[CANCER RESEARCH 34, 92–95, January 1974]

Inhibitory Effect of Manganese upon Muscle Tumorigenesis by Nickel Subsulfide[1]

F. William Sunderman, Jr.,[2] Thomas J. Lau, and Lewis J. Cralley[3]

Department of Laboratory Medicine, University of Connecticut School of Medicine, Farmington, Connecticut 06032

SUMMARY

Fischer rats in five experimental groups were given a single i.m. injection of penicillin suspension containing carcinogenic Ni_3S_2 dust (2.5 mg), alone or in combination with equimolar amounts of aluminum, copper, chromium, or manganese dusts. Rats in five control groups were treated identically, except that the Ni_3S_2 dust was omitted. After 24 months, the incidence of sarcomas at the injection site was 63% in the group that received the combination of Ni_3S_2 and manganese dusts, compared with incidences of 96 to 100% in the groups that received Ni_3S_2 alone or in combination with aluminum, copper, or chromium dusts ($p < 0.001$). No sarcomas occurred at the injection site in control groups that did not receive Ni_3S_2. The finding that the addition of equimolar amounts of manganese dust to Ni_3S_2 dust significantly depresses Ni_3S_2-induced tumorigenesis provides an experimental system for investigations of metal interactions in carcinogenesis.

INTRODUCTION

Investigations in our laboratory have been concerned with the carcinogenicity of nickel compounds in experimental animals and humans (8, 9, 14). In previous studies, attention has been focused particularly upon carcinogenic interrelationships between nickel compounds and polycyclic aromatic hydrocarbons, such as 3,4-benzpyrene (9, 13). In this study, we examined the effects of 4 metals upon nickel carcinogenesis. Our interest in this subject was stimulated by the reports of Gunn *et al.* (6, 7) that zinc inhibits cadmium carcinogenesis in rodents, and by the hypothesis of Cralley (4) that electromotive interactions between metals may influence asbestos carcinogenesis. As the experimental system in which to search for metal interactions in nickel carcinogenesis, we have tested 4 dusts containing pure metals and oxides of varying concentrations for their effect on induction of sarcomas in rats by the i.m. injection of nickel subsulfide (Ni_3S_2) (5).

MATERIALS AND METHODS

The experimental animals were 228 male albino rats of the Fischer strain (Charles River Breeding Laboratories, Inc., North Wilmington, Mass.), maintained on Purina laboratory rat chow. The rats were 8 weeks old at the time that the metal dusts were injected. The rats were divided into 10 experimental groups (Table 1). Groups N-1 to N-5 received injections of 2.5 mg of Ni_3S_2 dust, alone or mixed with dusts of aluminum, copper, chromium, or manganese, respectively. The remaining 5 groups, C-1 to C-5, were treated identically, except that the Ni_3S_2 was omitted. Group C-1 received the injection vehicle; Groups C-2 to C-5 received the 4 individual metal dusts. The injection vehicle was 0.5 ml of penicillin G procaine, 3,000,000 units/ml (Wycillin; Wyeth Laboratories, Inc., Philadelphia, Pa.). Aliquots of each suspension of metal dust in penicillin were analyzed by atomic absorption spectrometry, for verification of their metal content (Table 1). A single injection of a suspension of metal dust in penicillin was made deep into the musculature of a rat's hindleg, at the midlength of the thigh. No acute mortality, morbidity, or severe local inflammatory reaction occurred in any of the rats.

Physical examinations of the rats were performed each week by an animal caretaker skilled in detecting muscle tumors and in gauging the progression of tumor growth. During the study, the rats either died spontaneously or were killed when tumors became so large that the rats could not move about their cages and hence could not obtain food or water. Rats that survived 104 weeks after the injection were killed by inhalation of diethyl ether at 112 weeks of age. All rats were autopsied, and their tissues were examined by light microscopy. Classification of sarcomas was based upon the histological criteria of Stout and Lattes (12). Statistical comparisons of tumor incidences and survival data were performed by the χ^2 test with Yates' correction (1) and by the actuarial nonparametric log-rank test of Peto *et al.* (10, 11).

RESULTS

The survival data and incidences of sarcomas at the injection sites are listed in Table 1. Curves for cumulative sarcoma incidences in the experimental groups are shown in Chart 1. In Groups N-1 to N-4 there were no significant differences between the cumulative incidence curves for development of palpable tumors at the injection site. In Group N-5, which received both Ni_3S_2 and manganese dusts, the cumulative incidence curve for tumor development was shifted to the

[1] Sponsored by Research Grants EHS C 0109, HSM-99-72-24 from the National Institute for Occupational Safety and Health, and AT(11-1)-3140 from the Atomic Energy Commission.

[2] To whom requests for reprints should be addressed, at P. O. Box G, Farmington, Conn. 06032.

[3] Formerly with the National Institute for Occupational Safety and Health, Cincinnati, Ohio 45201.

Received July 9, 1973; accepted October 8, 1973.

Table 1

Survival data and sarcoma incidences

Fischer rats were given a single i.m. injection of 0.5 ml of penicillin suspension of metal dusts in the specified dosages and combinations.

Group	No. of 2-yr survivors	Rats with sarcomas at injection site/no. treated
Control groups		
C-1	16/24	0/24
C-2 (Al)[a]	20/24	0/24
C-3 (Cu)[b]	7/10	0/10
C-4 (Cr)[c]	22/24	0/24
C-5 (Mn)[d]	17/24	0/24
All control groups	82/106	0/106
Ni_3S_2 groups		
N-1 (Ni_3S_2)[e]	1/40	39/40
N-2 (Ni_3S_2 + Al)[a,e]	1/24	23/24
N-3 (Ni_3S_2 + Cu)[b,e]	0/10	10/10
N-4 (Ni_3S_2 + Cr)[c,e]	0/24	24/24
N-5 (Ni_3S_2 + Mn)[d,e]	9/24[f]	15/24[f]

[a] Aluminum oxide dust, 3.0 mg/rat; mean particle diameter, 0.7 μm; elemental Al, 12%; aluminum oxides (as Al_2O_3), 88%; Ni, Cu, Cr, Mn, and Co, <0.1%.

[b] Copper dust, 2.0 mg/rat; mean particle diameter, 8.0 μm; elemental Cu, 88%; copper oxides (as CuO), 12%; Ni, Al, Cr, Mn, and Co, <0.1%.

[c] Chromium dust, 2.0 mg/rat; mean particle diameter, 1.6 μm; elemental Cr, 65%; chromium oxides (as Cr_2O_3), 35%; Ni, Al, Cu, Mn, and Co, <0.1%.

[d] Manganese dust, 2.1 mg/rat; mean particle diameter, 1.4 μm; elemental Mn, 62%; manganese oxides (as MnO_2), 36%; Ni, Cu, Cr, and Co, <0.1%; Al, 2%.

[e] Ni_3S_2 dust, 2.5 mg/rat; mean particle diameter, 1.4 μm; Ni, 72%; S, 28%; Al, Cu, Cr, Mn, and Co, <0.1%.

[f] $p < 0.001$ *versus* Group N-1 (χ^2 test).

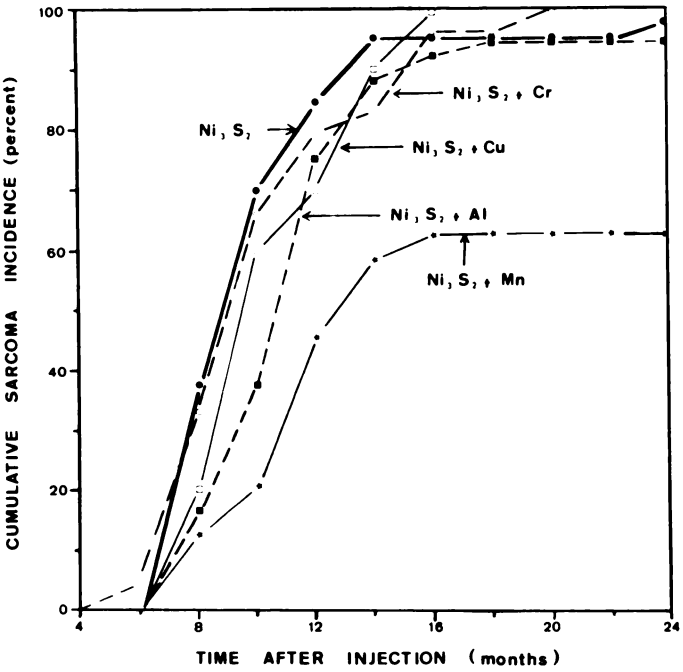

Chart 1. Cumulative incidence curves for sarcomas in the groups of rats that were given an i.m. injection of Ni_3S_2 dust, alone or in combination with aluminum, copper, chromium, or manganese dust.

right, in comparison to the curve for Group N-1 which received only Ni_3S_2 ($p < 0.025$).

Curves for cumulative mortality in the control and experimental groups are shown in Chart 2. The mortality curve for the control groups, combined, is shown at the bottom of the chart. In Groups N-1 to N-4 there were no significant differences between the mortality curves. The mortality curve for Group N-5 which received both Ni_3S_2 and manganese dusts was shifted to the right, in comparison to the curve for Group N-1 which received only Ni_3S_2 ($p < 0.05$).

Histological categorization of sarcomas at the injection sites was frequently difficult, owing to their pleomorphic character. In Table 2 are listed the proportions of tumors at the injection site that were unequivocally classified as rhabdomyosarcomas. The remaining tumors were predominently fibrosarcomas or undifferentiated sarcomas, as well as 2 liposarcomas and 2 neurofibrosarcomas. Leydig cell tumors were found in the testes of many of the rats but are not included in Table 3 since they are not malignant. Metastases to distant sites, including lungs, mediastinum, heart, liver, spleen, mesentery, and paraaortic lymph nodes, occurred in 21 to 73% of the rats that developed primary tumors at the injection site (Table 2).

Primary malignant tumors found at locations other than the injection site are listed in Table 3. In Groups C-1 to C-5, the incidences of all malignant neoplasms ranged from 8 to 13%, and there were no significant differences between any of these incidences. In Groups N-1 to N-5, the proportions of primary malignant tumors that developed distant from the injection sites ranged from 0 to 3%. The apparent differences in incidences of primary tumors at locations other than the

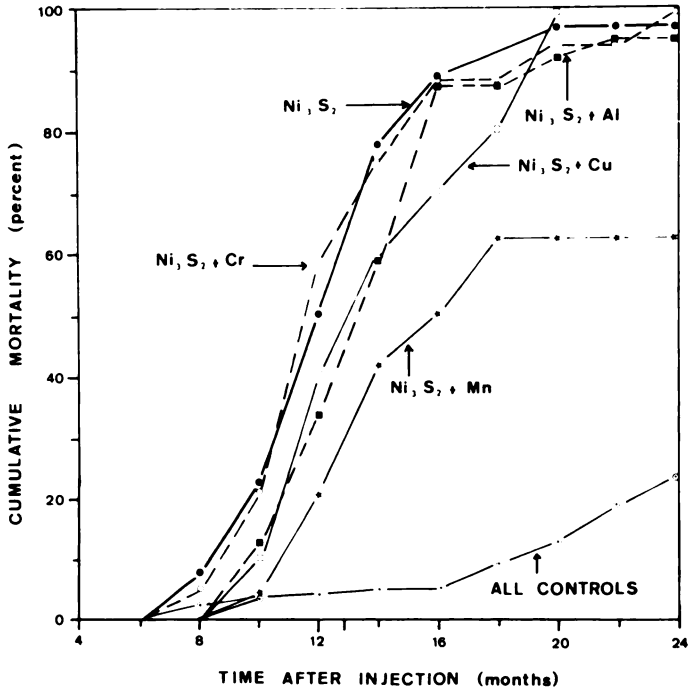

Chart 2. Cumulative mortality curves for the groups of rats that were given an i.m. injection of Ni_3S_2 dust, alone or in combination with aluminum, copper, chromium, or manganese dust. The mortality curve labeled *all controls* comprises the entire population of 106 rats in control Groups C-1 through C-6.

injection sites are attributable to the earlier mortality of rats in Groups N-1 to N-5. When these data were subjected to actuarial analysis, no significant differences in the incidences of primary tumors that developed distant from the injection sites were observed between any of the control (C-1 to C-5) or nickel-treated (N-1 to N-5) groups.

The outcome of this investigation became apparent 1 year prior to submission of this manuscript. A dose-response study was immediately begun in which graded dosages of Ni_3S_2 and manganese dusts were administered to 225 male Fischer rats, randomly distributed into 14 experimental groups. The findings of the dose-response study, after 60 weeks of observation, are listed in Table 4. No palpable sarcomas have developed at the injection sites in control Group A, or in Groups B, C, and D which received only manganese dust. Sarcomas have developed at the injection sites in Groups E, I,

Table 2

Characteristics of sarcomas at injection site

Fischer rats developed sarcomas at the sites of injection of Ni_3S_2 dust, alone or in combination with other metal dusts, as specified in Table 1. The characteristics of the sarcomas are summarized in this table.

Groups	No. of rhabdomyosarcomas/total no. of sarcomas	Rats with distant metastases/no. with sarcomas
N-1 (Ni_3S_2)	13/39	15/39
N-2 (Ni_3S_2 + Al)	10/23	6/23
N-3 (Ni_3S_2 + Cu)	6/10	4/10
N-4 (Ni_3S_2 + Cr)	15/24	5/24
N-5 (Ni_3S_2 + Mn)	6/15	11/15[a]
All groups	50/111	41/111

[a] $p = < 0.05$ *versus* Group N-1 (χ^2 test).

Table 4

Results of dose-response study

Fischer rats (225 males, 8 weeks old) were divided into 14 experimental groups and were given a single i.m. injection of Ni_3S_2 and manganese dusts, alone and in combination, at the specified dosages. The incidences of palpable sarcomas at the injection site by 60 weeks after the injection are listed below. (This experiment is in progress and will not be terminated until 104 weeks after the injection.)

Experimental group	Ni_3S_2 (mg)	Manganese (mg)	Rats with sarcomas at injection site/total no. treated, by Wk 60
A			0/30
B		0.5	0/15
C		1.0	0/15
D		2.0	0/15
E	0.6		4/15
F	0.6	0.5	0/15
G	0.6	1.0	0/15
H	0.6	2.0	0/15
I	1.2		11/15
J	1.2	0.5	2/15[a]
K	1.2	1.0	0/15[b]
L	2.5		13/15
M	2.5	0.5	6/15[c]
N	5.0		15/15

[a] $p < 0.005$ *versus* Group I (χ^2 test).
[b] $p < 0.001$ *versus* Group I (χ^2 test).
[c] $p < 0.025$ *versus* Group L (χ^2 test).

Table 3

Malignant tumors other than at injection site

Fischer rats in various experimental groups developed malignant neoplasms at locations that were distant from the injection site. These presumably spontaneous neoplasms are summarized below.

Groups	No. of rats with malignant tumors other than at injection site/ total no. tested	Tumors
Control groups		
C-1	3/24	2 squamous cell carcinomas (skin); fibrosarcoma (spleen)
C-2 (Al)	2/24	Mesothelioma (testis); rhabdomyosarcoma (neck)
C-3 (Cu)	1/10	Lymphosarcoma (spleen, liver)
C-4 (Cr)	2/24	Mesothelioma (testis); lymphosarcoma (spleen, liver)
C-5 (Mn)	3/24	2 squamous cell carcinomas (skin); lymphocytic leukemia
All control groups	11/106	
Ni_3S_2 groups		
N-1 (Ni_3S_2)	1/40	Lymphosarcoma (spleen, liver)
N-2 (Ni_3S_2 + Al)	0/24	
N-3 (Ni_3S_2 + Cu)	0/10	
N-4 (Ni_3S_2 + Cr)	0/24	
N-5 (Ni_3S_2 + Mn)	0/24	
All Ni_3S_2 groups	1/222	

L, and N, which received only Ni_3S_2 dusts. The incidences of sarcomas at the injection sites in Groups F, G, H, J, K, and M, which received various combinations of Ni_3S_2 and manganese dusts, are less than those of comparable groups that received only Ni_3S_2 dust. Thus, to date, the findings of this dose-response study are fully consistent with the results of the initial investigation.

DISCUSSION

This investigation has shown that the induction of sarcomas in Fischer rats by i.m. injection of Ni_3S_2 is significantly depressed by the simultaneous administration of manganese dust. Under the same conditions, aluminum, copper, and chromium dusts had no effect. These observations provide an experimental system for investigations of metal interactions in nickel carcinogenesis. A study is currently being initiated in which radioactive $^{63}Ni_3S_2$ is injected i.m. in rats, alone or in combination with manganese dust, and measurements are being made of the tissue retention of ^{63}Ni and of the elimination of ^{63}Ni in excreta. This study is designed to determine whether manganese dust influences the solubilization and mobilization of ^{63}Ni from the injection site. It is possible that manganese directly displaces nickel from Ni_3S_2. A 2nd possibility is that manganese stimulates a macrophage response that leads indirectly to the mobilization of Ni_3S_2. A 3rd hypothesis is that manganese may antagonize nickel inhibition of nucleolar RNA polymerase activity in rhabdomyocytes. This last possibility is consistent with the report of Webb *et al.* (16) that intracellular nickel in nickel-induced rhabdomyosarcomas is found predominantly within the nucleolar fraction, and with our finding that nickel inhibits manganese-activated RNA polymerase activity in hepatic nuclei (2, 3, 15).

ACKNOWLEDGMENTS

We are grateful for Richard Morang and Karen Parker for skillful animal care.

REFERENCES

1. Armitage, P. *In:* Statistical Methods in Medical Research, pp. 362–372. New York: John Wiley & Sons, 1971.
2. Beach. D. J., and Sunderman, F. W., Jr. Nickel Carbonyl Inhibition of ^{14}C-Orotic Acid Incorporation into Rat Liver RNA. Proc. Soc. Exptl. Biol. Med., *131:* 321–322, 1969.
3. Beach, D. J., and Sunderman, F. W., Jr. Nickel Carbonyl Inhibition of RNA Synthesis by a Chromatin-RNA Polymerase Complex from Hepatic Nuclei. Cancer Res., *30:* 48–50, 1970.
4. Cralley, L. J. Electromotive Phenomenon in Metal and Mineral Particulate Exposures: Relevance to Exposure to Asbestos and Occurrence of Cancer. Am. Ind. Hyg. Assoc. J., *32:* 653–661, 1971.
5. Gilman, J. P. W. Muscle Tumorigenesis. Proceedings of the Sixth Canadian Cancer Research Conference, pp. 209-233. Oxford, England: Permagon Press, Inc., 1964.
6. Gunn, S. A., Gould, T. C., and Anderson, W. A. D. Cadmium-induced Interstitial Cell Tumors in Rats and Mice and Their Prevention by Zinc. J. Natl. Cancer Inst., *31:* 745–759, 1963.
7. Gunn, S. A., Gould, T. C., and Anderson, W. A. D. Effect of Zinc on Cancerogenesis by Cadmium. Proc. Soc. Exptl. Biol. Med., *115:* 653–657, 1964.
8. Lau, T. J., Hackett, R. L., and Sunderman, F. W., Jr. The Carcinogenicity of Intravenous Nickel Carbonyl in Rats. Cancer Res., *32:* 2253–2258, 1972.
9. Maenza, R. M., Pradhan, A. M., and Sunderman, F. W., Jr. Rapid Induction of Sarcomas in Rats by Combination of Nickel Sulfide and 3,4-Benzypyrene. Cancer Res., *31:* 2067–2071, 1971.
10. Peto, R., and Peto, J. Asymptotically Efficient Rank Invariant Test Procedures. J. Roy. Statist. Soc. Ser. A, *135:* 185–206, 1972.
11. Peto, R., and Pike, M. C. Conservatism of the Approximation $\Sigma(O-E)^2/E$ in the Logrank Test for Survival Data or Tumour Incidence Data. Biometrika, in press.
12. Stout, A. P., and Lattes, R. Tumors of the Soft Tissues. Atlas of Tumor Pathology, Series 2, Fascicle 1, pp. 1-197. Washington, D. C.: American Registry of Pathology, 1967.
13. Sunderman, F. W., Jr. Inhibition of Induction of Benzpyrene Hydroxylase by Nickel Carbonyl. Cancer Res., *27:* 950–955, 1967.
14. Sunderman, F. W., Jr. The Current Status of Nickel Carcinogenesis. Ann. Clin. Lab. Sci., *3:* 156–180, 1973.
15. Sunderman, F. W., Jr., and Esfahani, M. Nickel Carbonyl Inhibition of RNA Polymerase Activity in Hepatic Nuclei. Cancer Res., *28:* 2565–2567, 1968.
16. Webb, M., Heath, J. C., and Hopkins, T. Intranuclear Distribution of the Inducing Metal in Primary Rhabdomyosarcomata Induced in the Rat by Nickel, Cobalt and Cadmium. Brit. J. Cancer, *26:* 274–278, 1972.