Reprinted from **Neuroimmunomodulation: The State of the Art**
Volume 741 of the *Annals of the New York Academy of Sciences*
November 25, 1994

Rotational Stress Reduces the Effectiveness of Antitumor Drugs in Mice[a]

T. GIRALDI,[b] L. PERISSIN,[b] S. ZORZET,[c] AND V. RAPOZZI[b]

[b]*Department of Biomedical Sciences and Technologies*
University of Udine
Via Gervasutta 48
I-33100 Udine, Italy

[c]*Institute of Pharmacology*
University of Trieste
Via L. Giorgieri 7
I-34100 Trieste, Italy

The existence of relationships between cancer and psychological factors was commonly accepted by eminent medical personalities from the time of Galenus until the late nineteenth century.[1,2] After 1900, the widely accepted medical viewpoint that cancer had definite roots in the psyche rapidly disappeared from the literature as the psychosomatic approaches developed in the nineteenth century went out of fashion, and this concept remained dormant for almost half a century. Since the 1950s, an upsurge has been noted in the number of investigations to determine with a scientifically acceptable method the possible relationships between psychosocial factors and the incidence and progression of clinical cancer.[3] Considerably heterogeneous methods were used in these studies, whose results were contradictory in some instances.[4] Despite methodologic difficulties and variable results, stressful life events, specific personality traits, and psychological modalities of coping with neoplastic disease were significantly related with the clinical cancer incidence and progression.[5,6] Among several notable published investigations concerning stressful life events,[7] depression,[8] bereavement,[9] and psychosocial support,[10] the studies by Greer and coworkers are worthy of mention. This group of researchers showed that the repression of emotions, anger in particular, is significantly higher in breast cancer patients than in subjects with nonneoplastic breast diseases.[11] Moreover, identified modalities of psychological adaptation to the diagnosis of cancer correlated in a highly significant way with the length of survival in women with breast cancer.[12–14] These findings are in good agreement with those independently obtained by Temoshok and colleagues[15] in patients with malignant melanoma. Finally, recent evidence supporting in a different manner the foregoing data was the demonstration by Spiegel *et al.*[16] that psychosocial

[a]This work was supported by the Italian National Research Council (CNR) Special Project "ACRO" contract no. 92.2192.PF39 and by MURST 40 and 60% to Prof. T. Giraldi, U.S.U. 1991.

support provided to patients with different types of advanced tumors significantly increased their survival. These reported observations apparently are of great interest. Generally they indicate that stress or rather difficulties or breakdown in adaptation to life events preceding the onset of a clinically diagnosed neoplastic disease as well as unfavorable modalities of adaptation to a diagnosis of cancer facilitate the progression of tumors. However, in very rare cases have the results of these studies been duplicated by independent researchers.

In parallel with clinical research, many experimental studies with laboratory animals have been performed to investigate the role of the central nervous system in the incidence and progression of cancer. Models of laboratory animals subjected to experimental stressors have been widely used to investigate the action of stress on tumor growth.[17–19] Critical review of the relevant reports showed a high heterogeneity for both the animal-tumor systems used and the characteristics of the stressors employed.[20] The occurrence and magnitude of the effects of stressors on tumor growth were correspondingly variable. Moreover, tumor metastasis was marginally considered despite its outstanding clinical relevance.[21] Among the numerous experimental stressors studied, the application of rotational stress (spatial disorientation) to mice housed in a stress-protected environment appears to be a carefully and widely characterized mild psychological stressor.[22]

Neoplastic diseases are almost invariably clinically treated with combined modalities which include antitumor drugs. Adjuvant chemotherapy for solid malignant tumors is currently directed against minimal residual disease (mainly metastatic tumor cells). The study of the kinetics of tumor cell kill by antineoplastic drugs indicates that currently available agents significantly reduce the number of clonogenic tumor cells; sterilization of the host from clonogenic tumor cells by antitumor chemotherapy apparently is generally not currently achievable.[23] An accepted explanation of ''cures'' induced by antitumor chemotherapy is that immune and natural antitumor resistance factors of the host succeed in totally eradicating tumor cells after their number is sufficiently reduced below a critical threshold by the treatment. These considerations appear crucial for the survival and cure of clinically treated cancer patients. At the same time, clinical reports of studies on psychooncology concentrate on cancer progression and patient survival, omitting analysis or discussion of the possible influence of psychosocial factors on the effectiveness and success of antitumor chemotherapy. Analysis of the literature similarly reveals a lack of experimental investigations on the possible modification by stress of the therapeutic efficacy of antitumor chemotherapy in experimental animal models.

Tumor progression in mice is affected by physical and psychological stressors via neuroendocrine modulation of immune and natural antitumor resistance factors of the host.[18,24] The aim of the present work was to determine if the neuroimmunomodulation caused by an experimental stressor, which affects tumor progression in untreated animals,[25] significantly affects the host's antitumor resistance factors which contribute to determining the overall magnitude of the effects of antitumor chemotherapy. The effects of rotational stress on primary tumor growth and the formation of spontaneous lung metastasis were examined in mice bearing Lewis lung carcinoma. In the same experimental setting, the magnitude of the antitumor and antimetastatic action of cyclophosphamide and razoxane was determined as a function of the application of the stressor.

MATERIALS AND METHODS

Animals and Tumor Transplantation

The animals used were female C57BL/6 and C57BL/6 × DBA/2F$_1$ (hereafter called BD2F1) mice weighing 18-20 g, purchased from Charles River, Calco, Como, Italy. Lewis lung carcinoma was originally provided by the National Cancer Institute, Bethesda, Maryland and was maintained in C57BL/6 mice by subcutaneous injection in the axillary region of 50 mm^3 of minced tumor tissue aseptically prepared from donors similarly inoculated 2 weeks before. For the experiments, the tumor was propagated in BD2F1 mice by subcutaneous or intramuscular injection as indicated, using a tumor cell suspension containing 10^6 viable tumor cells.[26]

Measurement of Tumor Growth and Metastasis Formation

Primary tumor weight 14 days after tumor inoculation was determined by caliper measurements of short (a) and long (b) axes (cm), taking tumor density as equal to 1:

$$\text{tumor weight} = \pi/6 \times a^2 \times b$$

The number of metastases was determined at sacrifice on day 22 from tumor inoculation by examining the surface of the lungs with a low-power stereomicroscope. The weight of metastases was determined as the sum of the individual weights calculated according to this equation after measurement of their dimensions by an ocular micrometer.[26]

Protected Environment and Rotational Stress

The animals were kept five per cage to avoid the effects of overcrowding or isolation on tumor progression.[22,27] The cages were placed in a protected environment for 2 weeks before tumor inoculation to allow the animals to recover from the stress of shipment and to adapt to the new housing conditions.[18,28] The protected environment consisted of a cabinet containing the animal cages with laminar air flow, minimizing acoustic, olfactory, and visual communication among the cages and also with the animal room. The cabinets were contained in a room distant from other animals' rooms, where staff entered only once every 5 days to check the animals for water and food supply, which were available *ad libitum.* Temperature and relative humidity were constant at 20°C and 60%, respectively. The light-dark cycle used was 12/12 and the intensity of illumination in the cages was 5 lux.

Rotational stress was applied to the animals maintained in the low stress environment, as indicated, by spinning the cages at 45 rpm for 10 minutes every hour from the time of tumor inoculation until sacrifice.[26]

Drug Treatment

Razoxane [(±)-1,2-di(3,5-dioxopiperazin-1-yl)propane] was supplied by the National Cancer Institute, Drug Synthesis and Chemistry Branch, Bethesda, Maryland,

and cyclophosphamide was generously provided by Schering S.p.A., Milan, Italy. To avoid the stress of repeated intraperitoneal injections, the drugs were administered orally admixed in powdered food. Drug concentration was selected to provide the daily dose indicated in the tables on the basis of a measured average daily food consumption of 5.0 ± 0.1 g per mouse.

RESULTS AND DISCUSSION

The use of laboratory animals for experimental cancer research and for the study of experimental stressors from the literature was performed with the animals in conventional animal rooms and laboratory conditions. The possibility that factors such as housing conditions, temporary permanence in the laboratory, and handling may constitute stressors capable of causing significant neuroimmunomodulation, and hence affect the experimental results, is largely neglected.[26,29] The stress from shipping, overcrowding, or isolation was considered in few instances,[22,27,30] and the most notable investigation on housing conditions is the detailed work of Riley *et al.*[18] Mice kept in conventional conditions show high and variable levels of stress, as indicated by their plasma levels of corticosterone; these levels can consistently be reduced by the use of a protected housing environment.[31] Experiments illustrated in the present work were consequently performed, maintaining animals in protected housing essentially identical to that devised by Riley[26] and maintaining stress levels consistently low, as determined by plasma corticosterone measurements.[32] Handling and intraperitoneal injections also acted as a stressor capable of significantly increasing tumor metastases in mice.[26] Drug treatment was consequently performed in the experiments herein reported by oral administration of the drugs admixed in the food provided to the animals.

Lewis lung carcinoma transplanted into syngeneic BD2F1 mice is a weakly immunogenic tumor. Its TD_{50} dose, previously determined in mice kept in conventional housing, is 8.6×10^4 cells per mouse; tumor takes after inoculation of 5×10^5 cells is 100%.[33] Data reported in TABLE 1 show that when mice were maintained in protected housing, a tumor inoculum of 5×10^5 cells in nonstressed mice did not result in the development of tumors in any of the animals examined. Using the same inoculum size, development of tumors increased from 0/10 to 5/10 (highly significant) after application of rotational stress. In the protected housing, an inoculum size of 10^6 cells caused 100% tumor takes in both stressed and nonstressed mice. Mice subjected to rotational stress displayed a significant increase in lung metastases formation in comparison with nonstressed mice (TABLE 1 and ref. 26). Therefore, the host's resistance to tumor progression is increased when the animals are maintained in the protected environment than in conventional housing and is reduced by the application of rotational stress. When tumor burden is limited, the effects of housing and rotational stress are reflected in differences in the fraction of tumor takes, whereas with larger tumor challenges, differences are mainly based on the number and weight of pulmonary metastases (TABLE 1 and ref. 26).

Cyclophosphamide treatment (240 mg/kg/day) of mice implanted with 10^6 tumor cells caused the absence of tumors in all (10/10) of the treated nonstressed mice. On application of rotational stress, the magnitude of the antitumor effects of cyclophos-

TABLE 1. Effects of Rotational Stress on Tumor Progression in Mice Implanted with a Different Inoculum Size of Lewis Lung Carcinoma*

Inoculum Size (Cells/ Mouse)	Rotational Stress	Primary Tumor Weight (g)	Metastasis		Tumor-Free Animals†
			Number	Weight (mg)	
5×10^5	–	–	–	–	10/10[c]
	+	1.2 ± 0.4	48 ± 14	76 ± 27	5/10[c]
1×10^6	–	2.0 ± 0.3	32 ± 2[a]	97 ± 14[b]	0/10
	+	2.6 ± 0.3	51 ± 7[a]	222 ± 41[b]	0/10

* Each value is the mean (± SE) obtained using groups of 10 mice implanted with the number of tumor cells indicated. Animals were subjected to rotational stress as indicated. Values marked with the same letter are significantly different, *t* test for unmatched data (*a,b*),[41] $p < 0.05$, and Fisher's exact test (*c*),[42] $p < 0.05$.

† No primary tumor or metastasis was detectable at necroscopy on sacrifice.

phamide was remarkably reduced. Indeed, primary tumor and lung metastases were observed in all stressed treated animals (10/10, highly significant). The weight of the primary tumor as well as the weight and number of pulmonary metastases were significantly reduced in comparison with stressed drug untreated controls (TABLE 2).

The effects of cyclophosphamide in dosages of 15 to 240 mg/kg/day were also examined. In mice not subjected to rotational stress, doses of 15 and 30 mg/kg/day were ineffective; a dose of 120 mg/kg/day permitted the development of tumor and metastases in all treated mice (10/10), with significant inhibition in comparison

TABLE 2. Effects of Rotational Stress on Tumor Response to Treatment with Cyclophosphamide in Mice Bearing Lewis Lung Carcinoma*

Rotational Stress	Treatment with CY	Primary Tumor Weight (g)	Metastasis		Tumor-Free Animals†
			Number	Weight (mg)	
–	–	4.1 ± 0.3	36 ± 3[b]	126 ± 19[d]	0/10[f]
–	+	–	–	–	10/10[f,g]
+	–	4.8 ± 0.5[a]	54 ± 8[b,c]	279 ± 53[d,e]	0/10
+	+	2.7 ± 0.2[a]	4 ± 0[c]	14 ± 2[e]	0/10[g]

* Each value is the mean (± SE) obtained in groups of 10 mice implanted with Lewis lung carcinoma. When indicated, animals received 240 mg/kg/day cyclophosphamide (CY) orally on days 1-6 and/or were subjected to rotational stress. Values marked with the same letter are significantly different, *t* test for unmatched data (*a-e*),[41] $p < 0.05$, and Fisher's exact test (*f,g*),[42] $p < 0.05$.

† No primary tumor or metastasis detectable at necroscopy on sacrifice.

TABLE 3. Effects of Rotational Stress on Tumor Response to Treatment with Different Dosages of Cyclophosphamide in Mice Bearing Lewis Lung Carcinoma*

Rotational Stress	Cyclophosphamide Dosage (mg/kg/day)	Primary Tumor Weight†	Metastasis		Tumor-Free Animals‡
			Number†	Weight†	
–	–	100 ± 24[a]	100 ± 15[b]	100 ± 15[c]	0/10
–	15	105 ± 18	87 ± 12	91 ± 15	0/10
–	30	94 ± 12	88 ± 22	99 ± 41	0/10
–	120	24 ± 6[a]	23 ± 19[b]	11 ± 10[c]	0/10
–	240	0‡	0‡	0‡	10/10§
+	–	100 ± 9[d-f]	100 ± 8[g,h]	100 ± 7[i,l]	0/10
+	15	71 ± 6[d]	102 ± 8	80 ± 8	0/10
+	30	65 ± 15	71 ± 17	71 ± 16	0/10
+	120	58 ± 12[e]	38 ± 22[h]	32 ± 10[i]	0/10
+	240	32 ± 9[f]	22 ± 3[g]	14 ± 2[l]	0/10

* Each value is the mean (± SE) obtained in groups of 10 mice implanted with Lewis lung carcinoma. Animals received the indicated daily dose of cyclophosphamide orally on days 1-6 and/or were subjected to rotational stress. Values marked with the same letter are significantly different, *t* test for unmatched data (*a-l*),[41] $p < 0.05$.

† Values are expressed as mean percent ratio (± SEM) for each treated group in comparison with drug untreated controls, separately for stressed and nonstressed groups.

‡ No primary tumor or metastasis was detectable at necroscopy on sacrifice.

§ Significantly different, Fisher's exact test,[42] $p < 0.05$.

with drug untreated controls. When rotational stress was applied, the effects of cyclophosphamide at dosages of 15-120 mg/kg/day were not (significantly) greater than those in nonstressed animals. The remarkable attenuation caused by the stressor on the tumor inhibitory action of cyclophosphamide at 240 mg/kg/day was confirmed (TABLE 3).

In the present investigation, cyclophosphamide was chosen as a reference antitumor drug, exerting a cytotoxic effect against tumor cells via its DNA cross-linking alkylating properties.[34] This drug exerts similarly pronounced cytotoxic effects on both subcutaneous and intramuscular primary tumors as well as on tumor deposits localized to the lungs. The latter characteristics are evident from data so far illustrated and from previous detailed examination.[35] The reduction caused by rotational stress on the magnitude of the antitumor effects by cyclophosphamide in mice bearing Lewis lung carcinoma is similarly pronounced on either primary tumor or lung metastasis.

The effects of rotational stress in untreated mice bearing Lewis lung carcinoma are more pronounced on metastasis than on primary tumor and occur on metastasis even in the absence of significant effects on the primary tumor (data just presented and ref. 26). The effects of this stressor have thus been examined also in relation to the action of the antimetastatic drug razoxane. Razoxane is a dioxopiperazine derivative that selectively inhibits the formation of metastases in laboratory animals bearing solid malignant tumors.[36,37] The antimetastatic action of razoxane occurs independently

TABLE 4. Effects of Rotational Stress on Tumor Response to Treatment with Razoxane in Mice Bearing Lewis Lung Carcinoma*

Rotational Stress	Treatment with Razoxane	Primary Tumor Weight (g)	Metastasis		Metastasis-Free Animals†
			Number	Weight (mg)	
–	–	1.8 ± 0.4	15 ± 3[a]	58 ± 15[c]	0/10[e]
–	+	1.0 ± 0.1	3	8	9/10[e,f]
+	–	2.4 ± 0.3	30 ± 3[a,b]	118 ± 14[c,d]	0/10
+	+	1.5 ± 0.6	4 ± 1[b]	9 ± 1[d]	2/10[f]

* Each value is the mean (± SE) in groups of 10 mice implanted with Lewis lung carcinoma. When indicated, animals received 25 mg/kg/day razoxane orally on days 1-21 and/or were subjected to rotational stress. Values marked with the same letter are significantly different, *t* test for unmatched data (*a-c*),[41] $p < 0.05$, and Fisher's exact test (*e,f*),[42] $p < 0.05$.

† No metastasis was detectable at necroscopy on sacrifice.

of the cytotoxic effects directed against tumor cells[35] and can be attributed to the drug's inhibition of tumor cell entry into the blood circulation[38] via normalization of blood vessels in the primary tumor.[39] Indeed, in nonstressed mice treated with razoxane, a remarkable reduction in the formation of metastases is observed in terms of the animals free of metastases. The absence of metastases is observed in 9 of 10 mice; this reduction is not accompanied by any significant reduction in primary tumor growth. Upon application of rotational stress, the antimetastatic action of razoxane is sharply attenuated, and the number of animals without metastases is reduced from 9 of 10 to 2 of 10 (highly significant). The number and weight of pulmonary metastases in the remaining 8 of 10 mice are significantly smaller than those observed in drug untreated stressed controls (TABLE 4).

CONCLUSIONS

The possible existence of relationships between psychological factors and the incidence and progression of cancer still constitutes a formidable, controversial, and largely unresolved scientific challenge. Laboratory animals have provided a large body of experimental evidence which clearly indicates that coping with physical and psychological stressor stimuli does cause distinct neuroimmunomodulation via neurovegetative and neuroendocrine pathways.[18] Stress-induced modulation of the immune system has been clearly reflected by a modified incidence and progression of either spontaneous or transplanted tumors in laboratory animals.[24]

A substantial aspect whose experimental examination appears from the literature to have been neglected is the relevance of stress-induced neuroimmunomodulation in the success of antitumor chemotherapy. Indeed, experimental evidence indicates that to achieve a cure, an antineoplastic drug must reduce the number of tumor cells, causing at the same time limited immunodepressive effects. In other words, the best

therapeutic response is obtained with optimal dosages, for which a good balance occurs between cytotoxic effects and limited immunodepression. Antitumor resistance factors of the host, spared by treatment, may thus significantly contribute to the overall success of treatment. A typical example is provided by the administration of cyclophosphamide in mice bearing a transplantable plasmocytoma, in which a maximum decrease in tumor growth is obtained with a dosage limited and much smaller than the maximum tolerated one.[40]

Experiments discussed in this paper were designed to determine the possible relevance of stress-induced neuroimmunomodulation in determining the magnitude of the effects of antitumor chemotherapy in mice. At first, stress appears of crucial importance in determining tumor rejection by the host. Tumor inocula which progress in mice maintained in conventional housing are rejected when the hosts are kept in a low stress environment; the application of rotational stress to these animals results in development of tumors. With a larger inoculum, tumor takes also occur in mice kept in low stress conditions, and the application of rotational stress increases tumor metastases to the lungs. On treatment with cyclophosphamide, dosages that are noncurative in conventional housing abrogate tumor take in the protected environment; this curative efficacy of the drug is remarkably reduced by rotational stress. Similar results were obtained when rotational stress was applied in mice treated with razoxane. The antimetastatic effects of this drug, which in contrast to cyclophosphamide are obtained with a noncytotoxic mechanism, are also reduced in magnitude by the application of the stressor.

These results are of interest because they show that host antitumor resistance factors crucial for determining the magnitude of the effects of antitumor drugs are amenable to neuroimmunomodulation by stress. Stress is therefore a significant parameter in determining the efficacy of the antitumor effects of antineoplastic drugs in experimental animal tumor systems. The data presented also suggest clinical implications. Stress in terms of the occurrence of stressful life events (including the communication of a diagnosis of cancer) and specific modalities of adaptation to them may be a significant factor, in addition to several already identified biological ones, in determining the clinical response to antitumor chemotherapy. Considering also that chemotherapy is performed in a limited and temporally defined (late) period of the natural history of a neoplastic disease, these data suggest an opportunity to analyze specifically the role of psychosocial factors in the effectiveness of clinical cancer chemotherapy.

ACKNOWLEDGMENT

The generosity of Fondazione C.e D. Callerio in providing the protected environment facility is gratefully appreciated.

REFERENCES

1. LE SHAN, L. 1959. Psychological states as factors in the development of malignant disease: A critical review. J. Natl. Cancer Inst. **22:** 1-18.

2. LE SHAN, L. 1989. Cancer As a Turning Point: A Handbook for People with Cancer, Their Families, and Health Professionals. Dutton. New York.
3. GREER, S. 1983. Cancer and the mind. Br. J. Psychiatry **143:** 535-543.
4. TEMOSHOK, L. & B. H. HELLER. 1984. On comparing apples, oranges and fruit salad: A methodological overview of medical outcome studies in psychosocial oncology. *In* Psychosocial Stress and Cancer. C. L. Cooper, Ed.: 231-260. John Wiley & Sons Ltd. Chichester.
5. TEMOSHOK, L. 1987. Personality, coping style, emotion and cancer: Towards an integrative model. Cancer Survey **6:** 545-567.
6. GREER, S. & M. WATSON. 1987. Mental adjustment to cancer: Its measurement and prognostic importance. Cancer Survey **6:** 439-453.
7. PAYKEL, S. & B. RAO. 1984. Methodology in studies of life events and cancer. *In* Psychosocial Stress and Cancer. C. L. Cooper, Ed.: 73-92. John Wiley & Sons Ltd. Chichester.
8. BIELIAUSKAS, L. A. 1984. Depression, stress and cancer. *In* Psychosocial Stress and Cancer. C. L. Cooper, Ed.: 37-48. John Wiley & Sons Ltd. Chichester.
9. EWERTZ, M. 1986. Bereavement and breast cancer. Br. J. Cancer **53:** 701-703.
10. SPIEGEL, D., J. BLOOM & I. YALOM. 1981. Group support for patient with metastatic breast cancer. Arch. Gen. Psychiatry **38:** 527.
11. MORRIS, T., S. GREER, K. W. PETTINGALE & M. WATSON. 1981. Patterns of expression of anger and their psychological correlates in women with breast cancer. J. Psychosom. Res. **25:** 111-117.
12. GREER, S. & T. MORRIS. 1975. Psychological attributes of women who develop breast cancer: A controlled study. J. Psychosom. Res. **19:** 147-153.
13. GREER, S. & K. W. PETTINGALE. 1979. Psychological response to breast cancer: Effect on outcome. Lancet **ii:** 785-787.
14. PETTINGALE, K. W., T. MORRIS, S. GREER & J. HAYBITTLE. 1985. Mental attitudes to cancer: An additional prognostic factor. Lancet **i:** 750.
15. TEMOSHOK, L., B. W. HELLER, R. SAGEBIEL, M. BLOIS, D. SWEET, D. DICLEMENTE & L. GOLD. 1985. The relationship of psychosocial factors to prognostic indicators in cutaneous malignant melanoma. J. Psychosom. Res. **2:** 139-154.
16. SPIEGEL, D., J. BLOOM, H. KRAEMER & E. GOTTHEIL. 1989. Effect of psychosocial treatment on survival of patients with metastatic breast cancer. Lancet **2:** 888-891.
17. PETERS, L. J. & K. A. MASON. 1979. Influence of stress on experimental cancer. *In* Mind and Cancer Prognosis. B. A. Stoll, Ed.: 103-124. John Wiley & Sons Ltd. Chichester.
18. RILEY, V., M. A. FITZMAURICE & D. H. SPACKMAN. 1981. Psychoneuroimmunologic factors in neoplasia: Studies in animals. *In* Psychoneuroimmunology. R. Ader, Ed.: 31-102. Academic Press. New York.
19. RILEY, V., M. A. FITZMAURICE & D. H. SPACKMAN. 1979. Cancer stress: Overview and critique. Cancer Detect. Prev. **2:** 163-195.
20. JUSTICE, A. 1985. Review of the effects of stress on cancer in laboratory animals: Importance of time of stress application and type of tumor. Psychol. Bull. **98:** 18-138.
21. BAMMER, K. 1981. Stress, spread and cancer. *In* Stress and Cancer. K. Bammer & B. H. Newberry, Eds.: 137-163. C. J. Hogrefe Inc. Toronto.
22. RILEY, V., M. A. FITZMAURICE & D. H. SPACKMAN. 1981. Animal models in biobehavioral research: Effects of anxiety stress on immunocompetence and neoplasia. *In* Perspectives on Behavioral Medicine. S. M. Weiss, J. A. Herd & B. H. Fox, Eds.: 371-400. Academic Press. New York.
23. STEEL, G. G. (Ed.) 1977. Growth Kinetics of Tumours. Clarendon Press. Oxford, England.
24. RILEY, V., M. A. FITZMAURICE & D. H. SPACKMAN. 1982. Immunocompetence and neoplasia: Role of anxiety stress. *In* Biological Mediators of Behavior and Disease: Neoplasia. S. M. Lewy, Ed.: 175-218. Elsevier Biomedical. New York.

25. GIRALDI, T., L. PERISSIN, V. RAPOZZI & S. ZORZET. 1991. Stress, tumor metastasis and effectiveness of antitumor chemotherapy. *In* Psychoneuroimmunology, Interaction between Brain, Nervous System, Behavior, Endocrine and Immune System. H. J. Schmoll, U. Tewes & N. P. Plotnikoff, Eds.: 216-236. Hoegrefe & Huber Publishers, Lewiston, Toronto, Bern, Göttingen.
26. GIRALDI, T., L. PERISSIN, S. ZORZET, P. PICCINI & V. RAPOZZI. 1989. Effects of stress on tumor growth and metastasis in mice bearing Lewis lung carcinoma. Eur. J. Cancer Clin. Oncol. **25:** 1583-1588.
27. LABARBA, R. C. 1970. Experiential and environmental factors in cancer. Psychosom. Med. **32:** 258-276.
28. RILEY, V. 1981. Psychoneuroendocrine influences on immunocompetence and neoplasia. Science **212:** 1100-1109.
29. VOGEL, W. H. 1987. Stress neglected variable in experimental pharmacology and toxicology. Trends Pharmacol. Sci. **8:** 35-38.
30. STEPLEWSKI, Z., P. ROBINSON GOLDMAN & W. H. VOGEL. 1987. Effects of housing stress on the formation and development of tumors in rats. Cancer Lett. **34:** 257-261.
31. RILEY, V., D. H. SPACKMAN, H. MCCLANAHAN & G. A. SANTISTEBAN. 1979. The role of stress in malignancy. Cancer Detect. Prev. **2:** 235-255.
32. PERISSIN, L., S. ZORZET, P. PICCINI, V. RAPOZZI & T. GIRALDI. 1989. Corticosterone does not mediate the effects of stress on tumor metastasis. Pharmacol. Res. **21:** 461-462.
33. PERISSIN, L., S. ZORZET, P. PICCINI, V. RAPOZZI & T. GIRALDI. 1991. Effects of rotational stress on the effectiveness of cyclophosphamide and razoxane in mice bearing Lewis lung carcinoma. Clin. Exp. Metastasis **9:** 541-549.
34. PRICE, C. C. 1975. Chemistry of alkylation. *In* Antineoplastic and Immunosuppressive Agents. Part II. A. C. Sartorelli & D. G. Johns, Eds.: 1-5. Springer-Verlag. Berlin.
35. GIRALDI, T., G. SAVA, R. CUMAN, C. NISI & L. LASSIANI. 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:** 2524-2528.
36. HELLMANN, K. & K. BURRAGE. 1969. Control of malignant metastases by ICRF 159. Nature **224:** 273-274.
37. HERMAN, E. H., D. T. WITIAK, K. HELLMANN & V. S. WARAVDEKAR. 1982. Biological properties of ICRF-159 and related bis(dioxopiperazine) compounds. *In* Advances in Pharmacology and Chemotherapy. S. Garattini, A. Goldin, F. Hawking & I. J. Kopin, Eds. Vol. **19:** 249-290. Academic Press. New York.
38. GIRALDI, T., G. SAVA, R. CHERUBINO, G. BOTTIROLI & G. MAZZINI. 1984. Effects of DTIC, DM-COOK and ICRF-159 on the number of circulating Lewis lung carcinoma cells detected by flow cytometry. Clin. Exp. Metastasis **2:** 151-154.
39. SALSBURY A. J., K. BURRAGE & K. HELLMANN. 1974. Histological analysis of the antimetastatic effect of (±)-1,2-bis(3,5-dioxopiperazin-1-yl)propane. Cancer Res. **34:** 843-849.
40. MOKYR, M. B. 1987. The MOPC-315 tumor as a model for the immunomodulatory effects of cyclophosphamide. Methods Find. Exp. Clin. Pharmacol. **9:** 579-588.
41. TALLARIDA, R. J. & R. B. MURRAY (Eds.) 1987. Manual of Pharmacologic Calculation with Computer Programs. Springer. New York.
42. SIEGEL, S. (Ed.) 1956. Non-parametric Statistics for the Behavioral Sciences. McGraw-Hill Book Company. New York.