disseminated malignant melanoma. Cancer Res 32: 2432
2. Ahmann DL, Hahn RG, Bisel HF (1974) Evaluation of 1-(2-chloroethyl-3-4-methylcyclohexyl)-1-nitrosourea (methyl-CCNU, NSC 95441) versus combined imidazole carboxamide (NSC 45388) and vincristine (NSC 67574) in palliation of disseminated malignant melanoma. Cancer 33: 615

3. Ajani JA, Cabanillas FF, Bodey GP (1982) Phase I trial of pentamethylmelamine. Cancer Treat Rep 66: 1227
4. Ames MM, Powis G, Kovach JS, Eagan RT (1979) Disposition and metabolism of pentamethylmelamine and hexamethylmelamine in rabbits and humans. Cancer Res 39: 5016
5. Ames MM, Sanders ME, Tiede WS (1981) Metabolic activation of hexamethylmelamine and pentamethylmelamine by liver microsomal preparations. Life Sci 29: 1591
6. Ames MM, Sanders ME, Tiede WS (1983) Role of N-methylolpentamethylmelamine in the metabolic activation of hexamethylmelamine. Cancer Res 43: 500
7. Audette RCS, Connors TA, Mandel HG, Merai K, Ross WCJ (1973) Studies on the mechanism of action of the tumour inhibitory triazenes. Biochem Pharmacol 22: 1855
8. Baggiolini M, Dewald B, Aebi H (1969) Oxidation of p-(N-methylhydrazinomethyl)-N-isopropylbenzamide (procarbazine) to the methylazo derivative and oxidative cleavage of the N-C bond in isolated perfused rat liver. Biochem Pharmacol 18: 2187
9. Barclay RK, Garfinkel E (1954) The influence of N-methylformamide on formate-^{14}C incorporation. I. In nucleic acids of rat liver. J Biol Chem 208: 875
10. Berd D, Cornog J, De Conti RC, Levitt M, Bertino JR (1975) Long term remission in diffuse histiocytic lymphoma treated with combination sequential chemotherapy. Cancer 35: 1050
11. Blum RH, Livingstone RB, Carter SK (1973) Hexamethylmelamine – a new drug with activity in solid tumours. Eur J Cancer 9: 195
12. Bolis G, D'Incalci M, Belloni C, Mangioni C (1979) Hexamethylmelamine in ovarian cancer resistant to cyclophosphamide and adriamycin. Cancer Treat Rep 63: 1375
13. Bollag W., Grunberg E (1963) Tumour inhibitory effects of a new class of cytotoxic agents: methylhydrazine derivatives. Experientia 19: 130
14. Bollag W, Kaiser A, Langemann A, Zeller P (1964) Methylazo and methylazoxy compounds: new types of antitumour agents. Experientia 20: 503
15. Bonadonna G, Zucali R, Monfardini S, De Lena M, Uslenghi C (1975) Combination chemotherapy of Hodgkin's disease with adriamycin, bleomycin, vinblastine and imidazole carboxamide versus MOPP. Cancer 36: 252
16. Bonomi PD, Mladineo J, Morrin B, Willbanks G, Slayton RE (1979) Phase II trial of hexamethylmelamine in ovarian carcinoma resistant to alkylating agents. Cancer Treat Rep 63: 137
17. Borden EC, Larson P, Ansfield FJ (1977) Hexamethylmelamine treatment of sarcomas and lymphomas. Med Pediatr Oncol 3: 401
18. Borm PJA, Mingels M-JJ, Frankhuijzen-Sierevogel AC, Van Graft M, Hulshoff A, Noordhoek J (1984) Cellular and subcellular studies of the biotransformation of hexamethylmelamine in rat isolated hepatocytes and intestinal epithelial cells. Cancer Res 44: 2820
19. Breithaupt H, Dammann A, Aigner K (1982) Pharmacokinetics of dacarbazine (DTIC) and its metabolite 5-aminoimidazole-4-carboxamide (AIC) following different dose schedules. Cancer Chemother Pharmacol 9: 103
20. Brindley CJ (1982) Investigation of the metabolism of N-alkyl agents with antineoplastic activity, PhD thesis, University of Aston, Birmingham
21. Brindley C, Gescher A, Langdon SP, Broggini M, Colombo T, D'Incalci M (1982a) Studies on the mode of action of antitumour triazenes and triazines – III. Metabolism studies on hexamethylmelamine. Biochem Pharmacol 31: 625
22. Brindley C, Gescher A, Harpur ES, Ross D, Slack JA, Threadgill M, Whitby H (1982b) Studies of the pharmacology of N-methylformamide in mice. Cancer Treat Rep 66: 1957
23. Broder LE, Carter SK (1973) Pancreatic islet cell carcinoma. II. Results of therapy with Streptozotocin in 52 patients. Ann Int Med 79: 109
24. Brookes P (1965) Studies on the mode of action of Ibenzmethyzin. In: Jelliffe AM, Marks J (eds) Natulan (ibenzmethyzin) Wright, Bristol, p 9
25. Bryan GT, Worzalla JF, Gorska RL, Ramirez G (1968) Plasma levels and urinary excretion of hexamethylmelamine following oral administration to human subjects with cancer. Clin Pharmacol Ther 9: 777
26. Casper ES, Gralla RJ, Lynch GR, Jones BR, Woodcock TM, Gordon C, Kelsen DP, Young CW (1981) Phase I and pharmacological studies of pentamethylmelamine administered by 24-hour intravenous infusion. Cancer Res 41: 1402
27. Chabner BA, Sponso R, Hubbard S, Canellos GP, Young RC, Schein PS, DeVita VT (1973) High-dose intermittent intravenous infusion of procarbazine (NSC-77213). Cancer Chemother Rep 57: 361
28. Clarke DA, Philips FS, Sternberg SS, Barcley RK, Stock CC (1953) Effects of N-methylformamide and related compounds in mouse sarcoma 180. Proc Soc Exp Biol Med 84: 203
29. Clarke DA, Barcley RK, Stock CC, Rondestvedt CS (1955) Triazenes as inhibitors of mouse sarcoma 180. Proc Soc Exp Biol Med 90: 484
30. Colombo T, D'Incalci M (1984) Comparison of the antitumour activity of DTIC and 1-p-(3,3-dimethyl-1-triazeno)benzoic acid potassium salt on murine transplantable tumours and their haematological toxicity. Cancer Chemother Pharmacol 13: 139
31. Comis RL (1976) DTIC (NSC 45388) in malignant melanoma: a perspective. Cancer Treat Rep 60: 165
32. Connors TA, Goddard PM, Merai K, Ross WCJ, Wilman DEV (1976) Tumour inhibitory triazenes: structural requirements for an active metabolite. Biochem Pharmacol 25: 241
33. Connors TA, Cumber AJ, Ross WCJ, Clarke SA, Mitchley BCV (1977) Regression of human lung tumour xenografts induced by water-soluble analogues of hexamethylmelamine. Cancer Treat Rep 61: 927
34. Cooksey PG, Gate EN, Gescher A, Hickman JA, Langdon SP, Stevens MFG (1983) The formation and metabolism of N-hydroxymethyl compounds. IV. Cytotoxicity and antitumour activity of N-hydroxymethylformamide, a putative metabolite of N-methylformamide (NSC 3051). Biochem Pharmacol 32: 3037
35. Cummings SW, Guengerich FP, Prough RA (1982) The characterisation of N-isopropyl-p-hydroxymethylbenzamide formed during the oxidative metabolism of azoprocarbazine. Drug Metab Dispos 10: 459
36. D'Incalci M, Bolis G, Mangioni C, Morasca L, Garattini S (1978) Variable oral absorption of hexamethylmelamine in man. Cancer Treat Rep 62: 2117
37. D'Incalci M, Erba E, Balconi G, Morasca L, Garattini S (1980) Time dependence of the in vitro cytotoxicity of hexamethylmelamine and its metabolites. Br J Cancer 41: 630
38. D'Incalci M, Beggiolin G, Sessa C, Mangioni C (1981) Influence of ascites on the pharmacokinetics of hexamethylmelamine and N-demethylated metabolites in ovarian cancer patients. Eur J Cancer Clin Oncol 17: 1331
39. D'Incalci M, Farina P, Sessa C, Mangiun C, Garattini S (1982) Hexamethylmelamine distribution in patients with ovarian and other pelvic cancers. Cancer Treat Rep 66: 231
40. Dawson WB (1965) Ibenzmethyzin in the management of late Hodgkin's disease. In: Jelliffe AM, Marks J (eds) Natulan (ibenzmethyzin) Wright, Bristol, p 31
41. De Milo AB, Borkovec AB (1968) Insect chemosterilants. VII. Oxidative degradation of hexamethylmelamine. J Med Chem 11: 961
42. DeVita VT, Hahn MA, Oliverio VT (1965) Monoamine oxidase inhibition by a new carcinostatic agent, N-isopropyl-alpha-(2-methylhydrazino)-p-toluamide (MIH). Proc Soc Exp Biol Med 120: 561
43. DeVita VT, Serpick AA, Carbone PP (1970) Combination chemotherapy in the treatment of advanced Hodgkin's disease. Ann Intern Med 73: 881

44. DeVita VT, Canellos GP, Chabner B, Schein P, Hubbard SP, Young RC (1975) Advanced diffuse histiocytic lymphoma, a potentially curable disease. Lancet 1: 248
45. Dexter DL, Spremulli EN, Matook GM, Diamond I, Calabresi P (1982) Inhibition of the growth of human colon cancer xenografts by polar solvents. Cancer Res 42: 5018
46. Dost FN, Reed DJ (1967) Methane formation in vivo from N-isopropyl-α-(2-methylhydrazino)-p-toluamide hydrochloride, a tumour-inhibiting methylhydrazine derivative. Biochem Pharmacol 16: 1741
47. Dunn DL, Lubet RA, Prough RA (1979) Oxidative metabolism of N-isopropyl-α-(2-methylhydrazino)-p-toluamide hydrochloride (procarbazine) by rat liver microsomes. Cancer Res 39: 4555
48. Eidinoff ML, Knoll JE, Marano BJ, Klein D (1961) Pyrimidine studies II. Effect of several compounds with antitumour activity on utilisation of precursors for synthesis of nucleic acid pyrimidines. Cancer Res 21: 1377
49. Emanuel NM, Vermel EM, Ostrovskaya LA, Korman NP (1974) Experimental and clinical studies of the antitumour activity of 1-methyl-1-nitrosourea (NSC 23909). Cancer Chemother Rep 58: 135
50. Ettinger DS, Orr DW, Rice AP, Donehower RC (1985) Phase I study of N-methylformamide in patients with advanced cancer. Cancer Treat Rep 69: 489
51. Evans JS, Gerritsen GC, Mann KM, Owen SP (1965) Antitumour and hyperglycemic activity of streptozotocin (NSC 37917) and its cofactor, U-15,774. Cancer Chemother Rep 48: 1
52. Fabian CJ, Rasmussen S, Stephens R, Haut A, Smith F, Balcerzak S, Tranum B (1979) Phase II evaluation of hexamethylmelamine in advanced breast cancer: a Southwest Oncology Group study. Cancer Treat Rep 63: 1359
53. Finklestein JZ, Albo V, Ertel I, Hammond D (1975) 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (NSC 45388) in the treatment of solid tumours in children. Cancer Chemother Rep 59: 351
54. Frei E, Luce JK, Talley RW, Vaitkevicius VK, Wilson HE (1972) 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (NSC 45388) in the treatment of lymphoma. Cancer Chemother Rep 56: 667
55. Furst A, Cutting WC, Gross H (1955) Retardation of growth of Ehrlich ascites tumour by formamides and related compounds. Cancer Res 15: 294
56. Gad-El-Mawla N, Ziegler JL, Hamza R, Elserafi M, Khaled H (1984) Randomized phase II trial of hexamethylmelamine versus pentamethylmelamine in carcinoma of the bilharzial bladder. Cancer Treat Rep 68: 793
57. Garattini E, Broggini M, Coccia I, Colombo T, Rossi C, D'Incalci M (1984) Biochemical studies of the ability of pentamethylmelamine to interact in vivo with DNA and proteins in a sensitive murine reticular cell sarcoma. Biochem Pharmacol 33: 2715
58. Gerulath AH, Loo TL (1972) Mechanism of action of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide in mammalian cells in culture. Biochem Pharmacol 21: 2335
59. Gescher A, D'Incalci M, Fanelli R, Farina P (1980) N-Hydroxymethylpentamethylmelamine, a major in vitro metabolite of hexamethylmelamine. Life Sci 26: 147
60. Gescher A, Hickman JA, Simmonds RJ, Stevens MFG, Vaughan K (1981) Studies of the mode of action of antitumour triazenes and triazines. II. Investigation of the selective toxicity of 1-aryl-3,3-dimethyltriazenes. Biochem Pharmacol 30: 89
61. Gescher A, Gibson NW, Hickman JA, Langdon SP, Ross D, Atassi G (1982) N-Methylformamide: antitumour activity and metabolism in mice. Br J Cancer 45: 843
62. Gersel Pedersen A, Sorenson S, Aabo K, Dombernowsky P, Hansen HH (1982) Phase II study of procarbazine in small cell carcinoma of the lung. Cancer Treat Rep 66: 273
63. Giraldi T, Houghton PJ, Taylor DM, Nisi C (1978) Antimetastatic action of some triazene derivatives against the Lewis lung carcinoma in mice. Cancer Treat Rep 62: 721
64. Giraldi T, Sava G, Cuman R, Nisi C, Lassiani L (1981) Selectivity of the antimetastatic and cytotoxic effects of 1-p-(3,3-dimethyl-1-triazeno)benzoic acid potassium salt, (±)1,2-di(3,5-dioxopiperazin-1-yl) propane, and cyclophosphamide in mice bearing Lewis lung carcinoma. Cancer Res 41: 2524
65. Goldberg RS, Griffin JP, McSherry JW, Krakoff IH (1980) Phase I study of pentamethylmelamine. Cancer Treat Rep 64: 1319
66. Goldin A, Venditti JM, MacDonald JS, Muggia FM, Henney JE, De Vita VT (1981) Current results of the screening programme at the Division of Cancer Treatment, National Cancer Institute. Eur J Cancer 17: 129
67. Gorsen RM, Weiss AJ, Manthei RW (1980) Analysis of procarbazine and metabolites by gas chromatography-mass spectrometry. J Chromatog (Biomed Appl) 221: 309
68. Gottlieb JA et al (1976) Role of DTIC (NSC 45388) in the chemotherapy of sarcomas. Cancer Treat Rep 60: 199
69. Gutterman J, Huang A, Hochstein P (1969) The mode of action of N-isopropyl-α(2-methylhydrazine)p-toluamide. Exp Biol Med 130: 797
70. Hansch C, Hatheway GJ, Quinn FR, Greenberg N (1978) Antitumour 1-(x-aryl)-3,3-dialkyltriazenes. 2. On the role of correlation analysis in decision making in drug modification. Toxicity quantitative structure activity relationships of 1-(x-phenyl)-3,3-dialkyltriazenes in mice. J Med Chem 21: 574
71. Hendry JA, Rose FL, Walpole AL (1951) Cytotoxic agents: I, Methylolamides with tumour inhibitory activity, and related inactive compounds. Br J Pharmacol 6: 201
72. Hickman JA (1978) Investigation of the mechanism of action of antitumour dimethyltriazenes. Biochimie 60: 997
73. Hill DL (1975) Microsomal metabolism of triazenylimidazoles. Cancer Res 35: 3106
74. Hope-Stone HF (1965) Ibenzmethyzin in the treatment of reticuloses. In: Natulan (ibenzmethyzin), Marks J Jelliffe AM (eds), Wright, Bristol p 15
75. Householder GE, Loo TL (1969) Elevated urinary excretion of >4-aminoimidazole-5-carboxamide in patients after intravenous injection of 4-(3,3-dimethyl-1-triazeno)imidazole-5-carboxamide. Life Sci 8: 533
76. Householder GE, Loo TL (1971) Disposition of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide, a new antitumour agent. J Pharmacol Exp Ther 179: 386
77. IARC Monograph (1978a) N-Nitroso-N-ethylurea. In: Evaluation of the carcinogenic risk of chemicals to humans, volume 17. Some N-nitroso compounds. IARC, Lyon, p 191
78. IARC Monograph (1978b) N-Nitroso-N-methylurea. In: Evaluation of the carcinogenic risk of chemicals to humans, volume 17. Some N-nitroso compounds. IARC, Lyon p 227
79. IARC Monograph (1981) Dacarbazine and procarbazine hydrochloride. In: Evaluation of the carcinogenic risk of chemicals to humans, volume 26. Some antineoplastic and immunosuppressive agents. IARC, Lyon, p 203 and p 311
80. Ihde DC, Dutcher JS, Young RC, Cordes RS, Barlock AL, Hubbard SM, Jones RB, Boyd MR (1981) Phase I trial of pentamethylmelamine: a clinical and pharmacologic study. Cancer Treat Rep 65: 755
81. Johnson BL, Fisher RI, Bender RA, DeVita VT, Chabner BA, Young RC (1978) Hexamethylmelamine in alkylating agent-resistant ovarian carcinoma. Cancer 42: 2157
82. Johnston TP, McCaleb GS, Montgomery JA (1963) The synthesis of antineoplastic agents. XXXII. N-nitrosoureas 1. J Med Chem 6: 669
83. Judson IR, Rutty CJ, Abel G, Gumbrell L, Harrap KR, Calvert AH (1985) Phase I trial of N^2,N^4,N^6-trihydroxymethyl-N^2,N^4,N^6-trimethylmelamine (Trimelamol), a new antitumour s-triazine. Proc Am Soc Clin Oncol 4: 31
84. Kaye SB, Juttner CA, Smith IE, Barrett A, Austin DE, Peckham MJ, McElwain TJ (1979) Three years' experience with

ChlVPP (a combination of drugs of low toxicity) for the treatment of Hodgkin's disease. Br J Cancer 39: 168
85. Kestell P, Gescher A, Slack JA (1985) The fate of N-methylformamide in mice. Routes of elimination and characterisation of metabolites. Drug Metab Dispos 13: 587
86. Kestell P, Gledhill AP, Threadgill MD, Gescher A (1986) S-(N-Methylcarbamoyl)-N-acetylcysteine: a urinary metabolite of the hepatotoxic experimental antitumour drug N-methylformamide (NSC 3051) in mouse, rat and man. Biochem Pharmacol 35: 2283
87. Klippert PJM, Hulshoff A, Mingels M-JJ, Hofman G, Noordhoek J (1983) Low oral bioavailability of hexamethylmelamine in the rat due to simultaneous hepatic and gastrointestinal metabolism. Cancer Res 43: 3160
88. Kohn KW (1977) Interstrand cross-linking of DNA by 1,3-bis(2-chloroethyl)-1-nitrosourea and other 1-(2-haloethyl)-1-nitrosoureas. Cancer Res 37: 1450
89. Kolar GF, Carubelli R (1979) Urinary metabolite of 1-(2,4,6-trichlorophenyl)-3,3-dimethyltriazene, with an intact diazoamino structure. Cancer Lett 7: 209
90. Kolar GF, Maurer M, Wildschütte M (1980) 5-(3-Hydroxymethyl-3-methyltriazeno)imidazole-4-carboxamide is a metabolite of 5(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide (DIC, DTIC, NSC 45388). Cancer Lett 10: 235
91. Kreis W (1970) Metabolism of an antineoplastic methylhydrazine derivative in a P815 mouse neoplasm. Cancer Res 30: 82
92. Kushner BS, Lazar M, Furman M (1969) Resistance of rabbits and guinea pigs to the diabetogenic effect of streptozotocin. Diabetes 18: 542
93. Kuttab SH, Tanglerpaibul S, Vouros P (1982) Studies on the metabolism of procarbazine by mass spectrometry. Biomed Mass Spectrom 9: 78
94. Lake LM, Grunden EE, Johnson BM (1975) Toxicity and antitumour activity of hexamethylmelamine and its N-demethylated metabolites in mice with transplantable tumours. Cancer Res 35: 2858
95. Langdon SP (1983) The synthesis and antitumour evaluation of methyl amino compounds PhD thesis, University of Aston, Birmingham
96. Lawley PD (1980) DNA as a target for alkylating carcinogens. Br Med Bull 36: 19
97. Legha SS, Slavik M, Carter SK (1976) Hexamethylmelamine. An evaluation of its role in the therapy of cancer. Cancer 38: 27
98. Legha SS, Buzdar AU, Hortobagyi GN, DiStefano A, Wiseman CL, Yap H-Y, Blumenschein GR, Bodey EGP, (1979) Phase II study of hexamethylmelamine alone and in combination with mitomycin C and vincristine in advanced breast carcinoma. Cancer Treat Rep 63: 2053
99. Livingstone RB, Carter SK (1971) In: Carter SK (ed) Proceedings of the Chemotherapy Conference on Procarbazine: Development and Application, U. S. Government Printing Office, Washington, p 329
100. Loo TL, Luce JK, Jardine JH, Frei E (1968) Pharmacologic studies of the antitumour agent 5-(dimethyltriazeno)imidazole-4-carboxamide. Cancer Res 28: 2448
101. Louis J, Louis NB, Linman JW (1967) The clinical pharmacology of hexamethylmelamine: a phase I study. Clin Pharmacol Ther 8: 55
102. Luce JK, Thurman WG, Isaacs BL, Talley RW (1970) Clinical trials with the antitumour agent 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC 45388). Cancer Chemother Rep 54: 119
103. Luce JK, Gamble JF, Wilson HE, Monto RW, Isaacs BL, Palmer RL, Coltman CA, Hewlett JS, Gehan EA, Frei E (1971) Combined cyclophosphamide, vincristine and prednisone therapy of malignant lymphoma. Cancer 28: 306
104. Mathe G, Schweisguth O, Schneider M, Amiel JL, Berumen L, Brule G, Cattan A, Schwarzenberg L (1963) Methyl hydrazine in the treatment of Hodgkin's disease. Lancet 2: 1077
105. McVie JG, ten Bokkel Huinick WW, Simonetti G, Dubbelman R (1984) Phase I trial of N-methylformamide. Cancer Treat Rep 68: 607
106. Mizuno NS, Decker RW (1976) Alteration of DNA by 5-(3-methyl-1-triazeno)imidazole-4-carboxamide (NSC 407347). Biochem Pharmacol 25: 2643
107. Mizuno NS, Humphrey EW (1972) Metabolism of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC 45388) in human and animal tumour tissue. Cancer Chemother Rep 56: 465
108. Moertel CG, Hanley JA, Johnson LA (1980) Streptozotocin alone compared with streptozotocin plus fluorouracil in the treatment of advanced islet cell carcinoma. N Engl J Med 303: 1189
109. Moertel CG (1975) Clinical management of advanced gastrointestinal cancer. Cancer 36: 675
110. Molony SJ, Prough RA (1983) Studies on the pathway of methane formation from procarbazine, a 2-methylbenzyl hydrazine derivative, by rat liver microsomes. Arch Biochem Biophys 221: 577
111. Morimoto M, Green D, Rahman A, Goldin A, Schein PS (1980) Comparative pharmacology of pentamethylmelamine and hexamethylmelamine in mice. Cancer Res 40: 2762
112. Morkhansen M, Hertz H, Videbaek A (1966) Use of a methyl hydrazine derivative (Natulan) especially in Hodgkin's disease. Acta Med Scand 18: 211
113. Morrison SS, Higgins GM (1956) An attempt to block sources of methyl groups in the therapy of mouse leukaemia. Cancer Res 16: 292
114. Muindi JRF, Rutty CJ, Harrap KR (1981) DNA cross-linking induced by pentamethylmonomethylolmelamine in vitro. Br J Cancer 44: 281
115. Muindi JRF, Newell DR, Smith IE, Harrap KR (1983) Pentamethylmelamine (PMM): phase I clinical and pharmacokinetic studies. Br J Cancer 47: 27
116. Murray-Lyon IM, Eddleston A, Williams R, Brown M, Hogbin BM, Bennett A, Edwards JC, Taylor KW (1968) Treatment of multiple-hormone-producing malignant islet cell tumours with streptozotocin. Lancet 2: 895
117. Myers WPL, Karnofsky DA, Burchenal JH (1956) The hepatotoxic action of N-methylformamide in man. Cancer 9: 949
118. Nagasawa HT, Shirota FN, Mizuno NS (1974) The mechanism of alkylation of DNA by 5-(3-methyl-1-triazeno)-imidazole-4-carboxamide (MIC), a metabolite of DIC (NSC 45388). Non-involvement of diazomethane. Chem Biol Interact 8: 403
119. Newbold RF, Warren W, Medcalf ASC, Amos J (1980) Mutagenicity of carcinogenic methylating agents is associated with a specific DNA modification. Nature 283: 596
120. Nicholson WM, Beard MEJ, Crowther D (1970) Combination chemotherapy in generalised Hodgkin's disease. Br Med J 3: 7
121. O'Dwyer PJ, Donehower M, Sigman LM, Fortner CL, Aisner J, Van Echo DA (1985) Phase I trial of N-methylformamide (NMF, NSC 3051). J Clin Oncol 3: 853
122. Oliverio VT, Denham C, DeVita VT, Kelly MG (1964) Some pharmacologic properties of a new antitumour agent, N-isopropyl-α-(2-methylhydrazino)-p-toluamide, hydrochloride (NSC 77213). Cancer Chemother Rep 42: 1
123. Omura GA, Greco FA, Brich R (1981) Hexamethylmelamine in mustard-resistant ovarian adenocarcinoma. Cancer Treat Rep 65: 530
124. Omura GA, Broun GO, Papps J, Birch R (1981) Phase II study of hexamethylmelamine in refractory Hodgkin's disease, other lymphomas and chronic lymphocytic leukaemia. Cancer Treat Rep 65: 1027
125. Palmer RL, Kroening PM (1978) Comparison of low dose radiation therapy alone or combined with procarbazine (NSC-77213) for unresectable epidermoid carcinoma of the lung, stage T3, N1, N2 or M1. Cancer 42: 424
126. Panasci LC, Fox PA, Schein PS (1977) Structure activity

studies of methylnitrosourea antitumour agents with reduced murine bone marrow toxicity. Cancer Res 37: 3321
127. Preussman R, Ivankovic S, Landschultz C, Gimmy J, Flohr E, Friesbach U (1974) Karzinogene Wirkung von 13 Aryldialkyltriazenen an BD-Ratten. Z Krebsforsch 81: 285
128. Prough RA, Brown MI, Dannan GA, Guengerich FP (1984) Major isozymes of rat liver microsomal cytochrome P-450 involved in the N-oxidation of N-isopropyl-alpha-(2-methylazo-p-toluamide), the azo derivative of procarbazine. Cancer Res 44: 543
129. Prough RA, Brown MI, Moloney SJ, Wiebkin P, Cummings SW, Spearman ME, Guengerich FP (1985) The activation of hydrazines to reactive intermediates. In: Boobis AR, Caldwell J, De Mattis F, Elcombe CR (eds) Microsomes and drug oxidations, Taylor and Francis, London, p 330
130. Raaflaub J, Schwartz DE (1965) Über den Metabolismus eines cytostatisch wirksamen Methylhydrazin-Derivates (Natulan). Experientia 21: 44
131. Ross WCJ (1962) In: Biological alkylating agents. Butterworth, London
132. Ross WE, McMillan DR, Ross CF (1981) Comparison of DNA damage by methylmelamines and formaldehyde. J Natl Cancer Inst 67: 217
133. Rutty CJ, Abel G (1980) In vitro cytotoxicity of the methylmelamines. Chem Biol Interact 29: 235
134. Rutty CJ, Connors TA (1977) In vitro studies with hexamethylmelamine. Biochem Pharmacol 26: 2385
135. Rutty CJ, Connors TA, Nam NH, Thang DC, Hoellinger H (1978) In vivo studies with hexamethylmelamine. Eur J Cancer 14: 713
136. Rutty CJ, Abel G, Harrap KR (1979) In vitro cytotoxicity of hexamethylmelamine and its analogues. Br J Cancer 40: 317
137. Rutty CJ, Newell DR, Munidi JRF, Harrap KR (1982) The comparative pharmacokinetics of pentamethylmelamine in man, rat and mouse. Cancer Chemother Pharmacol 8: 105
138. Rutty CJ, Newell DR, Vincent RB, Abel G, Goddard PM, Harland SJ, Calvert AH (1983) The species dependent pharmacokinetics of DTIC. Br J Cancer 48: 140
139. Sartorelli AC, Le Page GA (1958) The development and biochemical characterisation of resistance to azaserine in a TA3 ascites carcinoma. Cancer Res 18: 457
140. Sava G, Giraldi T, Lassiani L, Nisi C (1979) Mechanism of the antimetastatic action of dimethyl triazenes. Cancer Treat Rep 63: 93
141. Sava G, Giraldi T, Lassiani L, Nisi C (1982) Metabolism and mechanism of action of the antileukemic action of isomeric aryldimethyltriazenes. Cancer Treat Rep 66: 1751
142. Sava G, Giraldi T, Nisi C, Bertoli G (1983) Prophylactic antimetastatic treatment with aryldimethyl triazenes as adjuvants to surgical tumour removal in mice bearing Lewis lung carcinoma. Cancer Treat Rep 66: 115
143. Schwartz DE, Bollag W, Obrecht P (1967) Distribution and excretion studies of procarbazine in animals and man. Arzneimittelforsch 17: 1389
144. Schein PS, Cooney DA, Vernon ML (1967) The use of nicotinamide to modify the toxicity of streptozotocin diabetes without loss of antitumour activity. Cancer Res 27: 2324
145. Schein PS, Kahn R, Gordon P (1973) Steptozotocin for malignant insulinomas and carcinoid tumour. Report of eight cases and review of the literature. Arch Int Med 132: 555
146. Schein PS, O'Connell MJ, Blom J, Hubbard S, Magrath IT, Bergevin P, Wiernik PH, Ziegler JL, DeVita VT (1974) Clinical antitumour activity and toxicity of streptozotocin (NSC-85998). Cancer 34: 993
147. Schein PS, DeVita VT, Hubbard S, Chabner BA, Canellos GP, Berard C, Young RC (1976) Bleomycin, adriamycin, cyclophosphamide, vincristine and prednisone (BACOP) combination chemotherapy in the treatment of advanced diffuse histiocytic lymphoma. Ann Intern Med 85: 417
148. Selawry OS (1973) Monochemotherapy of bronchogenic carcinoma with special reference to cell type. Cancer Chemother Rep Pt [3] 4: 177
149. Shealy YF, Struck RF, Holum LB, Montgomery JA (1961) Synthesis of potential anticancer agents. XXIX. 5-Diazoimidazole-4-carboxamide and 5-diazo-v-triazole-4-carboxamide. J Org Chem 26: 2396
150. Shealy YF, Krauth CA, Montgomery JA (1962) Imidazoles. I. Coupling reactions of 5-diazoimidazole-4-carboxamide. J Org Chem 27: 2150
151. Shealy YF, Montgomery JA, Laster WR (1962) Antitumour activity of triazenoimidazoles. Biochem Pharmacol 11: 674
152. Shiba DA, Weinkam RJ (1983) The in vivo cytotoxic activity of procarbazine and procarbazine metabolites against L1210 ascites leukaemia cells of CDF1 mice and the effects of pretreatment with procarbazine, phenobarbital, diphenylhydantoin, and methylprednisolone upon in vivo procarbazine activity. Cancer Chemother Pharmacol 11: 124
153. Shiba DA, Weinkam RJ (1982) Quantitative analysis of procarbazine, procarbazine metabolites and chemical degradation products with application to pharmacokinetic studies. J Chromatogr Biomed Appl 229: 397
154. Shiloh Y, Becker Y (1981) Kinetics of O^6-methylguanine repair in human normal and ataxia telangiectaria cell lines and correlation of repair capacity with cellular sensitivity to methylating agents. Cancer Res 41: 5114
155. Sicher K (1965) Short report: In: Jelliffe AM, Marks J (eds) Natulan (ibenzmethyzin). Wright, Bristol, p 40
156. Sinha BK (1984) Metabolic activation of procarbazine. Evidence for carbon-centred free-radical intermediates. Biochem Pharmacol 33: 2777
157. Skibba JL, Ramirez G, Beal DD, Bryan GT (1969) Preliminary clinical trial and the physiological disposition of 4(5)-(3,3-dimethyl-1-triazeno)imidazole-5(4)-carboxamide in man. Cancer Res 29: 1944
158. Skibba JL, Ramirez G, Beal DD, Bryan GT (1970) Metabolism of 4(5)-(3,3-dimethyl-1-triazeno)imidazole-5-carboxamide to 4(5)-amino-imidazole-5(4)-carboxamide in man. Biochem Pharmacol 19: 2043
159. Skibba JL, Beal DD, Ramirez G, Bryan GT (1970) N-Demethylation of the antineoplastic agent 4(5)-(3,3-dimethyl-1-triazeno)imidazole-5(4)-carboxamide by rats and man. Cancer Res 30: 147
160. Skibba JL, Bryan GT (1971) Methylation of nucleic acids and urinary excretion of ^{14}C-labelled 7-methylguanine by rats and man after administration of 4(5)-(3,3-dimethyl-1-triazeno)-imidazole 5(4)-carboxamide. Toxicol Appl Pharmacol 18: 707
161. Skipper HE, Schabel FM Jr, Binns V, Thomson JR, Wheeler GP (1955) Studies on the mechanism of action and anticancer activity of N-methylformamide. Cancer Res 15: 143
162. Smulson ME, Schein P, Mullins DW, Sudhakar S (1977) A putative role for nicotinamide adenine dinucleotide – promoted nuclear protein modification in the antitumour activity of N-methyl-N-nitrosourea. Cancer Res 37: 3006
163. Spivack SD (1974) Procarbazine. Ann Intern Med 81: 795
164. Spremulli EN, Dexter DL (1984) Polar solvents: a novel class of antineoplastic agents. J Clin Oncol 2: 227
165. Stevens MFG, Hickman JA, Stone R, Gibson NW, Baig GU, Lunt E, Newton CG (1984) Antitumour imidazotetrazines. 1. Synthesis and chemistry of 8-carbamoyl-3-(2-chloroethyl) imidazo (5,1-d)-1,2,3,5-tetrazin-4(3H)-one, a novel broad-spectrum antitumour agent. J Med Chem 27: 196
166. Stolinsky D, Sadoff L, Braunwald J, Bateman J (1972) Streptozotocin in the treatment of cancer: phase II study. Cancer 30: 61
167. Tauer K, Kemeny N, Hollander P (1985) Phase II trial of N-methylformamide (NMF) in patients with advanced colorectal carcinoma. Proc Am Ass Cancer Res 26: 169
168. Takeuchi M, Ogiu T, Nakadate M, Odashima S (1980) Induction of duodenal tumours and thymomas in Fischer rats

by continuous oral administration of 1-propyl-1-nitrosourea. Gann 71: 231
169. Todd IDH (1965) Further experience with ibenzmethyzin: In: Jelliffe AM, Marks J (eds) Natulan (ibenzmethyzin), Wright Bristol, p 20
170. Van Echo DA, Chiuten DF, Whitacre M, Aisner J, Lichtenfeld JL, Wiernik PH (1980) Phase I trial of pentamethylmelamine in patients with previously treated malignancies. Cancer Treat Rep 64: 1335
171. Vavra JJ, de Boer C, Dietz A, Hanka LJ, Sokolski WT (1959–60) Streptozotocin, a new antibacterial antibiotic. Antibiot Ann 230
172. Vogel CL, Primack A, Owor R, Kyalwazi SK (1973) Effective treatment of Kaposi's sarcoma with 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC 45388). Cancer Chemother Rep 57: 65
173. Vogl SE, Pagano M, Davis TE, Einhorn N, Tunca JC, Kaplan BH, Arseneau JC (1982) Hexamethylmelamine and cisplatin in advanced ovarian cancer after failure of alkylating agent therapy. Cancer Treat Rep 66: 1285
174. Wagner DE, Ramirez G, Weiss AJ (1972) Combination Phase I–II study of imidazole carboxamide (NSC 45388). Oncology 26: 310
175. Weinkam RJ, Shiba DA (1978) Metabolic activation of procarbazine. Life Sci 22: 937
176. Weinkam RJ, Shiba DA, Chabner BA (1982) Nonclassical alkylating agents. In: Chabner B (ed) Pharmacologic principles of cancer treatment. Saunders, Philadelphia, p 340
177. Wharton JT, Rutledge F, Smith JP, Herson J, Hodge MP (1979) Hexamethylmelamine: an evaluation of its role in the treatmen of ovarian cancer. Am J Obstet Gynecol 133: 833
178. Wheeler GP, Grammer MG (1960) Prevention of the inhibitory effects of urethan, formamide and N-methylformamide on the growth of Escherichia Coli. Biochem Pharmacol 3: 316
179. Wiebkin P, Prough RA (1980) Oxidative metabolism of N-isopropyl-alpha-(2-methylazo)-p-toluamide (Azoprocarbazine) by rodent liver microsomes. Cancer Res 40: 3524
180. Wiemann MC, Cummings FJ, Posner MR, Weens JH, Crabtree GW, Birmingham BK, Moore A, Calabresi P (1985) Phase I clinical and pharmacological trial of oral N-methylformamide. Proc Am Soc Clin Oncol 4: 38
181. Wilson WL, De La Garza JG (1965) Phase I study of hexamethylmelamine (NSC 13875). Cancer Chemother Rep 48: 49
182. Worzalla JF, Johnson BM, Ramirez G, Bryan GT (1973) N-Demethylation of the antineoplastic agent hexamethylmelamine by rats and man. Cancer Res 33: 2810
183. Worzalla JF, Kaiman BD, Johnson BM, Ramirez G, Bryan GT (1974) Metabolism of hexamethylmelamine-ring-^{14}C in rats and man. Cancer Res 34: 2669
184. Zeller P, Gutmann H, Hegedus B, Kaiser A, Langemann A, Muller M (1963) Methylhydrazine derivatives, a new class of cytotoxic agents. Experientia 19: 129

Received February 10, 1986/Accepted July 22, 1986