Inhibition of Solid Tumors by Nitrosoureas. 1. Lewis Lung Carcinoma

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The utility of the Lewis lung carcinoma as a secondary screen for the evaluation of nitrosoureas as anticancer agents has been assessed. The activity of this series of compounds was determined against both the early (before metastasis) and late (after metastasis) forms of the disease. Although some exceptions were noted, compounds most active against the early form of the disease were most active against the established tumor. A differentiation in activity based on the Lewis lung system was evident with nitrosoureas equally active against leukemia L1210, although the significance of this differentiation with respect to the human disease has not yet been established.

Many *N*-(2-chloroethyl)-*N*-nitrosoureas are highly active against leukemia L1210 implanted intraperitoneally[2-5] in the usual manner or intracerebrally[3-5] to mimic metastatic leukemia in the brain. Because of the large number of

Table I. Nitrosoureas Active against Advanced Lewis Lung Carcinoma[a]

$RNHCON(NO)(CH_2)_2X$

No.	R	X	Dose,[b] mg/kg	% ILS[c] of dying animals	Complete tumor regressions/ total no. of animals	Cures[d]/ total no. of animals
1	Cyclohexyl	Cl	50	15	7/30	0/30
			40	31	1/10	0/10
2	*cis*-2-Chlorocyclohexyl	Cl	90	62	1/10	0/10
3	*trans*-2-Chlorocyclohexyl	Cl	55	18	0/10	0/10
4	2-Fluoroethyl (2-Chloroethyl)	Cl (F)	32	44	0/10	0/10
5	*trans*-4-[*N'*-(2-Chloroethyl)-*N'*-nitrosoureido]-cyclohexyl	Cl	171	146	9/10	2/10
6	2-Norbornyl	F	9	40	0/6	0/6
7	*trans*-4-(1,1-Dimethylethyl)cyclohexyl	Cl	316	41	1/10	0/10
8	*trans*-4-Methylcyclohexyl	Cl	36	46	14/50	5/50
9	2,6-Dioxo-3-piperidyl	Cl	15	49	1/10	0/10
10	*cis*-3-(Ethoxycarbonyl)cyclohexyl	Cl	45	0	3/10	0/10
			30	62	0/10	0/10
11	*trans*-4-(Ethoxycarbonyl)cyclohexyl	Cl	30	68	11/20	2/20
			29	75	4/10	3/10
12	Tetrahydro-2*H*-thiopyran-4-yl (*S,S*-dioxide)	Cl	11	27	1/10	0/10
13	(4-Methylphenyl)sulfonyl	Cl	93	28	0/10	0/10
			62	28	0/10	0/10
14	Tetrahydro-2*H*-thiopyran-4-yl (*S,S*-dioxide)	F	33	0	1/10	0/10
15	Tetrahydro-2*H*-thiopyran-4-yl	F	36	87	1/6	0/6
16	*trans*-4-(Acetyloxy)cyclohexyl	Cl	36	34	0/10	0/10
17	*trans*-4-Methylcyclohexyl	F	44	94	3/10	0/10
18	1,3-Dithian-5-yl	Cl	22	36	2/10	0/10
19	*trans*-4-Ethylcyclohexyl	Cl	67	17	8/10	0/10
20	2-Adamantyl	Cl	144	19	5/10	0/10
21	1,3-Dithian-5-yl	F	41	21	0/10	0/10
			27	40	0/10	0/10
22	4,4-Dimethylcyclohexyl	F	52	100	3/10	0/10
23	*cis*-4-Carboxycyclohexyl	Cl	30	79	4/20	0/20
			20	90	4/20	0/20
24	*trans*-4-Carboxycyclohexyl	Cl	30	67	6/20	0/20
25	*trans*-4-(Methoxycarbonyl)cyclohexyl	Cl	32	86	1/10	0/10
			21	113	1/10	0/10
26	*trans*-4-Methoxycyclohexyl	Cl	37	29	0/10	0/10
			25	29	0/10	0/10
			16	20	0/10	0/10
27	*cis*-4-(Methoxycarbonyl)cyclohexyl	Cl	54	100	1/10	0/10
			36	120	2/10	0/10
28	*trans*-4-[(Acetyloxy)methyl]cyclohexyl	Cl	18	65	0/10	0/10
			12	26	0/10	0/10
29	*trans*-4-(Carboxymethyl)cyclohexyl	Cl	26	24	1/10	1/10
30	2-Deoxy-D-glucos-2-yl	Cl	13	25	0/10	0/10
31	*cis*-2-Methyl-1,3-dithian-5-yl (*S,S,S',S'*-tetraoxide)	Cl	32	10	0/10	0/10
32	*trans*-4-(Chloromethyl)cyclohexyl	Cl	60	109	0/10	0/10
			40	51	0/10	0/10
33	*trans*-4-Carboxycyclohexyl	F	45	60	2/10	2/10
34	*trans*-4-[(Acetyloxy)methyl]cyclohexyl	F	48	26	0/10	0/10
			32	13	0/10	0/10
			21	26	0/10	0/10

[a] See Methods. [b] Single ip injection on the seventh day after tumor implantation. The dose shown is equal to or less than the LD_{10} in normal nontumor-bearing mice. [c] Increase in life-span. [d] Animals were observed for at least 80 days posttreatment.

Table II. Inactives against Advanced Lewis Lung Carcinoma[a]

No.	RNHCON(NO)$(CH_2)_2$X R	X
35	Cyclopentyl	Cl
36	*cis*-3-Methylcyclohexyl	Cl
37	*trans*-4-[*N'*-(2-Chloroethyl)-*N'*-nitrosoureido]-cyclohexyl	F
38	2,4,6-Trimethylphenyl	Cl
39	2-Deoxy-1,3,4,6-tetra-*O*-acetyl-D-glucopyranos-2-yl	Cl
40	5-Nitro-2-thiazolyl	Cl
41	Tetrahydro-3-thienyl (*S,S*-dioxide)[b]	Cl
42	*trans*-4-(Acetyloxy)cyclohexyl	F
43	3-Thiochroman-4-yl	F
44	Cyclododecyl	F
45	*trans*-4-Ethylcyclohexyl	F
46	*trans*-(1-Methylethyl)cyclohexyl	F
47	2-Adamantyl	F
48	2-Cyclohexen-1-yl	Cl
49	*cis*-4-Methylcyclohexyl	Cl
50	*cis*-4-[*N'*-(2-Chloroethyl)-*N'*-nitrosoureido]-cyclohexyl	Cl
51	*cis*-3-Carboxycyclohexyl	Cl
52	*cis*-2-Methyl-1,3-dithian-5-yl	Cl
53	*trans*-2-Methyl-1,3-dithian-5-yl (*S,S,S',S'*-tetraoxide)	Cl
54	1-Methylhexyl	Cl
55	*cis*-4-Carboxycyclohexyl	F
56	*trans*-3-Methylcyclohexyl	Cl
57	*trans*-4-Hydroxycyclohexyl	Cl
58	*cis*-4-Hydroxycyclohexyl	Cl
59	2-Chloroethyl (BCNU)	Cl

[a] See Methods. [b] Mixture with *N'*-nitroso isomer.

nitrosoureas capable of killing 10^5 cells inoculated ip or 10^4 cells ic, it was not possible to select a small number of superior candidates for consideration as possible clinical agents based on the data obtained in this test system. This problem led to the selection of Lewis lung carcinoma as a secondary screen for these highly active compounds in the hope of obtaining a greater spread of activity and of finding an animal test system better able to predict for compounds active against solid tumors in humans.

The Lewis lung carcinoma implanted subcutaneously in the subaxillary region of BDF_1 mice for these evaluations metastasizes to the lungs and other sites.[6,7] Surgical removal of the primary tumor more than 5 days postimplant has no effect on the death pattern of the host, indicating that metastasis has occurred by that time and that death must be associated with the metastatic disease rather than the primary tumor. Furthermore, the metastatic tumors have a shorter doubling time than the primary tumors except in those animals that survive well past the median day of death. The cell cycle and S phase at day 21 are shorter in the metastases than in the primary tumor at day 5, and the thymidine index is higher at any time that it can be measured. Both factors are consistent with greater drug sensitivity, which is known to result from a combination of kinetic characteristics generally found in very small tumors.[6] Thus, an assessment of drug activity in this complex system is much more difficult than in the simpler systems such as the murine leukemias or the solid tumors that do not metastasize. For this reason, a number of parameters have been observed for each compound, and evaluations were made against the early tumor by treating 1 or 2 days postimplant and against the established tumor (~400 mg) by treating 7 days postimplant when the growth characteristics of the primary tumor have changed and metastases have occurred. In order to simplify interpretation of the results, single-dose therapy was used for comparisons, although later the optimal dose and treatment schedule were sought for the most active compounds.

A pilot study with 14 nitrosoureas showed that, except for one aromatic structure and three carboxylic acids that were inactive, a parabolic relationship exists between the logarithms of partition coefficients (octanol–water) and the ability of these compounds to delay the growth of the Lewis lung carcinoma (log $1/C$) if they are administered 24 h after tumor implantation.[8] Unfortunately, the log P_0 (optimal partition coefficient) derived from this parabolic relationship is essentially the same as that derived from the relation of toxicity (log $1/LD_{10}$) to the logs of the partition coefficients, so no separation of activity from toxicity can be made on the basis of partition coefficients.[9] Furthermore, increase in life-span was not a satisfactory parameter for this type of study nor was the response of the advanced disease.[8]

Materials and Methods. The syntheses of the compounds used in this study have been described.[2,4,5,10-12]

BDF_1 mice (C57Blk/6 ♀ × DBA/2 ♂) were implanted subcutaneously by trocar with Lewis lung carcinoma tumor fragments weighing approximately 40 mg. Bacteriological cultures were done on a sample of each donor tumor, and only mice implanted from bacteriologically sterile tumors were used in the chemotherapy trials. Mice were randomly distributed into stainless steel cages and were given Wayne Lab Blox and water ad libitum.

In groups where advanced tumors were to be treated, mice were selected with tumors approximately 400 mg in size (day 6–7 post tumor implant). Tumor sizes were estimated using caliper measurements, which were converted to weight using the formula, $w = ab^2/2$, where a = length in millimeters, b = width in millimeters, and w = weight in milligrams. During chemotherapy trials, individual tumor measurements were made at least twice weekly.

Each drug was given by intraperitoneal injections as a solution (or suspension) in distilled water (CMC or gum acacia) or physiological saline (HPC or Tween 80) with the volume being ≤0.5 ml per injection. Normal nontumor-bearing mice of the same sex, strain, and source were given the same doses of test agents as the tumor-bearing mice and were observed for body-weight change and deaths (drug toxicity controls). The median survival time of the untreated controls was 27 ± 7 days.

Results and Discussion

In this study the effects of early treatment were assessed by observing the increase in life-span of dying animals, the delay in days of the growth of the primary tumor, and the number of animals cured of this disease. In the case of late treatment, the increase in life-span and number of complete regressions and cures were recorded. In general, even though there are notable exceptions, the compounds most active against the early tumor (Table III) were most active against the established tumor (Table I). All of these highly active compounds contain a cyclohexane ring and most of them are substituted at the 4 position of the cyclohexyl group with a substituent trans to the *N'*-(2-chloroethyl)-*N'*-nitrosoureido function. The most active of these compounds gave 50–70% cures (see Table III) of the early disease while delaying growth of the primary tumor in the dying animals by 9–23 days and increasing their life-span by 34–115% as compared to untreated controls. Certain compounds (**7**, **14**, and **34**) delayed growth of the primary tumor by 12–22 days but did not increase the life-span of the treated animals significantly. Although these results could indicate a much greater effect

Table III. Nitrosoureas Active against Early Lewis Lung Carcinoma[a]

No.	R	X	Dose,[b] mg/kg	% ILS[c] of dying animals	Delay in tumor growth[d]	Cures[e]/ total no. of animals
		RNHCON(NO)$(CH_2)_2$X				
3	*trans*-2-Chlorocyclohexyl	Cl	57	38	2.5	0/10
			38	43	3.3	0/10
5	*trans*-4-[*N'*-(2-Chloroethyl)-*N'*-nitrosoureido]cyclohexyl	Cl	152	41	4.0	0/10
			67	34	2.0	0/10
7	*trans*-4-(1,1-Dimethylethyl)cyclohexyl	Cl	315	19	22.0	0/10
			210	24	4.1	0/10
8	*trans*-4-Methylcyclohexyl	Cl	36	44	14.0	23/50
			24	37	6.5	5/50
9	2,6-Dioxo-3-piperidyl	Cl	17	28	3.8	0/10
			11	32	1.4	0/10
14	Tetrahydro-2*H*-thiopyran-4-yl (*S,S*-dioxide)	F	32	0	15.0	2/10
			21	18	8.3	2/10
17	*trans*-4-Methylcyclohexyl	F	148	0	1.4	0/10
			99	34	2.6	0/10
18	1,3-Dithian-5-yl	Cl	15	42	4.5	0/10
			10	24	3.5	0/10
23	*cis*-4-Carboxycyclohexyl	Cl	20	0		7/10
			13	35	23.0	7/10
24	*trans*-4-Carboxycyclohexyl	Cl	30	115	20.0	13/20
			13	33	7.6	4/20
26	*trans*-4-Methoxycyclohexyl	Cl	60	18	9.5	3/10
			40	0	2.0	0/10
			26	18	0	0/10
29	*trans*-4-(Carboxymethyl)cyclohexyl	Cl	34	23	11.0	5/10
			26	34	10.0	4/10
52	*cis*-2-Methyl-1,3-dithian-5-yl	Cl	30	42	18.0	1/10
			20	0	8.1	0/10
60	*cis*-4-(Carboxymethyl)cyclohexyl	Cl	38	42	9.2	7/10
			25	59	8.2	0/10
32	*trans*-4-(Chloromethyl)cyclohexyl	Cl	60	87	4.3	1/10
			40	74	5.2	0/10
34	*trans*-4-[(Acetyloxy)methyl]cyclohexyl	F	48	7	12.0	2/10
			21	24	1.3	0/10
56	*trans*-3-Methylcyclohexyl	Cl	113	50	6.1	2/10
			50	47	4.0	0/10
57	*trans*-4-Hydroxycyclohexyl	Cl	38	146	10.2	2/10
			26	84	3.3	1/10
58	*cis*-4-Hydroxycyclohexyl	Cl	45	110	18.0	2/10
			30	92	5.1	0/10

[a] See Methods. [b] Single ip injection on day 1 or 2. [c] Increase in life-span. [d] To reach 1 g, days. [e] Animals were observed for at least 80 days posttreatment.

Table IV. Compounds Inactive against Early Lewis Lung Carcinoma

No.	R	X
	RNCON(NO)$(CH_2)_2$X	
20	2-Adamantyl	Cl
61	*cis*-4-Methoxycyclohexyl	Cl
30	2-Deoxy-D-glucos-2-yl	Cl
31	*cis*-2-Methyl-1,3-dithian-5-yl (*S,S,S',S'*-tetraoxide)	Cl
62	*trans*-2-Methyl-1,3-dithian-5-yl (*S,S,S',S'*-tetraoxide)	Cl
54	1-Methylhexyl	Cl

on the growth of the primary tumor than on the metastases (and, incidentally, a greater dependence of metastasis on tumor age than size), it seems more likely that they are simply a manifestation of toxicity. These same compounds, as might be expected, had little effect on either life-span or tumor growth in the advanced disease. Complete regressions of established tumors appear to always result in increases in life-span; some compounds such as **32** and **28** gave large increases in life-span without any complete regressions of tumor, indicating that they may be more effective on metastases than on the primary tumor.

2-Fluoroethyl compounds, in the main, are less active than the corresponding 2-chloroethyl compounds, in contrast to results in the L1210 system in which no such relationship was evident.[2,4,5] The introduction of heteroatoms into the six-membered ring is clearly detrimental to activity, but the most striking structure–activity relationship—and one not easily explained—is the necessity for a 4-methyl or 4-[*N'*-(2-chloroethyl)-*N'*-nitrosoureido] group to be trans to the *N'*-(2-chloroethyl)-*N'*-nitrosoureido function as in **8** and **5** for significant activity. The corresponding cis compounds, **49** and **50**, are inactive against the advanced disease. In the case of the cyclohexanecarboxylic acids **24** and **23**, the trans compound is also more active than the cis, but in this case the cis is quite active. The activity of *N'*-(4,4-dimethylcyclohexyl)-*N*-(2-fluoroethyl)-*N*-nitrosourea (**22**), which is very active, even though it is a fluoro compound, would point to the beneficial effect of the trans grouping rather than the detrimental effect of the cis grouping, an observation also supported by the lower activity of CCNU (**1**) compared to MeCCNU (**8**) and its congeners. Compounds inactive against the advanced disease are listed in Table II and those inactive against the early disease in Table IV. The comparison of Lewis lung activity with L1210 activity shown in Table V clearly indicates the difference in response of these two types of murine cancer to the various nitrosoureas. This comparison is summarized in Table VI. Tables VII–IX summarize structure–activity correlations. The activity of various types of ring and acyclic compounds

Table V. Comparison of the Activity of Nitrosoureas against Advanced and Early Lewis Lung Carcinoma and Leukemia L1210

RNHCON(NO)$(CH_2)_2$X				
R	X	Advanced Lewis lung carcinoma[a]	Early Lewis lung carcinoma[b]	Leukemia L1210[c]
trans-4-[*N'*-(2-Chloroethyl)-*N'*-nitrosoureido]cyclohexyl	Cl	+++	+	++±
trans-4-Methylcyclohexyl	Cl	++±	+++	++
trans-4-Carboxycyclohexyl	Cl	+++	+++	+
2-Adamantyl	Cl	++	−	±
trans-4-Methylcyclohexyl	F	++	+	+
cis-4-Carboxycyclohexyl	Cl	++	+++	+
1,3-Dithian-5-yl	Cl	++	±	++±
2,6-Dioxo-3-piperidyl	Cl	++	±	++
trans-(1,1-Dimethylethyl)cyclohexyl	Cl	++	+	+
trans-2-Chlorocyclohexyl	Cl	+	+	+
cis-2-Methyl-1,3-dithian-5-yl (*S,S,S',S'*-tetraoxide)	Cl	+	−	+
trans-4-[(Acetyloxy)methyl]cyclohexyl	F	+	+	−
2-Deoxy-D-glucos-2-yl	Cl	±	−	−+
Tetrahydro-2*H*-thiopyran-4-yl (*S,S*-dioxide)	F	+	+	+++
trans-4-(Carboxymethyl)cyclohexyl	Cl	+	+++	+++
trans-4-(Chloromethyl)cyclohexyl	Cl	±	++	+
cis-2-Methyl-1,3-dithian-5-yl	Cl	−	+	++
cis-4-(Carboxymethyl)cyclohexyl	Cl	−	+++	++
trans-2-Methyl-1,3-dithian-5-yl (*S,S,S',S'*-tetraoxide)	Cl	−	−	++
1-Methylhexyl	Cl	−	−	−
trans-3-Methylcyclohexyl	Cl	−	++	++
trans-4-Hydroxycyclohexyl	Cl	−	++	++
cis-4-Hydroxycyclohexyl	Cl	−	++	−

[a] Single-dose treatment on day 6 or 7 when tumor is approximately 400 mg in weight. Rating: +++, cures; ++, complete regressions but no cures; +, no cures or complete regressions but >25% increase in life-span; −, no regressions and <25% increase in life-span. [b] Single-dose treatment on day 1 or 2. Rating: +++, >50% cure rate; ++, 10-30% cures and/or >50% increase in life-span; +, no cures but >25% increase in life-span or 10-30% cures and <25% increase in life-span; −, no cures and <25% increase in life-span. [c] 10^5 cells implanted intraperitoneally and single-dose therapy administered intraperitoneally on day 2. Rating: +++, ED_{50}/LD_{10} = 0.3 (ED_{50} = dose for 50% cure rate and LD_{10} = lethal dose for 10%); ±, ED_{50}/LD_{10} = 0.4-0.5; +, ED_{50}/LD_{10} = 0.6-0.8; −, no cures.

Table VI. Comparison of the Activity of Nitrosoureas against Advanced and Early Lewis Lung Carcinoma and Leukemia L1210. Summary

	Advanced Lewis lung carcinoma rating[a] (no. of compounds)	Rating against early Lewis lung carcinoma				Rating against leukemia L1210			
		+++	++	+	−	+++	++	+	−
	+++ (3)	2	0	1	0	1	1	1	0
	++ (7)	1	1	4	1	1	2	4	0
	+ (8)	1	1	3	3	2	1	4	1
	− (8)	1	3	2	2	0	6	1	1
Totals	(26)	5	5	10	6	4	10	10	2

[a] See Table V for rating scale.

Table VII. Activity of Nitrosoureas against Advanced Lewis Lung Carcinoma. Structural Correlations I

RNHCON(NO)$(CH_2)_2$Cl	
R	Activity[a]
2-Chloroethyl	−
Cyclohexyl	++
2-Adamantyl	++
2-Norbornyl	+
Cyclopentyl	−
2-Cyclohexen-1-yl	−
1-Methylhexyl	−
2,6-Dioxo-3-piperidyl	+
2-Deoxy-D-glucos-2-yl	− to +
2-Deoxy-1,3,4,6-tetra-*O*-acetyl-D-glucopyranos-2-yl	− to +
(4-Methylphenyl)sulfonyl	+

[a] See Table V for rating scale.

Table VIII. Activity of Nitrosoureas against Advanced Lewis Lung Carcinoma. Structural Correlations II

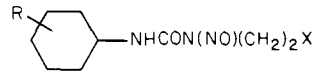
RNHCON(NO)$(CH_2)_2$X		
	Activity[a]	
R	X = Cl	X = F
Tetrahydro-2*H*-thiopyran-4-yl		+
Tetrahydro-2*H*-thiopyran-4-yl (*S,S*-dioxide)	+	+
1,3-Dithian-5-yl	++	+
cis-2-Methyl-1,3-dithian-5-yl	−	
cis-2-Methyl-1,3-dithian-5-yl (*S,S,S',S'*-tetraoxide)	+	
trans-2-Methyl-1,3-dithian-5-yl (*S,S,S',S'*-tetraoxide)	−	
Tetrahydro-3-thienyl (*S,S*-dioxide)	−	

[a] See Table V for rating scale.

is shown in Table VII. Five- and six-membered rings containing heteroatoms are given in Table VIII, and, finally, the highly active compounds—all ring-substituted derivatives of CCNU (1)—are shown in Table IX.

Of the 59 compounds tested against the advanced Lewis lung carcinoma in this study only five, when given as a single dose, failed to cure at least 50% of the mice given leukemia L1210.[2,4,5,10-12] Of these, only four cured mice with advanced Lewis lung carcinoma and with these the cure rate was 10–30%. Eighteen other compounds caused complete regressions of the established tumor and 12 gave

Table IX. Activity of Nitrosoureas against Advanced Lewis Lung Carcinoma. Structural Correlations III



R	Activity rating[a] 4, X = Cl Trans	4, X = Cl Cis	4, X = F Trans	4, X = F Cis	3, X = Cl Trans	3, X = Cl Cis	2, X = Cl Trans	2, X = Cl Cis
H	++				++		++	
Me	+++	−	++		−	−		−
Et	++		−					
4,4-Me_2			++					
Me_2CH			−					
Me_3C	+							
$ClCH_2$	+							
HO_2C	+++	++	+++	−		−		
MeO_2C	++	++						
EtO_2C	+++					++		
HO_2CCH_2	++	+++ (?)						
$X(CH_2)_2N(NO)CONH$	+++	−	−					
Cl							+	+
HO	−	−						
MeO	+							
AcO	+		−					
$AcOCH_2$	+		+					
HCO_2CH_2	+							

[a] See Table V for rating scale.

no complete regressions but did increase the life-span of the mice. Twenty-five compounds had essentially no effect on the course of the disease. Thus, it is clear that the Lewis lung carcinoma is able to differentiate between compounds of equal activity against leukemia L1210. The basis of the selectivity is not clear, although it is logical to assume that drug transport may be an important factor, and, if so, the structural features leading to optimal transport across the blood-brain barrier[3-5] are different from those leading to optimal activity against the Lewis lung carcinoma. Whether this system will be a good predictive system for solid tumors in man is not yet established, but a number of these nitrosoureas have also been evaluated for the activity against other solid tumors in rodents, including the Walker adenocarcinoma 256, the B16 melanotic melanoma, the C3H mammary adenocarcinoma, and the colon adenocarcinoma No. 36. In general, the compounds most active against the Lewis lung tumor were the most active against these other solid tumors,[13] indicating that the structural characteristics necessary for activity against solid tumors are probably fairly general. These features do not ensure activity, however, since, for example, none of the nitrosoureas are significantly active against the Ridgway osteogenic sarcoma.

Acknowledgment. This investigation was supported by the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare, through Contracts NO1-CM-43762 and NO1-CM-43756. The authors are indebted to Mrs. Anne Brazier and Mrs. Kay Johnson for technical assistance.

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