

# Tumor Rejection in Experimental Animals Treated with Radioprotective Thiols<sup>1</sup>

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## SUMMARY

In experimental animals, a systemic treatment with thiols of the mercaptoalkylamine type has affected all of five solid tumors so far investigated. (Three of the tumors were transplanted into the strain of origin.) There was either inhibition of growth or "oncodieresis," *i.e.*, a necrosis and sloughing of tumors conducive to full recovery and repair. Mercaptoalkylamines and derivatives of the type used in our experiments are known to bind to cellular sites by a two-point attachment involving both thiol and amino groups. One of these compounds, cysteamine, was active in its native, unsubstituted form, but did not bring about oncodieresis when either the amino or thiol group, or both, were alkylated. Mercaptopropylamine, the 3-carbon homolog of cysteamine, was less active. Cystamine, a disulfide dimer of cysteamine that has no free reactive sulfhydryl, did not induce any reaction. Thioglycerol, lacking a terminal amino group, had only negligible activity. Rejection was much more striking when treatment was started on the day of inoculation than when started 7 days later. Male mice rejected better than females. Results were inferior when two of the agents were given simultaneously or together with other radioprotectants, such as L-cysteine, glutathione, dimethyl sulfoxide, or reserpine. Tumor rejection was enhanced when the phosphorylated thiols, *S*-2-(3-amino-propylamino)ethylphosphorothioic acid or *S*-(2-ethylguanidine)phosphorothioic acid, were given simultaneously with the radioprotective serotonin, but there was no synergy of serotonin with the nonphosphorylated compounds *S*-2-aminoethylisothiuronium bromide or cysteamine. Serotonin alone did not affect the tumors.

## INTRODUCTION

Previous studies (3-6) have led us to conclude that the proliferation of (murine) tumor cells is dependent upon the unhindered interchange between thiols and disulfides. We have assumed that this metabolic feature might be exploitable for chemotherapy of neoplasia, and early efforts were aimed at blocking reactive sulfhydryls with alkylating

agents such as iodoacetate or *N*-ethyl-maleimide. However, these compounds cross the plasma and nuclear membranes (C. A. Apffel, J. E. Walker, and G. Theriault, unpublished radiochemical studies), are toxic for normal as well as for tumorous cells, and offer no useful therapeutic margin. Therefore, we decided upon an altogether different approach, *i.e.*, to bring the electron shuttle between thiols and disulfides to a slowdown or even to a standstill by the formation of mixed disulfides. This approach can be implemented with agents relatively innocuous for normal cells but capable of binding strongly to the membrane system of tumor cells. Various radioprotective thiol compounds, all mercaptoalkylamines or derivatives, seem to fulfill the requirements. They are known to bind with sites on particulate fractions of cells by a 2-point attachment involving both thiol and amino groups (8, 15, 16). Their designation, name, and structure are listed in Table 1.

The radioprotective properties of cysteamine, *i.e.*, MEA,<sup>2</sup> were demonstrated by Bacq *et al.* in 1953 (7). WR-638 is the *S*-phosphorylated cysteamine. MPA has one more carbon link between amino and thiol group. AET, in aqueous solutions above pH 7, is converted to the thiol  $\beta$ -mercaptoethylguanidine by a rearrangement involving transguanylation (14, 19, 21). WR-2721, as well as WR-638, belongs to a series of phosphorothioate radioprotectors initiated by Åkerfeldt (2), where phosphorylation protects the thiol function. Its biochemical and pharmacological properties have been studied by Yuhás (23), Yuhás and Storer (24), and Harris and Philipps (18). EGP is the *S*-phosphorylated form of  $\beta$ -mercaptoethylguanidine. It was synthesized by us, considering that the nonphosphorylated form of WR-2721 is only marginally radioprotective (23), whereas the equally nonphosphorylated  $\beta$ -mercaptoethylguanidine, *i.e.*, the active form of AET, is one of the best radioprotectants.

We are presenting evidence that, under appropriate experimental conditions, MEA, MPA, AET (via  $\beta$ -mercaptoethylguanidine), WR-2721, and EGP,<sup>3</sup> administered

<sup>1</sup>Supported in part by American Cancer Society (Massachusetts Division) Grant 1408-C, and by the Pondville Hospital Trust Fund for Cancer.

Received April 26, 1974; accepted November 5, 1974.

<sup>2</sup>The abbreviations used are: MEA,  $\beta$ -mercaptoethylamine namely, cysteamine; WR-638, *S*-(2-aminoethyl)phosphorothioic acid; MPA,  $\beta$ -mercapto-propylamine; AET, *S*-2-aminoethylisothiuronium bromide; WR-2721, *S*-2-(3-aminopropylamino)ethyl phosphorothioic acid; EGP, *S*-(2-ethylguanidine) phosphorothioic acid; 5-HT, 5-hydroxytryptamine.

<sup>3</sup>To the best of our knowledge, none of these compounds has been previously assayed for antitumor activity on their own merit, *i.e.*, other than as radioprotectants, by single injection before X-irradiation or

daily i.p., do bring about the inhibition or rejection of otherwise lethal solid tumors.

## MATERIALS AND METHODS

**Animals and Tumors.** C57BL/P mice were from our inbred Pondville subline of C57BL. C3H/HeJ and A/J mice originated from the Jackson Laboratory, Bar Harbor, Maine, and Swiss mice were from the Charles River Breeding Laboratory, Wilmington, Mass. Syrian golden hamsters were provided by Dennen Animal Industries, Gloucester, Mass. All strains were maintained by brother × sister mating in our colony. Five solid tumors were used: (a) Krebs-2 in Swiss mice, (b) MC sarcoma in C57BL/P mice (5), (c) sarcoma 1 in A/J mice, (d) BAC/P in C3H/HeJ (6), and (e) MC tumor in Syrian golden hamsters. Since the s.c. inoculation of ascites tumor is often followed by rejection (5), Krebs-2 solid was maintained, as were the other solid murine tumors, by the s.c. trocar inoculation (between the shoulder blades) of 2- x 4-mm pieces of solid tumor, 9 to 15 days old. All experimental animals were 10 to 14 weeks old. Experimental groups consisted of 10 untreated controls and 10 treated mice for each assay. Fifty mice were treated for histology versus 30 untreated controls. MC sarcoma was originally methylcholanthrene-induced and maintained in the C57BL/P strain of origin (5). BAC/P (6) originated from a spontaneous breast adenocarcinoma in a C3H/HeJ mouse and has since then been carried in the strain of origin. The MC tumor of hamsters (methylcholanthrene induced by G. P. Fulton at Boston University in 1951) was maintained by serial transplantation of 4- x 4-mm pieces under the skin of the scapular region. Groups included 8 untreated and 8 treated hamsters. The Krebs-2 tumor is allogeneic. The other 3 murine tumors are close to syngeneity, insofar as they are carried in the strain of origin. The case of the hamsters is special, since all hamsters used in this country stem from littermates at the Hebrew University of Jerusalem in 1953 (22). For MC tumors in C57BL/P mice or hamsters, for BAC/P in C3H/HeJ mice, and for Krebs-2 in Swiss mice, the rates of takes and of lethality in untreated hosts were 100%; for sarcoma 1 in A/J mice, they were 80% ± 2 [ratios established on the basis of 1383 inoculates (345 sarcoma 1) over a period of 42 months.]

**Compounds.** Cysteamine, used by us, was 2-aminoethanethiol hydrochloride (Aldrich Chemical Co., Milwaukee, Wis.). MPA was a gift from Dr. R. C. Clapp, United States Army Laboratories, Natick, Mass.<sup>4</sup> AET hydrobromide was purchased from Calbiochem (La Jolla, Calif.). WR-2721 and WR-638 were supplied by the Division of

administration of radiomimetic drugs; cf., cumulative indexes of "Cancer Chemotherapy Screening Data LVIII," i.e., CANCER RESEARCH, 27(12), 1967; "Cancer Chemotherapy Abstracts" (NCI), and *Cancer Chemotherapy Reports*, 1967-1974.

<sup>4</sup> Batches of dry MEA or MPA, and solutions thereof in distilled water at low concentrations, undergo autooxidation, particularly, at room temperature and when exposed to light. Since disulfide compounds are inactive or even tumor-enhancing, oxidized dimers should be eliminated from batches of the dry substances. For treatment, fresh solutions are to be prepared weekly in isotonic saline, refrigerated and stored in amber glass under nitrogen or under mineral oil.

Medicinal Chemistry, Walter Reed Army Institute of Medical Research (courtesy of Dr. M. I. Varon, Dr. K. Kinnamon, Dr. M. H. Heiffer, and Dr. P. S. Loizeaux). EGP, the guanidinium derivative of WR-638, was prepared following a method of Habeeb (17) by reacting WR-638 in aqueous solution with 1-guanyl-3,5-dimethyl pyrazole nitrate. The product was crystallized from ethanol, and its infrared spectrum was compared with that of WR-638 and of guanidine. Introduction of the guanidine group into the new molecule was thus ascertained (M.W., 199.17; m.p., 225°). (A complete structural analysis will be reported elsewhere.)

**Treatment.** All agents were dissolved in 0.9% NaCl solution. The pH was adjusted to 7.8 in the case of AET, and to 7.4 for all other compounds. Dosage was based on the amount lethal to 50% of the animals, as determined by radiobiologists, and on the posology adopted for radioprotective usage (single doses of 1 mg corresponding approximately to one-tenth of the 50% lethal dose). Dose response was explored by administering daily doses of 0.5, 1, 1.5, 2, and 4 mg (AET and WR-2721) to groups of 10 tumor-bearing mice. The dose of 1 mg/day/mouse in 0.1 ml 0.9% NaCl solution was adopted as being closest to the optimum. It was injected i.p., daily, starting on the day of inoculation (1 to 2 hr later), the next day, or later. Treatment was terminated on Day 12 for C57BL/P mice with MC sarcoma and for C3H/HeJ mice with BAC/P adenocarcinoma. Swiss mice, with Krebs-2 solid tumor growing at a slower pace, were treated during 18 days. Hamsters, with slow growing MC tumors, and more susceptible to i.p. infection, were given injections every other day (10 mg MEA in 0.1 ml i.p.) for 24 days.

**Synergy.** Serotonin, i.e., 5-HT, has been recognized as an efficient radioprotectant (20) either alone or in synergy with mercaptoalkylamines (15, 16). Serotonin creatinin sulfate, monohydrate (Nutritional Biochemicals Co., Cleveland, Ohio), was added to the solutions of the other agents at a concentration of 0.1%, each 0.1-ml injection conveying 100 µg. Ten controls (C57BL/P with MC sarcoma) were treated with 5-HT alone (100 µg/day).

**Histology.** For histological follow-up, 80 male C57BL/P mice were inoculated with MC sarcoma that had grown for 16 days in the donor. Thirty animals were untreated controls. Fifty were treated daily with 1 mg WR-2721 + 0.1 mg 5-HT i.p. for 12 days. Three treated and 2 control mice were sacrificed by cervical dislocation after 24, 48, and 72 hr. From then on, the same number of treated and control animals were sacrificed every 2nd day until Day 21 after inoculation. Hair overlying the zone of inoculation was clipped, and skin flaps, 30 to 44 mm in diameter, were cut out. Inocula, or tumors, surrounded by a 8- to 10-mm large zone of healthy tissue, were excised but left adhering to the flaps and fixed in 10% formalin. Sections were stained with hematoxylin and eosin.

## RESULTS

A discrete phenomenon often brought about by treatment with the various active mercaptoalkylamines and derivatives

was oncodieresis, *i.e.*, necrosis, sloughing and, in most favorable cases, total rejection and lasting recovery (Fig. 1; Chart 1). When hair overlying the inocula was clipped, a discoloration of the skin could be observed as early as 3 to 4 days after initiation of treatment. There were purpuric patches and petechiae on a background of yellowish pallor. A few days later, the discoloration took on a greenish cast; the tumor takes, instead of remaining acuminate (Fig. 2), tended to spread and became soft (Fig. 3). Between Days 8 and 10, the tumors appeared blighted, and the overlying skin began to exhibit the dark appearance and hard consistency of a mummified scab. Breaks occasionally occurred between scab and healthy skin, giving passage to a pale-yellow, pasty, slightly granulous pus. Between Days 12 and 30, the scabs were shed, leaving clean ulcerations. At that point, 1 of 3 things happened. (a) A remaining, unaffected area of the tumor pursued its growth. (b) After seemingly total rejection, growth would resume (particularly, in the precaudal region, at the point of penetration of the tumor-loaded trocar). (c) After total rejection, the resulting ulceration would retract, undergo healing, and leave a small, inapparent scar. These animals remained tumor free for 2 to 5 months and were then challenged. Necrosis, scabs and ulcerations have always covered an area much more extensive than early takes (often 20 x 35 mm).

The experiment designed for gathering histological information ensured the statistical significance of oncodieresis. Included were 50 treated (1 mg WR-2721 + 0.1 mg 5-HT per day) and 30 untreated male C57BL/P mice inoculated with the MC tumor. Extensive to complete reaction was observed in 44 of the treated mice. Six had been sacrificed after 24 and 48 hr, *i.e.*, before the phenomenon could be

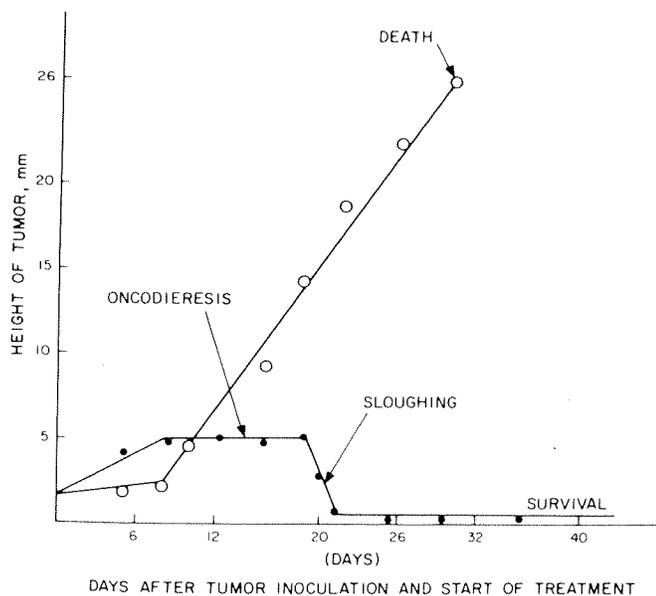


Chart 1. Height of tumors (mm above level of surrounding skin) in function of time after inoculation. O, mean for 20 untreated C57BL/P male mice; ●, mean for 48 C57BL/P males, treated with an active thiol or thiophosphate (1 mg/day *i.p.*) and having undergone oncodieresis with total tumor rejection. Tumor growth interrupted by effective oncodieresis (●) contrasts with progressive, lethal tumor growth in untreated controls (O).

evident.

Other results are summarized in Tables 2 and 3. Of the 7 substances assayed, 6 previously had been found to be effective radioprotectants. All these were active in bringing about reactions against tumors, as was EGP, which fulfills the structural requirements to be a radioprotectant but was never tested as such. One discrepancy appeared, however. WR-638, the phosphorylated form of cysteamine, was less active than native cysteamine. Either oncodieresis or inhibition was brought about in all host-tumor systems investigated, but the various tumors were not affected to the same extent. MC sarcoma in C57BL/P males responded best, and definitely better than in corresponding females. When treatment was started 7 days after inoculation of MC tumors, oncodieresis occurred in only 4 of 10 C57BL/P males and did not lead to recovery. AET did not affect BAC/P in C3H/HeJ, but EGP did. Although BAC/P tumors in C3H/HeJ were not rejected by oncodieresis in response to WR-2721, there was 60% inhibition of growth and histological evidence of reaction when the tumors were excised, weighed, and examined on Day 20 after inoculation. In hamsters, too, there was inhibition rather than rejection. Whereas 8 tumors developed in 8 controls, only 2 tumors were found in 8 treated animals on Day 20.

Dose-response experiments with C57BL/P mice bearing MC sarcoma indicated that the adopted dosage of 1 mg/day/mouse was close to the optimum. With doses of 2 to 4 mg/day, toxicity of the compounds (AET and WR-2721) became apparent by weight loss and occasional death, while efficiency was not significantly increased. When thiol compounds were given alone at the rate of 1 to 2 mg/day, no animals died during treatment, and weight change varied from +3.0 to -9.8% (-4.9% with MEA; -9.8% with WR-638).

Sixty mice, that had undergone tumor rejection, followed by total recovery and repair (48 C57BL/P and 12 Swiss), were challenged with fresh MC and Krebs-2 tumors 2 to 5 months after tumor rejection. The newly inoculated tumors grew as usual, without significant impairment by acquired immunity.

Treatment of MC sarcoma-bearing C57BL/P mice with the disulfides, dithiodiethanol or L-cystine, brought about a respective 214 or 187% enhancement of tumor growth within the 12-day period of treatment. This corollary finding agrees with the observation of Broome and Jeng (11) that disulfides of certain thiols promote the growth of lymphoma cells.

**Histology.** Differences between treated and untreated tumors became evident after 72 hr (Figs. 4 and 5). Sections stemming from experimental animals displayed marked acute inflammation with necrosis. In 2 specimens, the area of inflammation contained rare scattered tumor cells. A 3rd specimen was free of tumor. From then on, the differences were as follows. In control specimens, the tumors were larger, acuminate, and well circumscribed (Fig. 6). There was occasional scant inflammation in the surrounding connective tissue. Necrosis, when observed, was central and of the ischemic type.

On sections from treated animals, the lesions were spread

Table 1

*Mercaptoalkylamines, mercaptoalkylguanidine, and derived thiophosphates with radioprotective and antitumoral activity*

Designation	Chemical name	Structure
MEA	$\beta$ -mercaptoethylamine (cysteamine)	$H_2NCH_2CH_2SH$
WR-638	S-(2-aminoethyl)phosphorothioic acid	$H_2NCH_2CH_2SPO_3H_2$
MPA	$\beta$ -mercaptopropylamine	$H_2NCH_2CH_2CH_2SH$
AET	S-2-aminoethylisothiuronium bromide	$H_2NCH_2CH_2SC(=NH)_2 \cdot HBr$
MEG	$\beta$ -mercaptoethylguanidine	$NH_2C(=NH)NHCH_2CH_2SH$
WR-2721	S-2-(3-aminopropylamino)ethyl phosphorothioic acid	$H_2NCH_2CH_2CH_2NHCH_2CH_2SPO_3H_2$
EGP	S-(2-ethylguanidine)phosphorothioic acid	$H_2NC(=NH)NHCH_2CH_2SPO_3H_2$

Table 2

*Effect on tumors of some radioprotective thiol compounds*

The compounds were administered as follows: 1 mg/day i.p. for 12 days, except animals with Krebs-2 were treated for 18 days. Ratios of 9/10 are significant at the 0.5% level. Ratios of 5% or less are not significant. Statistical evaluation from table for use with binomial samples (D. Mainland, L. Herrera, and M. Sutcliffe, 1956; Department of Medical Statistics, New York University, College of Medicine).

Compound	Tumor	No. with oncodi- eresis <sup>a</sup> /10 animals	No. of tumor-free survivors <sup>b</sup> > 60 days/10 animals
MEA	MC sarcoma	10	9
MEA	Sarcoma 1 <sup>c</sup>	2	2
MEA	Krebs-2	0	0
MEA	BAC/P	0	0
AET	MC sarcoma	9	8
AET	Sarcoma 1	0	2
AET	Krebs-2	8	3
AET	BAC/P <sup>c</sup>	0	0
WR-638	MC sarcoma	8	2
WR-2721	MC sarcoma	10	6
WR-2721	Sarcoma 1	0	2
WR-2721	Krebs-2	6	4
WR-2721	BAC/P <sup>c</sup>	1	0
EGP	MC sarcoma	9	5
EGP	Sarcoma 1	1	2
EGP	Krebs-2 <sup>c</sup>	1	0
EGP	BAC/P	0	0
0.9% NaCl solution	MC sarcoma	0	0
0.9% NaCl solution	Sarcoma 1	0	2
0.9% NaCl solution	Krebs-2	0	0
0.9% NaCl solution	BAC/P	0	0

<sup>a</sup> Necrosis and sloughing.<sup>b</sup> Twenty mice survived 150 days without recurrence before being challenged.<sup>c</sup> Significant inhibition of tumor growth during the period of treatment: 60% and above by weight in mg. of excised tumors.

over a larger area (Fig. 7). There were inflammatory exudates with polymorphonuclear leukocytes and occasional floating tumor cells. Vacuolization (Fig. 8), pyknosis, and loss of cohesion were observed among the tumor cells. Subsequent necrosis was massive and peripheral (Figs. 9 and 10), starting in the deep s.c. layers and progressing toward the epidermis. The last tumor cells to hold out were in a shallow band closest to the epidermis. Only a few scarce lymphocytes and plasma cells were seen at the periphery, but not more than in controls. The overall image was not suggestive of an immune reaction of the allograft type. The fibroblastic reaction found in controls was absent from treated tumors. Vascular changes like those characterizing the Shwartzman phenomenon were not seen, and calcification was proportional to the extent of necrosis.

## DISCUSSION

Thiol compounds, which we have found to induce tumor rejection, are known radioprotectants. According to radiobiologists (7, 15, 16), they operate essentially in 2 ways, by radical scavenging and by forming mixed disulfides. Evidence has been gathered (15) that indicates that the radioprotective compounds attach themselves to cellular sites at 2 points. Admittedly, their basic amino groups form hydrogen bonds with anionic sites (carboxyl or phosphoryl groups), while their thiols interact with corresponding disulfides and perhaps sulfhydryls in close vicinity (4.6 Å in the case of cysteamine). A free sulfhydryl and a free amino group were necessary for oncodieresis to occur. The 2-point attachment appears to be a prerequisite of tumor rejection as well as of radioprotection.<sup>5</sup> When the chain length

<sup>5</sup> Significant antitumor effects have been achieved by M. A. Apple and D. M. Greenberg (*Cancer Chemotherapy Reports*, 53: 195-198, 1969) with the related compound DL-2-mercapto-3-hydroxypropanal, a structural analog of DL-glyceraldehyde. In this case, an aldehyde function replaces the amino group of MPA at one end of the molecule, and can form a covalent bond.

Table 3

Results of treatment with radioprotective thiols:<sup>a</sup> effect of structural changes and association with other radioprotectants

Ratios of 9/10 are significant at the 0.5% level. Ratios of 5% or less are not significant. Statistical evaluation from table for use with binomial samples (D. Mainland, L. Herrera, and M. Sutcliffe, 1956; Department of Medical Statistics, New York University, College of Medicine).

Compounds	No. with oncodieresis <sup>b</sup> /10 animals	No. with tumor-free survival > 60 days/10 animals <sup>c</sup>
MEA alone	10	9
SH-blocked MEA	0	0
NH <sub>2</sub> -blocked MEA	0	0
Double-blocked MEA	0	0
Cystamine	0	0
Thioglycerol	2	0
MPA	5	1
MEA + L-cysteine	5	0
MEA + GSH <sup>d</sup>	4	0
MEA + DMSO <sup>e</sup>	0	0
MEA + reserpine	0	0
MEA + 5-HT	5	2
AET alone	9	8
AET + 5-HT	2	0
WR-2721 alone	10	6
WR-2721 + 5-HT	10	7
EGP alone	9	5
EGP + 5-HT	10	9
0.9% NaCl solution	0	0

<sup>a</sup> C57BL/P mice bearing MC sarcoma treated with 1 mg/day i.p. for 12 days.

<sup>b</sup> Necrosis and sloughing.

<sup>c</sup> Forty-seven mice listed in this column remained free of tumor growth. Twenty were challenged with fresh tumor after an interval of 60-150 days.

<sup>d</sup> GSH, reduced glutathione.

<sup>e</sup> DMSO, dimethyl sulfoxide.

between the 2 points was increased by 1 carbon as in MPA, activity was markedly decreased.

Radioprotective thiols bind to sites in the nuclei and mitochondria of normal cells (8, 15). In tumors, however, they may act on the plasma membrane where basic amino or guanidino endings would be retained by the increased density of anionic groups (1). A plausible explanation of oncodieresis is that the compounds complex with surface antigens of the tumor cells, thus evoking a humoral immune response to the transplanted tumor. However, sulfhydryl-bearing substances do inhibit complement action (12, 13), and the possibility of a selective, nonimmunological cytotoxicity has to be envisaged. —SH groups and disulfides are important for the maintenance of membrane structure (9). A cytolytic action may involve an interaction with membrane disulfides or —SH groups, following attachment to anionic binding sites. Alternatively, the agents, once anchored by their thiol group, may momentarily alter the surface charge of tumor cells.

Strictly speaking, our results do not depend on the use of compounds endowed with free reactive —SH groups, inasmuch as the agents WR-2721, WR-638, and EGP, in which the thiol is phosphorylated, performed as well as agents in which the thiol is immediately reactive. Nevertheless, the contradiction with our basic hypothesis about the

role of the free —SH group is more apparent than real. Yuhás (23) and Harris and Philipps (18) have established that WR-2721 must be dephosphorylated in order to become active, and the other 2 compounds can be assumed to behave in the same way. Dephosphorylation of the thiophosphates takes place within the cells but also extracellularly, as enzymes leak from the cells into the medium (18). Normal and malignant cells do not differ significantly in their ability to dephosphorylate WR-2721 (18).

During our investigation, it became evident that big, well-established tumors were poorly accessible to treatment with mercaptoalkylamines. If a clinical application is ever to be possible, an explanation has to be found for this failing. Since direct injection of the agents into such tumors was ineffective, we do not regard the inadequate tumor size/dose ratio as a satisfactory explanation. We are exploring the possibility that an inhibitor may hamper the activity of the agents as well as the possibility of a more active enzymatic breakdown in females and in advanced tumors.

Why has the ability of radioprotective thiols and thiophosphates to induce tumor rejection never been observed before? The reasons appear to be complex. First, with few exceptions,<sup>6</sup> they were given to nontumor-bearing animals. Second, they were not administered in a sustained way, by daily injections, but in single doses, minutes before or hours after X-irradiation. Finally, because the protectants were administered in combination with X-irradiation, their thiol groups bound free radicals and were no longer available to form mixed disulfides at sites of tumor cells.

In conclusion, dramatic effects can be exerted on early tumors with mercaptoalkylamines and related compounds, a fact which may open new avenues toward chemotherapy and perhaps chemoprophylaxis of neoplastic diseases.

## ACKNOWLEDGMENTS

We gratefully acknowledge support by the Pondville Hospital Trust Fund for Cancer. Our thanks are extended to Professor S. Marglin, H.I.E.R., Harvard University, for his careful revision of the manuscript, to Professor F. Homburger for his observations and advice, as well as to Dr. M. I. Varon, Cpt., MC, U.S.N., Director of the AFRR, Bethesda, Md., and Dr. R. C. Clapp (United States Army Laboratories, Natick, Mass.) for contributing some of the compounds. We further thank J. Donohue for technical assistance and D. E. Noonan for constant clerical help.

## REFERENCES

1. Abercrombie, M., and Ambrose, E. J. The Surface Properties of Cancer Cells. A Review. *Cancer Res.*, 22: 525-548, 1962.
2. Åkerfeldt, S. Radioprotective Effects of S-phosphorylated Thiols. *Acta Radiol. Therap. Phys. Biol.*, 1: 465-469, 1963.

<sup>6</sup> AET and WR-638 (cystaphos) have been administered to rats and mice bearing experimental tumors as protectants against the toxicity of radiomimetic drugs. The 2 agents were given by single injection 30 min before the drug was administered. Toxicity was repeatedly decreased and survival was prolonged. In one instance, pretreatment with AET increased tumor regression, whereas cystamine decreased antitumor activity (*Cancer Chemotherapy Abstracts*, 68-2206, 69-1563, 69-2348, 69-2444, 69-2665).

3. Apffel, C. A. Deactivation of Tumor Cells by Blocking of Sulfhydryls. *Proc. Am. Assoc. Cancer Res.*, *13*: 1, 1972.
4. Apffel, C. A., Arnason, B. G., and Peters, J. H. Induction of Tumour Immunity with Tumour Cells Treated with Iodoacetate. *Nature*, *209*: 694-696, 1966.
5. Apffel, C. A., and Peters, J. H. Rejection of Lethal Ascites Tumors after Subcutaneous Inoculation: A Phenomenon of Antigenic Expression? *J. Natl. Cancer Inst.*, *39*: 1129-1139, 1967.
6. Apffel, C. A., and Walker, J. E. Tumor Growth and Disulfide Reduction: Possible Dependence on Protein Disulfide Reductase. *J. Natl. Cancer Inst.*, *51*: 575-583, 1973.
7. Bacq, Z. M., Dechamps, G., Fischer, P., Hervé, A., Le Bihan, H., Lecomte, J., Pirotte, M., and Rayet, P. Protection against X-rays and Therapy of Radiation Sickness with  $\beta$ -Mercaptoethylamine. *Science*, *117*: 633-636, 1953.
8. Bacq, Z. M., and Goutier, R. Mechanisms of Action of Sulfur-containing Radioprotectors. In: *Recovery and Repair Mechanisms in Radiobiology*. Brookhaven Symposia in Biology #20, pp. 241-262 Upton, N.Y.: Brookhaven National Laboratory, Associated Universities, Inc., 1967.
9. Benesch, R. E., and Benesch, R. Relation between Erythrocyte Integrity and Sulfhydryl Groups. *Arch. Biochem.*, *48*: 38-42, 1954.
10. Bradford, R. H., Shapira, R., and Doherty, D. G. Selective Intracellular Binding of Radiation Protective Agents by Mammalian Tissues. *Federation Proc.*, *16*: 157, 1957.
11. Broome, J. D., and Jeng, M. W. Promotion of Replication in Lymphoid Cells by Specific Thiols and Disulfides *in vitro*. Effects on Mouse Lymphoma Cells in Comparison with Splenic Lymphocytes. *J. Exptl. Med.*, *138*: 574-592, 1973.
12. Cushman, W., Becker, E. L., and Wirtz, G. Concerning the Mechanism of Complement Action. III. Inhibitors of Complement Activity. *J. Immunol.*, *79*: 80-83, 1957.
13. Dierich, M. P., Ferrone, S., Pellegrino, M. A., and Reisfeld, R. A. Chemical Modulation of Cell Surfaces by Sulfhydryl Compounds: Effect on C3b Receptors. *J. Immunol.*, *113*: 940-947, 1974.
14. Doherty, D. G., and Burnett, W. T., Jr. Protective Effect of S, $\beta$ -aminoethylisothiuronium. Br. HBr and Related Compounds against X-radiation Death in Mice. *Proc. Soc. Exptl. Biol. Med.*, *89*: 312-314, 1955.
15. Eldjarn, L., and Pihl, A. Mechanism of Protective and Sensitizing Action. In: M. Errera and A. Forsberg (eds.), *Mechanisms in Radiobiology*, p. 278. New York: Academic Press, Inc., 1968.
16. Fabrikant, J. I. *Radiobiology*, pp. 208-211. Year Book Medical Publishers, Chicago: 1972.
17. Habeeb, A. F. Guanidination of Proteins. *Methods Enzymol.*, *25*: 558-566, 1972.
18. Harris, J. W., and Philipps, T. L. Radiobiological and Biochemical Studies of Thiophosphate Radioprotective Compounds Related to Cysteamine. *Radiation Res.*, *46*: 362-379, 1971.
19. Khym, J. X., Shapira, R., and Doherty, D. G. Ion Exchange Studies of Transguanylation Reactions. I. Rearrangement of S-2-Aminoethyl-isothiourea to 2-Mercaptoethylguanidine and 2-Aminothiazoline. *J. Am. Chem. Soc.*, *79*: 5663-5666, 1957.
20. Rixon, R. H., and Baird, K. M. The Therapeutic Effect of Serotonin on the Survival of X-irradiated Mice. *Radiation Res.*, *33*: 395-402, 1968.
21. Shapira, R., Doherty, D. G., and Burnett, W. T., Jr. Chemical Protection against Ionizing Radiation. III. Mercaptoalkyl-guanidines and Related Isothiuronium Compounds with Protective Activity. *Radiation Res.*, *7*: 22-34, 1957.
22. Yerganian, G. History of Cytogenetics of Hamsters. *Progr. Exptl. Tumor Res.*, *16*: 2-34, 1972.
23. Yuhas, J. M. Biological Factors Affecting the Radioprotective Efficiency of S-2-(3-Aminopropylamino)ethylphosphorothioic Acid (WR-2721). LD<sub>50/30</sub> Doses. *Radiation Res.*, *44*: 621-628, 1970.
24. Yuhas, J. M., and Storer, J. B. Differential Chemoprotection of Normal and Malignant Tissues. *J. Natl. Cancer Inst.*, *42*: 331-335, 1969.

FIG. 1. Tumor rejection has occurred in Mice 1 and 3 (from left). Resulting ulcerations are retracting. Mice 2 and 4 are untreated controls. The 1st and 2nd are male C57BL/P mice inoculated 25 days earlier with MC sarcoma; the 3rd and 4th are male C3H/HeJ mice inoculated 28 days earlier with BAC/P. Mouse 1 was treated with WR-2721; the 3rd mouse, with EGP + 5-HT.

Fig. 2. Control group of 10 untreated C57BL/P males bearing MC sarcoma on Day 11 after inoculation. The tumors are acuminate.

Fig. 3. Group of 10 C57BL/P males with MC sarcoma (on Day 11 after inoculation) treated with WR-2721. Inflammation and necrosis are in progress. The tumors have collapsed and are soft. There is discoloration of the overlying skin.

FIG. 4. Untreated control after 72 hr; MC sarcoma in C57BL/P males. Skin surface, not shown, is at bottom, beyond the layer of s.c. muscles. Tumor occupies the upper one-half of the section. H & E,  $\times$  400.

Fig. 5. MC sarcoma treated with WR-2721, after 72 hr. From upper to lower side, massive necrosis, zone of inflammation, shallow band of unaffected tumor cells, and s.c. muscles with interstitial polymorphonuclear leukocytes. H & E,  $\times$  400.

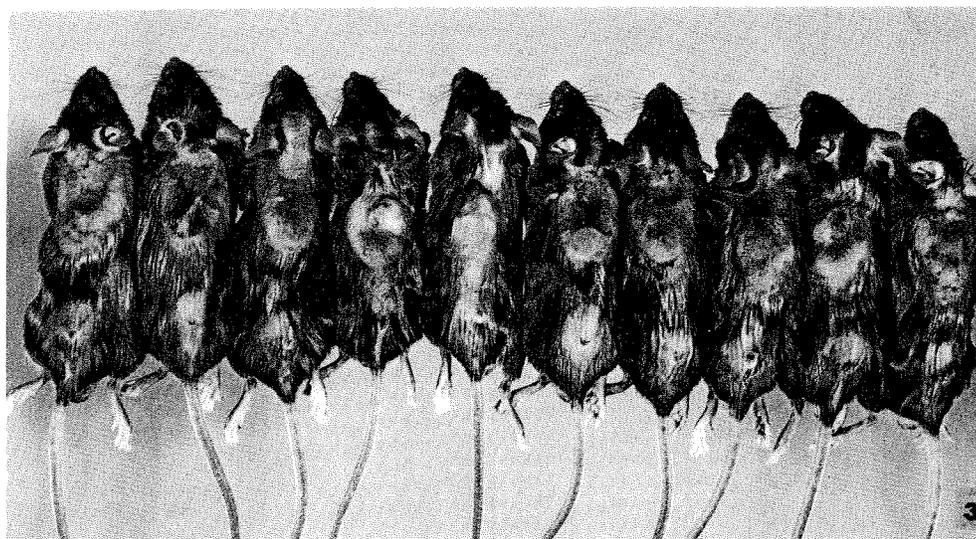
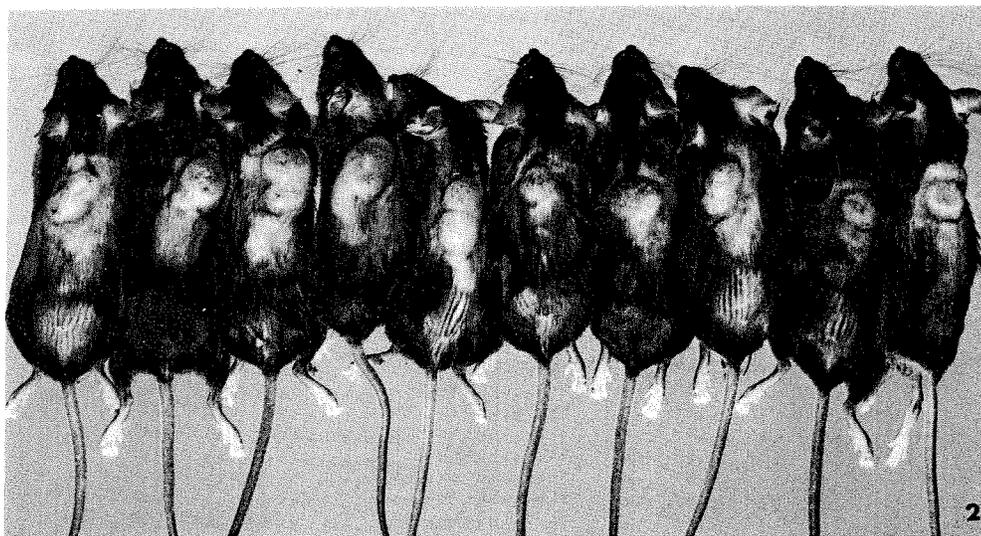
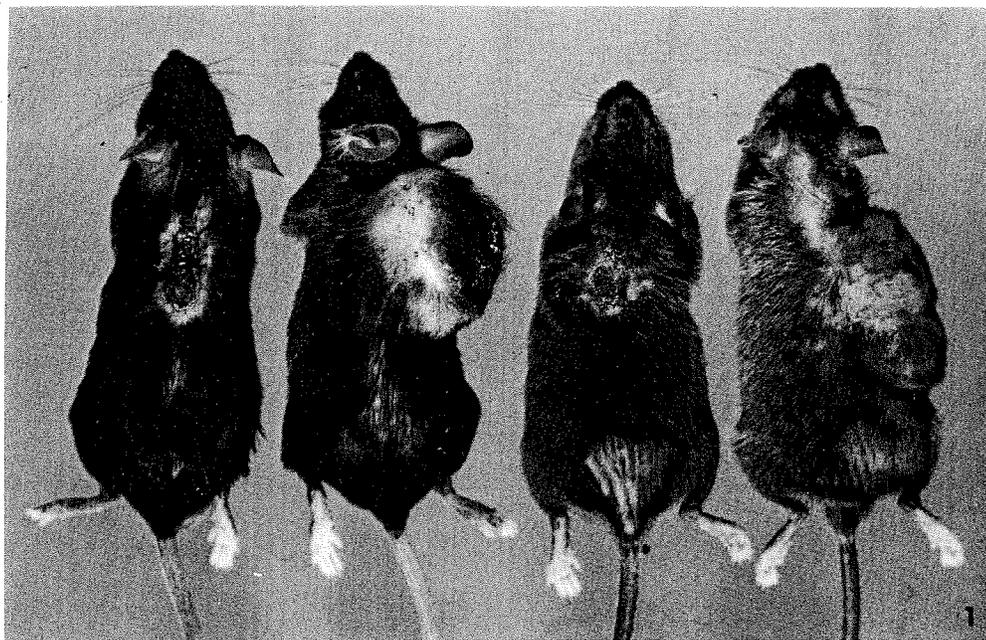
Fig. 6. Untreated control, 7 days after inoculation. Well-circumscribed, acuminate tumor. Mild peripheral inflammatory infiltrate and small areas of necrosis within the tumor. H & E,  $\times$  12.

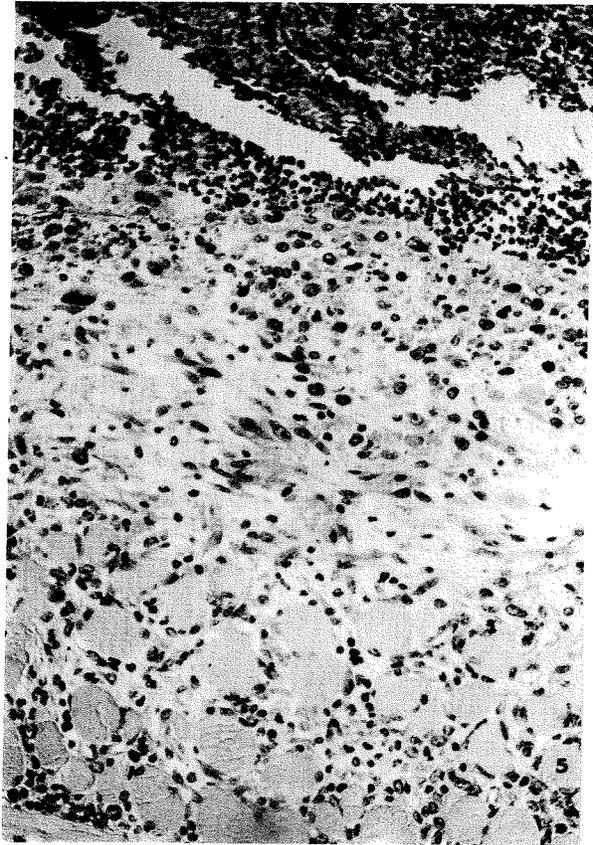
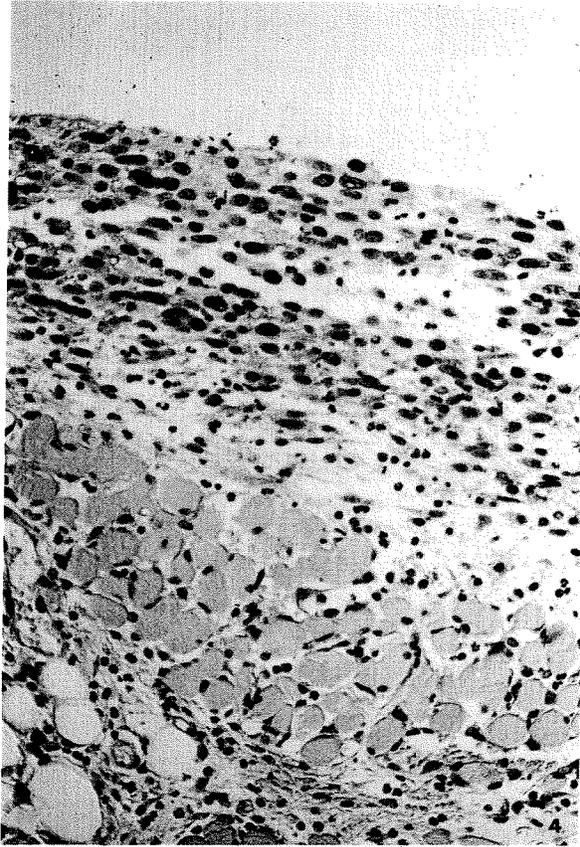
Fig. 7. Treated, 7 days after inoculation. The lesions are neither circumscribed nor acuminate, and necrosis is extensive. H & E  $\times$  12.

Fig. 8. Treated, 5 days after inoculation. Extensive necrosis in the deep layers of the tumor (right). Closer to the epidermis (left), many tumor cells appear to have lost cohesion and viability. There is pyknosis and vacuolization. H & E,  $\times$  400.

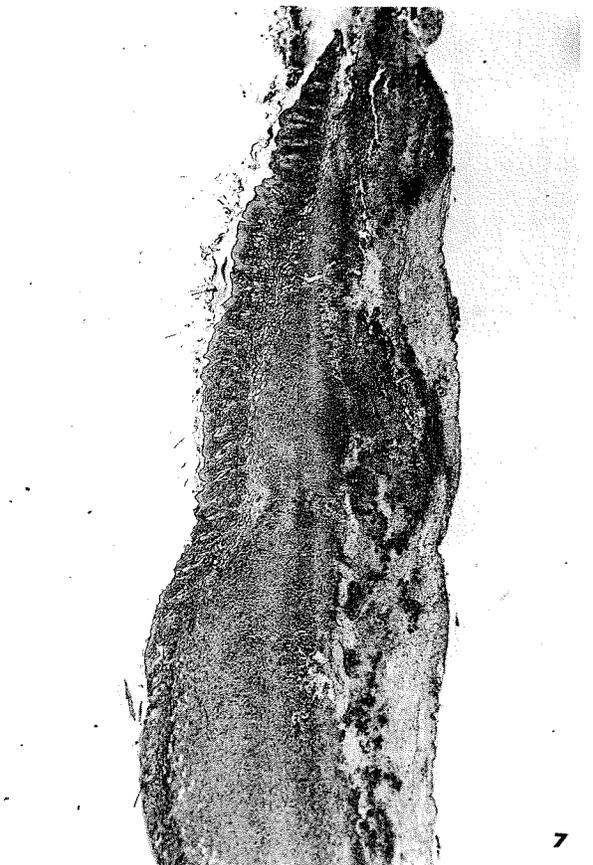
Fig. 9. Untreated control, 13 days after inoculation. H & E,  $\times$  400.

Fig. 10. Treated, 13 days after inoculation. In sharp contrast with Fig. 9, there is extensive necrosis with infiltrates of polymorphonuclear leukocytes. No viable tumor cells are detectable. H & E,  $\times$  400.





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