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Recent Research on Nickel Carcinogenesis

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Research on nickel carcinogenesis from 1975 to March 1980 is reviewed. Epidemiological studies have strengthened the evidence that workers in nickel refineries have increased risks of cancers of the nasal cavities and lungs. Clinical investigations have resulted in improved diagnosis, classification, and management of cancers of respiratory organs in nickel refinery workers. Carcinogenicity tests have demonstrated the carcinogenicity of nickel subsulfide (α-Ni_3S_2) in rodents following administration by a variety of parenteral routes. Radiotracer studies and x-ray diffractometry have clarified the metabolism of α-Ni_3S_2 in rodents. *In vitro* exposures of mammalian cells to certain nickel compounds have been shown to inhibit cellular uptake of thymidine-^{3}H, and to induce chromosomal aberrations, somatic mutations, and morphological transformation. Mutagenicity tests of nickel compounds in bacterial systems have consistently been negative. Ni(II) has been reported to impair the fidelity of viral and bacterial DNA polymerases for *in vitro* replication of synthetic nucleotide templates.

Introduction

Research on nickel carcinogenesis prior to 1975 has been comprehensively reviewed and critically evaluated by panels of scientific experts under the auspices of the U.S. National Academy of Sciences (NAS) (*1*) and the International Agency for Research on Cancer (IARC) (*2*). Both scientific panels concluded that increased incidences of lung cancer and nasal cancer have been demonstrated by epidemiological studies of nickel refinery workers, and that the carcinogenicity of certain nickel compounds has been definitely established by animal experiments (*1, 2*). In 1977, the U.S. National Institute of Occupational Safety and Health (NIOSH) stated that "An excess number of deaths from lung cancer and nasal cancer has been observed in nickel refinery workers. After review of the relevant data, it was concluded that a substantial portion of those excess deaths was caused by exposure to airborne nickel compounds" (*3*). Readers are referred to the NAS, IARC, and NIOSH monographs (*1-3*) and to several review articles (*4-9*) for the scientific background on nickel carcinogenesis. Relevant investigations from 1975 to March 1980 are summarized in the present article, so that readers may be informed about the many recent developments in nickel carcinogenesis. Emphasis is placed upon new experimental techniques to study nickel carcinogenesis *in vivo* and *in vitro*, and attention is focused upon prospects for future research on the mechanism(s) whereby nickel compounds initiate neoplastic transformation.

Epidemiological Studies

Several recent studies have analyzed the risks of respiratory tract cancers in workmen who have been exposed to inhalation of nickel compounds (*10-15*). Doll et al. (*10*) reinvestigated the causes of death of employees at a nickel refinery in Clydach, Wales, U.K. based upon a cohort of 967 men who began working before 1945. As shown in Table 1, men who entered employment prior to 1930 had excess risks of respiratory tract cancer. Pedersen et al. (*11*) reported a similar updated survey of mortality from respiratory cancers in workers at a nickel refinery in Kristiansand, Norway. Increased risks of cancers of the nasal cavities, lung and larynx occurred in a cohort of 2249 men who

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worked at the refinery for at least 3 years prior to 1953. Twenty cases of nasal cancer were observed (O) versus 0.81 expected (E) (O/E = 24.7); 69 cases of lung cancer were observed versus 18.64 expected (O/E = 3.7), and 6 cases of larynx cancer were observed versus 2.11 expected (O/E = 2.8). Workers in the roasting, smelting, and electrolysis departments of the nickel refinery had the highest incidence rates for cancers of respiratory organs. Kreyberg (*12*) studied the characteristics of lung cancers in workmen at the same nickel refinery in Kristiansand, Norway. A tabulation of 39 lung cancers included 26 epidermoid (squamous cell) carcinomas, 6 small cell anaplastic carcinomas, and 7 adenocarcinomas (including related histological types). Based upon detailed analyses of work chronologies and smoking histories, Kreyberg (*11*) concluded that tobacco smoking contributed to the development of lung cancers in the nickel-exposed workers. Lessard et al. (*13*) studied the influence of nickel exposure upon lung cancer mortality in Noumea, New Caledonia. Workers at a nickel refinery had three-fold risk of lung cancer, independent of the effects of age and cigarette smoking. Lung cancer risk was also increased three-fold in persons who lived in a zone less than 1 km from the nickel refinery, in comparison to persons who lived more than 3 km from the refinery. This excess risk was independent of employment in the refinery. Bernacki et al. (*14*) assessed the possible association between exposure to nickel-containing compounds and lung cancer mortality in workmen at an aircraft engine factory in Hartford, Connecticut, U.S.A. This case-control study was limited to men who died prior to retirement from work, and hence the possible latent period for development of lung cancer was restricted. The 42 nickel-exposed decedents comprised welders, electroplaters, metal-powder sprayers, grinders, polishers, and bench mechanics, and the 84 control decedents comprised workers in other factory trades who had minimal occupational exposures to nickel. The proportions of deaths from lung cancer were equal in the nickel-exposed decedents and in the controls. Godbold and Tompkins (*15*) performed a case-control study of mortality from respiratory cancer in 814 white men who had been employed prior to 1954 in the barrier department of a gaseous diffusion plant in Oak Ridge, Tennessee, U.S.A. The workers used nickel powder to fabricate a porous barrier for isotopic enrichment of uranium by gaseous diffusion. The control group included 1600 white men who worked in other departments and who had no record of nickel exposure. Employees and pensioners were traced for a minimum follow-up period of 19 years. The nickel-exposed cohort experienced lower mortality than the controls, both in deaths from respiratory cancer and in deaths from all causes, but neither of these differences was statistically significant (*15*).

Clinical Investigations

Recent advances in diagnosis, classification, and management of cancers of the nasal cavities and lungs in nickel refinery workers have been described in clinical reports (*16-23*). Nelen et al. (*16*) conducted a prospective study of sputum cytology in 268 asymptomatic men who had been employed prior to 1963 in the sintering department of a nickel refinery in Sudbury, Ontario, Canada. Eleven cases of lung cancer and one case of laryngeal cancer were discovered. Nelen et al. (*16*) concluded that prospective cytologic screening of sputum for neoplastic cells is an effective technique for detection of cancers of the respiratory tract in nickel refinery workers. They also noted that 11 of the 12 subjects with respiratory tract cancers were smokers and that the remaining subject was a former smoker (*16*). Barton (*17*) summarized the results of a cancer

Table 1. Deaths from respiratory tract cancers in a cohort of workmen at a nickel refinery in Clydach, Wales.[a]

	Deaths from cancer of the nasal sinuses[b]			Death from cancer of the lung[b]		
Year of first employment	O[c]	E	O/E	O	E	O/E
< 1910	14	0.036	389	24	2.389	10.0
1910-1914	24	0.037	649	34	3.267	10.4
1915-1919	11	0.025	440	20	3.070	6.5
1920-1924	7(1)	0.071	99	50	9.642	5.2
1925-1929	0(1)	0.026	0	9	3.613	2.5
1930-1944	0	0.034	0	8	5.463	1.5

[a]Data of Doll et al. (*10*).

[b]O = observed deaths based upon death certificates; E = expected deaths based upon national mortality rates; O/E = ratio of observed to expected deaths.

[c]Cases of nasal sinus cancer referred to as an associated cause of death are shown in parentheses.

detection program for approximately 1200 workmen at a nickel refinery in Kristiansand, Norway. The program consisted of periodic physical examinations (including rhinoscopy), x-ray examinations of the chest and nasal sinuses, and analyses of nickel concentrations in plasma and urine. In selected cases, sputum cytology and nasal mucosal biopsy were performed. During 5 years of surveillance, nearly 100 employees with suspicious signs or symptoms were subjected to nasal mucosal biopsies. The biopsies yielded the following significant findings: 4 cases of invasive squamous cell carcinoma, 2 cases of carcinoma *in situ*, and 16 cases of epithelial atypia. Fifteen of these subjects had worked in the roasting-smelting department of the nickel refinery. Barton (*17*) discussed the management of nasal cancers in nickel refinery workers and he recommended radical surgical resection, alone, or in combination with postoperative radiation therapy. Torjussen and co-workers (*18-23*) evaluated the rhinoscopic appearance, x-ray findings, histopathologic lesions, and nickel concentrations in body fluids and nasal biopsies of active and retired workers at the same nickel refinery in Kristiansand, Norway. Nasal polyps and hyperplastic rhinitis were more common in the nickel refinery workers than in controls, but no significant differences were observed between x-ray findings in nasal sinuses of nickel-exposed and control subjects (*18*). Histopathologic changes in nasal biopsies from active and retired nickel refinery workers and from controls were scored numerically, and the scores were found to correlate with duration of nickel exposure, type of nickel exposure, and tobacco consumption. Two workers from the roasting-sintering department, both employed 28 years at the nickel refinery, had nasal carcinomas. Epithelial dysplasia, an apparently precancerous lesion, was found in 38 of 318 active and 7 of 15 retired nickel workers, and in only 1 of 57 controls (*19, 20*).* Increased nickel concentrations were found in nasal biopsy specimens from all categories of nickel-exposed workers, but the highest concentrations were found in workers from the roasting-smelting department (*21*). Analyses of nickel concentrations in nasal biopsy specimens from pensioned workers showed that accumulated nickel was retained for several years after termination of nickel exposure, and was slowly released from the nasal mucosa with an estimated half-life of 3.5 years (*21*). Attempts to identify the cellular localization of nickel in nasal biopsy specimens by histochemical staining methods and by energy dispersive x-ray microanalysis were inconclusive, owing to insufficient analytical sensitivity (*22, 23*). Sunderman (*24*) reported an unusual case of polypoid squamous cell carcinoma of the nose in a 36-year-old man. The patient had worked in a cutlery factory for 12 years. For several years, he had immersed small nickel-plated objects (such as teapots) in a tank of HCl-HNO_3 at 85°C in order to remove old nickel plating (e.g., from battered hotel utensils). During this operation, the patient had chronically inhaled nickel-containing acid fumes from the nickel-stripping tank. In view of the patient's relative youth and occupational history, Sunderman (*24*) suspected that the patient's nasal cancer was caused by nickel. Bourasset and Galland (*25*) previously reported a similar case of nasal cancer in a cutlery worker who had been exposed to inhalation of nickel-containing fumes.

*In view of the propensity of wood-workers to develop nasal cancer, it is noteworthy that the control subject with epithelial dysplasia was a carpenter.

Carcinogenicity Tests in Animals

In order to bring up to date the IARC monograph on nickel and nickel compounds (*2*), this résumé of recent tests of the carcinogenicity of nickel compounds in experimental animals is patterned on the IARC format.

Inhalation and/or Intratracheal Administration

Saknyn and Blohkin (*20*) exposed nonpedigree albino rats to inhalation of feinstein dust (an intermediate product of nickel refining, which contains NiS, NiO, and metallic Ni) in atmospheric concentration of 70 mg dust/m^3 for 5 hr/day, 5 days/week during 6 months. Lung cancers (squamous cell carcinomas) were found in 2 of 5 rats that survived the treatment. The latent period for tumor development was 17 months. Saknyn and Blohkin (*20*) also administered black nickel monoxide (NiO) to albino rats as a single intratracheal injection (20 to 40 mg/rat). Lung cancer (squamous cell carcinoma) developed in 1 of 26 rats after a latent period of 17 months. No lung tumors were found in an untreated control group of 47 rats.

Mukubo (*27*) treated female albino rats by a single intratracheal injection of metallic nickel dust (10 mg/rat), alone, or in combination with methylcholanthrene (5 mg/rat). At 12 weeks, lung cancers (squamous cell carcinomas) were seen in (a) 3 of 5 rats that received nickel plus methylcholanthrene; (b) 2 of 7 rats that received methylcholanthrene alone, and (c) 0 of 7 rats that received nickel alone. The failure to detect lung cancers in the nickel-treated group should not be considered a negative

carcinogenicity test, owing to the short period of observation.

Yarita and Nettesheim (*28*) studied the carcinogenicity of nickel subsulfide (α-Ni_3S_2) in heterotopic tracheas that were transplanted (two tracheas/rat) under the dorsal skin of isogeneic female rats of the Fischer strain. Gelatin pellets were inserted into the transplanted tracheas at 4 weeks after grafting. Sixty tracheas received pellets that contained 1 mg of α-Ni_3S_2; 64 tracheas received pellets that contained 3 mg of α-Ni_3S_2, and 10 control pellets received gelatin pellets, alone. Surviving rats were killed after 20 months. At the 1 mg dose of α-Ni_3S_2, tumors developed in 9/60 tracheas (5 squamous cell carcinomas, 1 undifferentiated carcinoma, 2 fibrosarcomas, and 1 leiomyosarcoma). At the 3 mg dose of α-Ni_3S_2, tumors developed in 45/64 tracheas (1 squamous cell carcinoma, 12 fibrosarcomas, 10 leiomyosarcomas, 10 fibromyosarcomas, 2 rhabdomyosarcomas, 2 fibromyxosarcomas, 7 sarcomas of uncertain type, and 1 benign myoma). No tumors developed in the 10 control tracheas.

Oral Administration

Sunderman et al. (*29*) painted α-Ni_3S_2 in glycerol onto the buccal pouch mucosa of four groups of Syrian golden hamsters of the LVG/LAK strain. The dosage schedules were: (a) 1 mg α-Ni_3S_2, 3 times/week for 18 weeks in 6 hamsters; (b) 2 mg α-Ni_3S_2, 3 times/week for 18 weeks in 7 hamsters; (c) 5 mg α-Ni_3S_2, 3 times/week for 36 weeks in 15 hamsters; and (d) 10 mg α-Ni_3S_2, 3 times/week for 36 weeks in 13 hamsters. Surviving hamsters were killed at 24 months after the initial application. No tumors were found in the buccal pouch, oral cavity or gastrointestinal tract of any of these hamsters, or in 15 controls that received buccal pouch applications of the glycerol vehicle (0.2 ml, 3 times/week for 36 weeks). Cancers (squamous cell carcinomas) of the buccal pouch were found in 4/4 hamsters in a positive control group that received similar applications of dimethylbenzanthracene in glycerol (1 mg DMBA, 3 times/week for 18 weeks).

Intramuscular Injection

Sunderman (*30*) administered α-Ni_3S_2 to albino mice of both sexes by a single IM injection (2.5 mg α-Ni_3S_2/mouse). Within 100 weeks, sarcomas developed at the injection site in 6/9 mice of the DBA-2 strain (versus 0/9 vehicle controls) and in 5/10 mice of the C57-BL6 strain (versus 0/9 vehicle controls).

Sunderman et al. (*31*) gave single IM injections of six nickel compounds in equal doses (14 mg Ni/rat) to male Fischer rats. This experiment was a supplement to an earlier study (*32*) that was included in the 1976 IARC monograph (*2*). By 100 weeks after the IM injection, the incidences of sarcomas at the injection sites were: (a) metallic Ni dust: 13/20 rats; (b) crystalline nickel subselenide (Ni_3Se_2): 21/23 rats; (c) crystalline nickel monoselenide (NiSe): 8/16 rats; (d) crystalline nickel subsulfide (α-Ni_3S_2): 9/9 rats; (e) crystalline nickel monosulfide (β-NiS): 14/14 rats; (f) amorphous nickel monosulfide (NiS): 0/10 rats; and (g) vehicle controls: 0/44 rats. Based upon the marked differences in sarcoma incidences after an IM injection of crystalline β-NiS and amorphous NiS, Sunderman et al. (*31*) concluded that the physical form of nickel sulfides has a critical influence upon their carcinogenic activities. In the same experiment, sarcomas were observed at the injection site in 3/16 rats that received a lower dose (7 mg Ni/rat) of nickel carbonyl-cyclopentadiene dimer; $[Ni(CO)_2(C_5H_5)_2]_2$.

Sunderman (*30*) derived a dose-response curve for induction of sarcomas in male Fischer rats by single IM injection of α-Ni_3S_2, based upon four published and two previously unpublished experiments which involved a total of 383 rats. The experiments were all terminated at 100 to 104 weeks after the injection. Sarcoma incidence at 62 weeks after the injection was linearly related to the reciprocal of the α-Ni_3S_2 dose, and ranged from 24% (7/29) in rats that received 0.63 mg of α-Ni_3S_2, to 100% (9/9) in rats that received 20 mg of α-Ni_3S_2. There was no indication of a threshold carcinogenic dosage in this experimental system. The histologic types of 336 sarcomas induced by an IM injection of α-Ni_3S_2 included 161 rhabdomyosarcomas, 91 undifferentiated sarcomas, 72 fibrosarcomas, 9 liposarcomas, 2 neurofibrosarcomas, and 1 hemangiosarcoma. Metastases were found in 41% (137/336) of tumor-bearing rats.

Sunderman et al. (*33*) gave male Fischer rats a single IM injection of α-Ni_3S_2 (1.2 mg/rat), alone, or in combination with metallic Mn dust (1.0 mg/rat) or metallic Cr dust (1.0 mg/rat). Within 100 weeks, the sarcoma incidence in rats that received only α-Ni_3S_2 was 22/30. Addition of Mn dust to α-Ni_3S_2 reduced the sarcoma incidence to 1/14, whereas addition of Cr dust to α-Ni_3S_2 did not affect the sarcoma incidence (12/15). No sarcomas developed at the IM injection site in three control groups, including 39 rats that received the injection vehicle, 14 rats that received Mn dust alone (1.0 mg/rat), and 15 rats that received Cr dust alone (1.0 mg/rat).

Sunderman (*29*) administered α-Ni_3S_2 to male Syrian golden hamsters of the LVG/LAK strain by a single IM injection. By 24 months after the injection, the incidence of local sarcomas was 5/15 in hamsters that received 5 mg of α-Ni_3S_2 and 12/17 in

hamsters that received 10 mg of α-Ni_3S_2. Metastases were found in 10 sarcoma-bearing hamsters. No sarcomas occurred at the injection site in 14 control hamsters that received an IM injection of the NaCl vehicle.

Hildebrand and Biserte (*34, 35*) described 16 sarcomas (including unspecified numbers of rhabdomyosarcomas, fibrosarcomas, and 3 leiomyosarcomas) that developed in albino rabbits at the site of IM implantation of α-Ni_3S_2 in agar (80 mg α-Ni_3S_2/rabbit). These papers were concerned primarily with the ultrastructural features of the tumors. The numbers of treated and control rabbits were not specified.

Intraperitoneal Injection

Stoner et al. (*36*) and Shimkin et al. (*37*) described an experiment in which nickelous acetate was administered to 3 groups of strain A mice (20 mice/group) by IP injection, 3 times/week for 8 weeks, for total dosages of 72, 180, and 360 mg/kg, respectively. A control group was given similar IP injections of the vehicle. The mice were killed at 30 weeks after the first injection. The average number of lung tumors/mouse was 0.42 in controls, 0.67 at the 72 mg/kg dose; 0.71 at the 180 mg/kg dose, and 1.26 at the 360 mg/kg dose. At the highest dose level, the increase in lung tumors was statistically significant.

Saknyn and Blokhin (*26*) treated albino rats by single IP injection of feinstein dust at a dosage of 90-150 mg dust/rat. Sarcomas developed at the injection site in 6 of 39 rats after latent periods of 6 to 15 months.

Intrarenal Injection

Jasmin and associates (*38, 39*) administered α-Ni_3S_2 to female Sprague-Dawley rats by intrarenal (IR) injection in dosage of 10 mg/rat. In three separate experiments, cancer of the injected kidney developed in 7/16, 11/24, and 11/20 rats, respectively, within 12 months. In contrast, renal cancers did not develop in two control groups of 20 and 16 rats which received IR injection of the vehicle, or in two groups of 18 and 20 rats which received IR injection of either metallic Ni dust or NiS (10 mg/rat). The renal tumors in α-Ni_3S_2-treated rats were all classified as carcinomas, although many of the tumors were pleomorphic and included anaplastic spindle-cell varieties. Jasmin and Solymoss (*39*) mentioned unsuccessful attempts to induce renal tumors in mice, hamsters and rabbits by IR injection of α-Ni_3S_2, but they did not furnish experimental details.

Sunderman et al. (*40*) also tested the carcinogenicity of α-Ni_3S_2 following IR injection in rats. Tumors of the injected kidney developed within 2 years in 9/32 Fischer rats (14 female, 18 male) that received 5mg of α-Ni_3S_2, and in 23/38 rats (14 female, 24 male) that received 10 mg of α-Ni_3S_2. No renal tumors occurred in 52 control Fischer rats (17 female, 35 male) that received IR injection of the NaCl vehicle. Injection IR of α-Ni_3S_2 (5 mg/rat) induced renal tumors in 6/12 NIH black rats (6 female, 6 male), and 7/11 Wistar-Lewis rats (6 female, 5 male). In contrast, no renal tumors developed in 12 α-Ni_3S_2-treated Long-Evans rats (6 female, 6 male). In male Fischer rats that received an IR injection of α-Ni_3S_2 (10 mg/rat) combined with metallic Mn dust (7 mg/rat), the incidence of renal tumors was 17/28, which differed significantly from the corresponding incidences of 18/24 and 0/23 in male Fischer rats that received IR injections of α-Ni_3S_2 (10 mg/rat) alone or Mn dust (7 mg/rat) alone. The 54 renal tumors that were observed by Sunderman et al. (*40*) in α-Ni_3S_2-treated rats were all malignant, and metastases were found in 37/54 tumor-bearing rats. The authors were uncertain whether the renal cancers were epithelial or mesenchymal in origin (*40*).

Intratesticular Injection

Damjanov et al. (*41*) administered α-Ni_3S_2 to male Fischer rats by single intratesticular injection (10 mg α-Ni_3S_2/rat). Within 20 months, malignant testicular neoplasms developed in 16 of 19 α-Ni_3S_2-treated rats, and in 0 of 18 controls that received intratesticular injection of NaCl vehicle. The testicular neoplasms in α-Ni_3S_2-treated rats included 4 fibrosarcomas, 4 fibrous histiocytomas, and 4 rhabdomyosarcomas. Metastases were identified in 4/16 tumor-bearing rats.

Intraocular Injection

Albert et al. (*42*) injected α-Ni_3S_2 (0.5 mg/rat) into the vitreous cavity of the right eye of juvenile male Fischer rats (1 month old). Malignant ocular tumors developed in 14 of 15 treated rats by 8 months, and in 0 of 11 controls which received an intraocular injection of NaCl vehicle. In three α-Ni_3S_2-treated rats, the injected eye had two primary tumors, and in two α-Ni_3S_2-treated rats, the injected eye had three distinct primary tumors. The 22 ocular neoplasms included 11 amelanotic uveal melanomas, 4 retinoblastomas, 3 gliomas, 1 fibrosarcoma and 1 phakocarcinoma, and 3 unclassified malignant tumors. Extraocular extension, invasion of the optic nerve, and metastases to lung and brain were noted.

Intracerebral Injection

Sosinski (*43*) injected nickelic oxide (Ni_2O_3) into the cerebral cortex of 20 Wistar rats (10 male, 10 female) in a dosage of 3 mg Ni_2O_3/rat. Each rat also received an im injection of Ni_2O_3 (10 mg/rat) into the left gastrocnemius muscle. Control rats were not mentioned. Cerebral gliomas were observed in two rats that were killed at 14 and 21 months, respectively, and a meningioma was found in one rat that was killed at 21 months. No neoplasms developed at the sites of IM injection of Ni_2O_3.

Other Parenteral Routes

Jasmin and Solymoss (*39*) mentioned that IV administration of α-Ni_3S_2 (10 mg/rat) to 20 female Sprague-Dawley rats did not increase the incidence of benign or malignant tumors, and that intrahepatic administration of α-Ni_3S_2 (10 mg/rat) to eight female Sprague-Dawley rats did not induce any hepatic tumors. The periods of observation were not specified. Sunderman et al. (*29*) reported that no hepatic tumors developed in 13 male Fischer rats that received an intrahepatic injection of α-Ni_3S_2 (5 mg/rat), nor did any salivary gland tumors develop in 11 male Fischer rats that received an injection of α-Ni_3S_2 (2.5 mg/rat) into a submaxillary gland. The periods of observation were 2 years (*29*).

Relevant Experiments in Animals

X-Ray Diffractometry and Radiotracer Studies

The metabolism of α-Ni_3S_2 in rodents has been investigated by x-ray diffractometry (*44*, *45*) and by radiotracer studies (*33*, *44-46*). Applications of nickel radioisotopes in biological research have recently been comprehensively reviewed by Kasprzak and Sunderman (*47*). These authors emphasized that ^{63}Ni is an ideal radioisotope for investigations of metal carcinogenesis, because ^{63}Ni is available in high specific activity (up to 3.87 kCi/g-atom) and has a long half-life (92 years). Moreover, the soft beta emission of ^{63}Ni (67 keV) is readily counted by liquid scintillation spectrophotometry, and it provides autoradiograms with exceptionally high resolution. Kasprzak (*46*) administered α-Ni_3S_2 that was radiolabeled with ^{63}Ni or ^{35}S to Fischer rats by IM injection in both hind limbs (10 mg/injection). Local sarcomas developed in 8/8 α-$^{63}Ni_3S_2$-treated rats and in 4/7 α-$Ni_3{}^{35}S_2$-treated rats during 8 months of observation. Tumors developed at one injection site in 5/15 rats, and at both injection sites in 7/15 rats. The tumors were all pleomorphic rhabdomyosarcomas. Autoradiography showed extracellular particles of $\alpha^{63}Ni_3S_2$ and $\alpha Ni_3{}^{35}S_2$ at the injection sites. The particles of radiolabeled α-Ni_3S_2 eventually became surrounded by neoplastic tissue. Intracellular localization of ^{63}Ni or ^{35}S was not detected within muscle or tumor cells, but sparse α-$^{63}Ni_3S_2$ particles were seen within macrophages at the injection sites (*46*).

Sunderman et al. (*33*) elucidated the metabolism of ^{63}Ni following single IM injection of α-$^{63}Ni_3S_2$ in 10 male Fischer rats (1.2 mg/rat). The cumulative excretions of ^{63}Ni during 8 weeks after the injection averaged 67 (S.D. ± 2) % in urine and 7 ± 2 % in feces. At 2 to 10 weeks after IM injection of α-Ni_3S_2, the particles of α-Ni_3S_2 which remained at the injection site were predominantly intracellular, and were located primarily within cytoplasmic vesicles in fibroblasts and macrophages. Residual ^{63}Ni at the injection site averaged 19 ± 4% of the dose in rats killed at 20 to 24 weeks, and 14 ± 2% of the dose in rats killed at 31 weeks after the injection. Whole-body ^{63}Ni kinetic parameters which were computed by compartmental analysis were not affected by admixture of Mn dust based upon measurements of ^{63}Ni in urine, feces, injection site, and viscera of rats that received an IM injection of α-$^{63}Ni_3S_2$ (1.2 mg/rat), alone, or in combination with Mn dust (1.0 mg/rat). However, the subcellular distribution of ^{63}Ni derived from α-$^{63}Ni_3S_2$ was significantly changed by admixture of Mn dust (*33*). ^{63}Ni concentrations in ultrafiltrates of supernatant fractions of homogenates of injection sites averaged 2.8 ± 0.7 ng/ml at 20 to 24 weeks after injection of α-$^{63}Ni_3S_2$ plus Mn dust, versus 5.4 ± 2.0 ng/ml after injection of only α-$^{63}Ni_3S_2$.

Oskarsson et al. (*45*) administered α-$^{63}Ni_3S_2$ or α-$Ni_3{}^{35}S_2$ to NMRI mice by an IM or SC injection (10 mg/mouse). During the period from 2 to 14 months after the injection, local sarcomas developed in 11/16 mice that received a SC injection of α-Ni_3S_2 labelled with ^{63}Ni or ^{35}S, and in 8/16 mice that received a similar IM injection of α-Ni_3S_2. In the tumors, radiolabelled particles of α-Ni_3S_2 were mostly intracellular within fibroblasts and macrophages. X-ray diffractometry of the insoluble residue from lyophillized tumor tissue did not reveal α-Ni_3S_2, but distinctly demonstrated crystalline α-Ni_7S_6 and β-NiS. Whole-body autoradiography showed gradual mobilization of solubilized ^{63}Ni and ^{35}S from the injection site. There was also mobilization of nonsolubilized ^{63}Ni-labeled particles, which were located within phagocytes in liver, spleen, and regional lymph nodes (*45*). These *in vivo* findings are compatible with earlier observations of Kasprzak

and Sunderman (*44*), who employed x-ray diffractometry to elucidate the dissolution of α-Ni_3S_2 during *in vitro* incubation in rat serum. α-Ni_3S_2 was slowly oxidized to crystalline nickel monosulfide (β-NiS), which subsequently underwent further oxidation to yield soluble Ni(II) complexes and relatively insoluble particles of nickel hydroxide [$Ni(OH)_2$] (*44*).

Electron Microscopic Studies

Bruni (*48*) administered α-Ni_3S_2 to male Sprague-Dawley rats by unilateral or bilateral IM injection into the thigh muscles (20 mg/injection). The rats were sacrificed at intervals from 2 to 26 weeks, and tissue from the injection site was examined by electron microscopy. Mitotic activity was seen primarily in muscle satellite cells. Satellite cells in division were morphologically indistinguishable from dividing stem cells in α-Ni_3S_2-induced rhabdomyosarcomas. On the basis of these findings, Bruni (*48*) suggested that muscle satellite cells are progenitors of the α-Ni_3S_2-induced tumors.

Hildebrand and Biserte (*49*) performed electron microscopy of 12 rhabdomyosarcomas that were induced in an unspecified number of Wistar rats by IM implantation of α-Ni_3S_2 in agar (20 mg α-Ni_3S_2/rat). Successive stages of differentiation of tumor cells were described, and formation of microtubules in interphase rhabdomyoblasts was convincingly demonstrated. Hildebrand and Biserte (*49*) did not observe any satellite cells in the rhabdomyosarcomas. In another study, Hildebrand and Biserte (*35*) described cylindrical paracrystalline structures in rhabdomyoblasts of rhabdomyosarcomas that were induced in rabbits by IM injection of α-Ni_3S_2 in agar (80 mg α-Ni_3S_2/rabbit). The authors speculated that the laminated cylindrical bodies represented abnormal aggregates of contractile proteins that were synthesized during myofibrillar differentiation.

Jasmin et al. (*50*) treated 25 female Sprague-Dawley rats by IR injection of α-Ni_3S_2 in glycerol (5 mg α-Ni_3S_2/rat). Groups of five rats were killed at biweekly intervals until 2 months, and the injected kidneys were examined by electron microscopy. Unusual crystalline inclusions were observed in mitochondria of tubular cells that were located in the pars recta of the distal nephron. By goniometric analysis, the authors deduced that the crystalline inclusions were composed of cylindrical rods in a hexagonal array. Jasmin et al. (*50*) speculated that the crystalline inclusions might consist of abnormal assemblages of protein components of mitochondrial cristae.

Cytogenetic and Tumor Transplantation Studies

Yamashiro et al. (*51*) performed karyotypic analyses and transplantation experiments on rhabdomyosarcomas that were induced in Fischer and Long-Evans rats by single IM injection of α-Ni_3S_2 (10 mg/rat). The chromosomal complements of tumor cells from 12 primary rhabdomyosarcomas were usually in the diploid range, although 11 of the 12 tumors contained a few triploid and tetraploid cells. Abnormal chromosomes were found, including dicentric, triradial, and ring forms. Comparisons of chromosomes of primary and metastatic tumor cells suggested that tumors with diploid or near-diploid chromosomes were most likely to metastasize. Rhabdomyosarcoma cells were cultured *in vivo* in diffusion chambers which were implanted in the peritoneal cavity of syngeneic rats. Such cell cultures exhibited less myogenic differentiation than parallel cell cultures which were incubated *in vitro* in Leighton tubes (*51*).

Abandowitz (*52*) added neuraminidase to cultured cells from an α-Ni_3S_2-induced rat fibrosarcoma. The neuraminidase treatment inhibited tumor growth following inoculation of the fibroblasts into normal recipient rats. The recipient rats acquired enhanced resistance to subsequent inoculations of tumor cells.

Effects on DNA Synthesis *in Vivo*

Hui and Sunderman (*53*) measured *in vivo* incorporation of thymidine-^3H into DNA in rats at 28 hr after partial hepatectomy. Administration of nickel carbonyl [$Ni(CO)_4$] at 2 or 4 hr before sacrifice inhibited thymidine-^3H uptake into liver and kidney DNA. For example, in rats killed 4 hr after IV injection of $NI(CO)_4$ (2 mg Ni/100 g), ^3H-labeling of liver DNA averaged 54 (SE ± 10) % of controls, and ^3H-labeling of kidney DNA averaged 53 ± 6 % of controls. Injection of $NiCl_2$ (2 mg Ni/100 g, im) 4 hr before sacrifice did not significantly affect thymidine-^3H uptake into liver DNA, but did inhibit thymidine-^3H uptake into kidney DNA (65 ± 6% of controls). Binding of ^{63}Ni to DNA in liver and kidney of rats killed 4 hr after injection of $^{63}Ni(CO)_4$ or $^{63}NiCl_2$ ranged from 0.3 to 2.2 mole ^{63}Ni/mole of DNA nucleotides. The binding of ^{63}Ni to DNA that was observed by Hui and Sunderman (*53*) was consistent with previous reports by Heath and Webb (*54*) and Webb et al. (*55*) of nickel binding to nucleoli, chromatin, and deoxyribonucleohistones isolated from nickel-induced rhabdomyosarcomas. However, the presence of ^{63}Ni in the DNA prepara-

tions did not necessarily connote *in vivo* ^{63}Ni-binding, since the possibility of ^{63}Ni-binding to DNA during tissue homogenization and DNA isolation could not be excluded (*53*). Hui and Sunderman performed ultracentrifugal fractionations of liver DNA on alkaline sucrose gradients (*53*), and they did not observe any differences between sedimentation profiles of liver DNA from $Ni(CO)_4$-treated rats versus paired control rats.

Relevant Studies in Cell Cultures

In vitro exposure of mammalian cells to certain nickel compounds inhibits cellular uptake of thymidine-^{3}H and induces chromosomal aberrations, somatic mutations, and morphological transformation. In studies which were briefly mentioned in the NAS and IARC monographs (*1, 2*), Basrur and Gilman (*56*) and Swierenga and Basrur (*57*) showed that addition of α-Ni_3S_2 to cultures of rat embryo muscle cells profoundly inhibited thymidine-^{3}H uptake, suppressed cell division, and induced bizarre mitoses, including multipolar and distorted bipolar spindles, C-metaphase-like shapes, and lagging chromosomes. Mitotic arrest occurred in telophase and post-telophase, consistent with disturbed dissolution of mitotic spindles. In a recent study, Nishimura and Umeda (*58*) found that addition of nickel compounds, ($NiCl_2$, NiS, nickel acetate, and potassium cyanonickelate), to cultures of mouse mammary carcinoma cells inhibited thymidine-^{3}H uptake and increased the frequency of chromosomal aberrations. Anacher and Paillet (*59*) reported that exposure of mouse lymphoma cells to $NiCl_2$ caused dose-dependent increases in trifluorothymidine-resistant mutants, and Hsie et al. (*60*) noted that exposure of Chinese hamster ovary cells to $NiCl_2$ induced thioguanine-resistant mutants. Casto et al. (*61*), DiPaolo and Casto (*62*), Pienta et al. (*63*), Costa et al. (*64-66*), and Rivedal and Sanner (*67*) showed that *in vitro* exposures of Syrian hamster embryo cells to $NiSO_4$ or α-Ni_3S_2 resulted in morphological transformation. Casto et al. (*61*) failed to detect DNA damage by alkaline sucrose gradient ultracentrifugation of DNA from cultured hamster embryo cells that had been exposed *in vitro* to $NiSO_4$. Costa et al. (*66*) demonstrated that several clones of α-Ni_3S_2-transformed cells produced fibrosarcomas following SC injection in nude mice. DiPaolo and Casto (*62*) and Costa et al. (*66*) observed dose-dependent relationships between the concentration of α-Ni_3S_2 in the tissue culture medium and the incidence of morphological transformation of Syrian hamster fetal cells. Amorphous nickel monosulfide (NiS) did not induce morphological transformation under the same conditions (*62, 66*). Costa et al. (*67*) compared *in vitro* uptake of α-Ni_3S_2 and amorphous NiS by Chinese hamster ovary cells and Syrian hamster fetal cells. Both types of cells avidly engulfed α-Ni_3S_2 particles, whereas they only engulfed a few amorphous NiS particles under similar exposure conditions. Costa et al. (*67*) suggested that the striking disparity in carcinogenic activity of crystalline α-Ni_3S_2 and amorphous NiS may be attributed to marked differences in cellular uptake of the two compounds.

Rivedal and Sanner (*68*) employed *in vitro* morphological transformation and induction of somatic mutation to investigate synergism between nickel and polycyclic aromatic hydrocarbons. The transformation frequency of Syrian hamster embryo cells increased with increasing concentrations of $NiSO_4$, benzo(a)pyrene (BP), or methylcholanthrene (MC). When cells were exposed to combinations of $NiSO_4$ and BP, the transformation frequencies were much higher than when the compounds were tested separately. The greatest enhancement was found with 5 μg/ml of $NiSO_4 \cdot 6H_2O$ and 0.78 μg/ml of BP. The transformation frequency obtained with this combination was 10.7%, compared to frequencies of 0.5% and 0.6% that were obtained with the individual substances. No synergistic effect was detected between $NiSO_4$ and MC. In experiments that measured somatic mutations in Syrian hamster embryo cells by selection for ouabain-resistance, the mutation frequency was significantly higher than expected when the cells were exposed to mixtures of $NiSO_4$ and BP (*68*). Rivedal and Sanner's observation of mutagenic synergism between $NiSO_4$ and BP is consistent with an earlier report by Maenza et al. (*69*) of carcinogenic synergism between α-Ni_3S_2 and BP following IM injection in Fischer rats. Sunderman (*70*) observed that exposure of rats to $Ni(CO)_4$ by inhalation or IV injection inhibited phenothiazine induction of BP (arylhydrocarbon) hydroxylase activity in lung and liver. Sunderman and Roszel (*71*) administered BP to rats by IV injection and studied the effect of $Ni(CO)_4$ on the retention of BP in lung and liver. A single exposure of rats to $Ni(CO)_4$ inhibited BP mobilization from lung and liver for 48 hr (*70*). The inhibitory effects of nickel compounds on BP metabolism are of especial interest in view of the potentiating effect of cigarette smoking on the development of lung cancer in nickel refinery workers (*12, 13, 16*).

Mutagenicity Tests in Bacteria

Bacterial mutagenesis tests of nickel compounds have consistently been negative, despite several attempts by experienced workers to demonstrate

mutagenicity in *E. coli* or *S. typhimurium* (*7, 72-75*).*

Studies in Biochemical Systems

Sirover and Loeb (*77*) demonstrated that Ni(II), Co(II), and Mn(II) substituted for Mg(II) as activators of avian myeloblastosis virus (AMV) DNA polymerase for replication of synthetic polynucleotide templates. During DNA synthesis by AMV DNA polymerase in the presence of Mg(II), addition of Ni(II) (as well as soluble salts of other carcinogenic metals) decreased the fidelity of DNA replication (*77, 78*). Sirover and Loeb (*78*) suggested that impaired fidelity of DNA replication by AMV DNA polymerase might serve as an *in vitro* screening test to identify metal compounds that could potentially be carcinogenic and/or mutagenic. Miyaki et al. (*79*) found that Ni(II) increased misincorporation of deoxynucleotides by *E. coli* DNA polymerase I during transcription of synthetic polynucleotide templates. Loeb et al. (*80*) and Zakour et al. (*81*) speculated that carcinogenic metals may diminish the fidelity of DNA polymerase activity in target cells *in vivo*, and may thereby induce errors in selection of nucleotide bases during DNA synthesis. According to this hypothesis, decreased fidelity of DNA polymerase might initiate a cascade of random somatic mutations and evolve transformed cells that possess selective advantages for proliferation in the host. This hypothesis and related theories about possible molecular mechanisms of metal carcinogenesis have been considered in recent review articles (*7, 75, 81, 82*).

Prospects for Future Research

The demonstration by Sirover and Loeb (*80*) of metal-induced infidelity of DNA replication may possibly point to a fundamental mechanism of metal carcinogenesis. However, even if this is not the case, their research has attracted the attention of molecular biologists to the previously neglected area of metal carcinogenesis. Recent refinements of techniques to investigate derangements of nucleic acid synthesis, repair, and regulation in eukaryotic cells will undoubtedly facilitate mechanistic studies of metal carcinogenesis. α-Ni_3S_2 is an exceptionally advantageous compound for use in such studies, since α-Ni_3S_2 is inexpensively available in high purity and is readily labelled with ^{63}Ni, a beta-emitting radioisotope with long half-life that is well suited for liquid scintillation counting and autoradiography. The carcinogenic activity of α-Ni_3S_2 is apparently greater than any other metallic compound which has been investigated (*6*). A remarkable variety of animal species, routes of administration and cell culture systems can be employed for cancer research with α-Ni_3S_2. Furthermore, neoplastic transformation by α-Ni_3S_2 can be suppressed by manganese dust *in vivo* and *in vitro* (*30, 33, 40, 64*). This observation may serve as a clue to identify the biochemical effects of α-Ni_3S_2 that are specifically associated with neoplastic transformation.

*Dr. Samuel J. Rogers (Montana State College, Bozeman, MT) did not observe mutations of *S. typhimurium* in the Ames mutagenesis test system with α-Ni_3S_2, NiS, Ni_3S_4, NiTe, NiSb, NiO, and $NiSO_4$, (personal communications on December 3, 1976 and January 26, 1977). Dr. Goran Lofroth (University of California, Berkeley, CA) also obtained negative results in the Ames mutagenesis test system with α-Ni_3S_2, (personal communication on March 3, 1977).

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Role of Cobalt, Iron, Lead, Manganese, Mercury, Platinum, Selenium, and Titanium in Carcinogenesis

by George Kazantzis*

The possible carcinogenicity of cobalt, iron, lead, manganese, mercury, platinum, selenium, and titanium is reviewed, taking into account epidemiological data, the results of animal experimental studies, data on mutagenic effects and on other *in vitro* test systems. Of the great variety of occupations where exposure to one of these metals may occur, only haematite mining has been clearly shown to involve an increased human cancer risk. While the possibility that haematite might in some way act as a carcinogen has to be taken into consideration it is more likely that other carcinogens are responsible. Certain platinum coordination complexes are used in cancer chemotherapy, are mutagenic, and likely to be carcinogenic. Cobalt, its oxide and sulfide, certain lead salts, one organomanganese, and one organotitanium compound have been shown to have a limited carcinogenic effect in experimental animal studies, and except for titanium appear to be mutagenic. Certain mercury compounds are mutagenic but none have been shown to be carcinogenic. The presently available data are inadequate to assess the possible carcinogenicity of selenium compounds, but a few observations suggest that selenium may suppress the effect of other carcinogens administered to experimental animals and may even be associated with lower cancer mortality rates in man. Epidemiological observations are essential for the assessment of a human cancer risk, but the difficulty in collecting past exposure data in occupational groups and the complexity of multiple occupational exposures with changes over time, limits the usefulness of retrospective epidemiological studies.

Cobalt

Sources of Exposure

Cobalt is found in nature together with nickel and arsenic but is more often recovered from residues in the smelting of arsenical ores of nickel, copper and lead. World production had increased to about 33,000 tons by 1975. The principal uses of cobalt are in magnets, high temperature alloys, and cobalt steels. Cobalt is used as a binder for tungsten carbide cutting tools. A cobalt-chromium-molybdenum-nickel alloy, vitallium, is used in orthopaedic surgery as an implant.

Occupational exposure to cobalt occurs principally in the refining of cobalt, in the production of alloys and in the tungsten carbide hard metal industry. High exposure levels have been reported, with a concentration of up to 79 mg/m^3 in a plant where cobalt nitrate was calcined. Adverse respiratory effects have been reported at concentrations between 0.1 and 2 mg/m^3. The TLV-TWA for cobalt metal, dust, and fume (as cobalt) is 0.05 mg/m^3 as a tentative value, having previously been 0.1 mg/m^3.

Cobalt is present in low concentration in soil, with an average distribution in the earth's crust of 25 mg/kg, soil concentrations varying from less than one to 100 mg/kg. It is present in low concentrations in drinking water (0.1-5 μg/l.) and in many foods, in particular in sea foods. The average normal daily intake is of the order of 140-580 μg. Cobalt is an essential trace element in man and animals. A cobalt-containing compound, cyanocobalamin, or

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vitamin B12 was found effective in the treatment of pernicious anaemia, but three different cobalamins have now been identified in the body. Vitamin B12 is necessary for growing tissue, deficiency resulting in defective synthesis of DNA.

Cobalt as an integral part of the molecule of Vitamin B12 is used in the treatment of megaloblastic anaemias, and cobalt salts are also used for the prevention of cobalt deficiency in ruminants.

Mutagenic Effects

Cobalt compounds have been shown to affect the mitotic spindle causing C-mitosis, as have a number of other metals (*1*). Herich (*2*) found chromosome abnormalities in root tips exposed to cobalt nitrate. There is some evidence from observations on mitochondrial mutations in yeast cells, that cobalt is able to react with DNA (*3*). However, no effect has been shown on chromosomes of human leukocytes treated with cobalt nitrate (*4*). Cobalt chloride has been tested for its ability to affect the accuracy of DNA synthesis *in vitro* and has been shown to decrease the fidelity of DNA synthesis by at least 30% at a concentration of 4*mM* (*5*).

Carcinogenic Effects

Experimental. Finely divided cobalt metal powder, cobalt oxide, and cobalt sulfide, have given rise to injection site fibrosarcoma following subcutaneous injection, and to rhabdomyosarcoma following intramuscular injection in rats (*6*, *7*). About one year after a single injection of 20 mg cobalt oxide into rat thigh muscle, 50% of the injected group responded with sarcomas, but mice given doses twice as high did not develop any malignant tumors (*8*). Particles from surgical prostheses made from cobalt-chromium alloys have been shown to be carcinogenic to rat muscle (*9*, *10*). Sarcomata, both at the injection site and at distant sites have also been produced with multiple injections of a solution of cobalt chloride in physiological saline (*11*).

Clinical and Epidemiological. Although heavy occupational exposure has occurred to cobalt containing dusts, there have been very few reports of cancer developing in these workers. A single case of carcinoma of the bronchus in a worker with hard metal disease was reported as the first recorded case (*12*). In epidemiological surveys in nickel extraction plants in the USSR, an increased mortality from lung cancer was found in the cobalt recovery shops as well as in the nickel processing departments. Exposure to arsenic containing dusts was heavy, and it is not clear to what extent the cobalt workers had also been exposed to nickel dusts (*13*, *14*). Cobalt in the occupational environment has been declared a possible carcinogen in Germany and in Sweden.

While wear particles from surgical prostheses containing cobalt were found to be carcinogenic in the rat (*9*), there are no convincing reports of cancer arising in relation to such a surgical implant in man, even though raised blood and urinary cobalt levels have been observed in patients with vitallium prostheses. Again, no definitive epidemiological studies have been performed. Cobalt has been used as a therapeutic agent in the treatment of pernicious anaemia for close to 20 years, but again there are no reports of cancer related to therapy.

Comments and Evaluation

Cobalt, in the form of finely divided metal powder, the oxide and the sulphide has given rise to fibrosarcoma and rhabdomyosarcoma in rats. Following implantation, the metal slowly dissolves and disappears from the injection site. Heath et al. (*15*) have shown that metallic cobalt reacts slowly with serum proteins to form soluble nondialyzable complexes which are less toxic to rat myoblasts in culture than the equivalent amount of ionic cobalt. They have suggested that cobalt-protein complexes, absorbed on the surface of myoblasts, may enter the cell by endocytosis and that subsequent digestion of the carrier proteins by lysosomal proteinases leads to intracellular liberation and redistribution of cobalt. A high proportion of intracellular cobalt hasbeen found in the nuclei of muscle cells, more than half of this within nucleoli, where it would be well placed to exert an effect on DNA and RNA replication (*16*). Cobalt acetate has been shown to enhance viral transformation in embryonic cell culture (*17*). There is inadequate evidence at present to indicate that cobalt is a human carcinogen.

Research Needs

Further experimental work is required to determine the mutagenic potential of cobalt and the ability of the metal to induce transformation in cell culture. Metallic cobalt together with its oxide and sulphide are carcinogenic in the experimental situation, briefly reported above, but it is not known whether cobalt acts as an initiator or as a promoter of the carcinogenic process. It would be of interest to induce cancer in species other than the rat, and to investigate further the carcinogenicity of soluble compounds of cobalt.

There is a need for epidemiological studies of cancer incidence and mortality in occupational groups

exposed to respirable compounds and followed up for an adequate period. Lifetime follow-up studies would be useful of patients treated surgically with cobalt-containing implants for the development of cancer, together with appropriate controls.

Iron

Sources of Exposure

Iron, the most abundant metal in the earth's crust, is found principally in the minerals haematite Fe_2O_3, magnetite Fe_3O_4 and siderite $FeCO_3$. Used by man from ancient times, world production, mainly in the form of steel, is now measured in hundreds of millions of tons annually.

Iron is widely distributed in soil and water with great variation in concentration. Soil levels range between 7 g/kg and 550 g/kg, while in fresh water, levels range between 0.01 and 1.0 mg/l. Ambient air concentrations are low in rural areas, intermediate in urban areas and highest in the vicinity of iron and steel foundries where mean levels of up to 11 $\mu g/m^3$ have been reported. The daily intake of iron from the diet averages from about 9 to 35 mg per day, an average mixed diet containing about 12-15 mg iron. Meat, offal, eggs, and wholemeal cereals are rich, while refined high carbohydrate diets are poor in iron. Iron deficiency is an important cause of nutritional ill health in industrial countries. At the other extreme, the Bantu, who cook and brew in iron pots, have an intake of up to 100 mg/day and accumulate iron in the liver which may give rise to cirrhosis.

Iron is an essential element. It is present in the heme molecule in combination with a porphyrin, in myoglobin, and in certain enzymes such as cytochromes. In therapeutics, iron deficiency is treated orally with a variety of ferrous salts, the sulfate, gluconate, succinate, and fumarate being the most common. Certain iron carbohydrate complexes, detailed below, are given in parenteral therapy.

Occupational exposure to iron compounds, mainly oxides, is common in mining, iron and steel foundry work and in arc welding. The TLV-TWA for iron oxide fume is 5 mg/m^3.

Mutagenic Effects

Iron, in common with a number of other inorganic metal compounds, has been shown to affect the mitotic spindle, causing C-mitosis (*1*). Both ferrous chloride and ferrous sulfate enhanced the transformation frequency of hamster embryo cells with simian adenovirus (*17*). However, concentrations of 0.9*mM* or greater were required and the enhancement observed was small in contrast with other metals showing a positive response. Ferrous chloride showed no evidence of decreased fidelity of DNA synthesis (*5*).

Carcinogenic Effects

Experimental. The repeated intramuscular or subcutaneous injection of certain iron carbohydrate complexes, i.e., iron-dextran, iron dextrin, and saccharated iron oxide, in large doses has given rise to sarcoma at the injection site in rats, mice, hamsters and rabbits (*18-23*). No tumors were observed in squirrel monkeys (*24*), but only three animals survived more than 44 weeks after the last injection. The tumors obtained have been either fibrosarcomas or histiocytic sarcomas without much variation in histological type and characterized by an abundance of iron containing macrophages. Iron sorbitol, given to rats and mice under identical conditions to those in which iron dextran was administered, failed to produce tumors (*22, 25*). The iron complex is essential for the sarcomatous response, for neither the carbohydrate injected alone (*18, 19*) nor inorganic iron compounds (*26*) gave rise to sarcomas at the injection site. There is evidence, from the experiments quoted above, of a dose-response relationship, for while the latent period appears to be independent of dose, both the number of tumors and the grade of malignancy increased with the total dose given.

The repeated intratracheal instillation in golden hamsters of 3 mg ferric oxide suspended in normal saline did not give rise to any tumors of the lung (*27*). However, iron oxide has been shown to act in a synergistic manner when given intratracheally to hamsters together with benzo(a)pyrene (*28, 29*) and following inhalation, together with systemically administered diethylnitrosamine (*30*) when an increased tumor response in the lung was observed. A single dose of 37.5 mg benzo(a)pyrene with 12.5 mg ferric oxide produced lung cancer in 10% of exposed hamsters compared with no tumors in hamsters given a single dose of 50 mg ferric oxide alone (*28*). It has been suggested (*29*) that ferric oxide serves as a carcinogenic cofactor either by retarding clearance of inhaled carcinogens or by inducing cytopathological changes which make the cells of the respiratory tract more susceptible when exposed to carcinogens. Squamous cell carcinoma of the lung has been obtained in rats following the intratracheal instillation of a suspension of iron dusts from an open hearth furnace. An increased incidence of tumors was found when this suspension was given with benzo(a)pyrene, explained as synergism between the iron dust and benzo(a)pyrene

(*31*). In this experiment, the iron dusts were cleansed to remove tarry materials, but a variety of other metals were present in the dust, which contained 52% iron with less than 1% nickel, chromium, and arsenic.

Clinical and Epidemiological Observations. Organic complexes of iron have been given parenterally in the treatment of iron deficiency anaemia for nearly 30 years. Preparations used have been principally ferric hydroxide complexed with low molecular weight dextran (Imferon) given by deep intramuscular injection or by intravenous infusion, and more recently iron-sorbitol (Jectofer), iron dextrin (Astrafer), saccharated iron oxide (Ferrivenin), and iron-polymaltose (Ferrum-Hausmann). Iron dextran is now seldom given intramuscularly because of local irritation and because attention has been drawn to its sarcomatous potential in experimental animals.

About 13 million doses of iron carbohydrate complex had been administered in the first 3 years of its use (*32*). Sufficient time has now elapsed for its oncogenic potential to become apparent, but while there has been no large-scale epidemiological study, there is little evidence to suggest that tumors occurring at sites where intramuscular injections are usually given are becoming more common.

All cases of sarcoma of the buttock were identified from cancer registry entries in the UK over a 2-year period. Drug histories could only be obtained in 90 of these (46% of total) of which four had received intramuscular iron injections (*33*). Weinbren et al. (*34*) reviewed the histology and clinical reports of seven of eight published cases of tumors developing at the site of intramuscular injection of iron complex, including the four cases recorded by Greenberg (*33*). In two of these the histology was that of a benign rather than a malignant lesion (including Greenberg's case 2). The tumor types in the remaining five cases varied. Two were confirmed as fibrosarcoma, the others were diagnosed as reticulum cell sarcoma, rhabdomyosarcoma and hemangiopericytoma (Greenberg's case 1). In only two of these cases was the latent interval between injection and the appearance of the tumor greater than 6 years, and in one case (Greenberg's case 4) the latent interval was only a few months. A retrospective survey of all 72 cases of soft tissue sarcoma presenting in a defined area over a two year period showed no relationship with any history of parenteral therapy (*35*).

An excess mortality from lung cancer has been observed in haematite miners from a number of countries. The original observations first reported from Cumberland, England in 1956 (*36*) were confirmed in a further study reported in 1970 (*37*), where 36 lung cancer deaths were found among underground haematite workers compared with 21 expected from national and regional mortality figures. There was no evidence of any excess mortality for lung cancer among surface workers, and for iron miners as a whole, mortality was close to the national experience. In this study, Boyd et al. (*37*) found an average concentration of radon of 100 pCi/l. in the atmosphere of the mine, and the relationship between the level of radiation and the excess mortality from lung cancer was comparable to that found in other mines where radioactivity is considered to be responsible for an excess lung cancer mortality. An excess lung cancer mortality was found in a group of iron-ore miners in the Lorraine basin in France (*38*, *39*), in the USSR (*40*), in Minnesota, U.S.A. (*41*), and in the iron miners of Kiruna, Sweden (*42*). An increased incidence of lung cancer has also been reported in iron and steel foundry workers from Sheffield in England (*43*), where 94% of 149 subjects examined had pulmonary fibrosis and 43% had tuberculosis with 16 (11%) cases of lung cancer. Exposure to polycyclic hydrocarbons had also occurred. In a cohort study of nearly 4000 foundry workers in Finland, the standardized mortality rate for lung cancer was computed at 270, taking the national figures as a standard (*44*). The contribution made by cigarette smoking could not be assessed in these studies. No consistent increase in lung cancer has been found in studies carried out in welders where again complex exposures occur. Exposure to high levels of iron oxide in the production of sulfuric acid from iron pyrites showed, in a case-control study, no excess of cancer of the lung or of cancer at other sites (*45*).

Comment and Evaluation

In their evaluation of the carcinogenicity of iron carbohydrate complexes, the IARC (*46*) found the evidence acceptable for local sarcoma production with iron dextran in several animal species and for iron dextrin and saccharated iron oxide in mice, but not for iron-sorbitol-citric acid complex at the dose rates tested. At the time of the evaluation in 1973, only one case of sarcoma at the injection site had been reported in man. Since then there have been reports of a further small number of cases and two small epidemiological studies which showed no clear relationship between iron injections and soft tissue sarcoma. The total therapeutic dose given was small compared with that producing sarcoma in animal experiments. Furthermore, in therapy iron is given to a subject with pre-existing iron deficiency and therefore, likely to be mobilised more rapidly

from the injection site. The possibility that iron dextran injections may give rise to sarcoma cannot however, be entirely discounted, although the risk involved appears to be very small. The IARC in 1979 (*47*), on the basis of the experimental animal studies and on what they termed suggestive evidence in man, classified iron dextran as "possibly carcinogenic for humans."

In their 1972 evaluation of haematite and iron oxide, the IARC (*48*) commented that iron oxide had not been found to be carcinogenic when given intratracheally or by inhalation to experimental animals. Since that time, however, a number of investigators have demonstrated a synergistic effect of iron oxide on the lung when administered with other carcinogens.

With regard to occupational exposure, the IARC in 1979 (*47*) concluded, on the basis of epidemiological evidence, that underground haematite mining does increase the risk of lung cancer in man, but that the degree of evidence is inadequate to classify haematite as a carcinogen. This increased risk applies to underground workers only, who may also be exposed to radon and radon daughters, other carcinogens and to silica dust. Exposure to dusts containing nickel, chromium or arsenic may also occur. Concomitant exposure to carcinogenic polycyclic hydrocarbons may occur in smelting operations and in steel foundries.

Public Health Implications and Research Needs

In the present state of knowledge, exposure to dust in haematite mining should be kept to a minimum through the use of the most feasible and applicable controls (*49*).

The animal experimental work lends credence to the possibility that iron oxide particles can serve as carriers for other carcinogens, and this hypothesis requires further experimental exploration.

The role of free silica giving rise to pulmonary fibrosis inhaled concomitantly with iron oxide requires further investigation.

It is unlikely that iron oxide or any other compound of iron could act as an initiator of the carcinogenic process, but the possibility that a compound in certain circumstances may act as a promoter or potentiate the activity of another carcinogen requires further investigation.

In epidemiological studies on iron miners, foundry workers, welders and other workers exposed to iron oxide by inhalation, attempts should be made to estimate the exposure to ionising radiation and to other possible carcinogens.

Lead

Sources of Exposure

Lead is extracted from several minerals, the most abundant being galena, containing the sulfide, but it is also found as a carbonate, sulfate, phosphate, and chloride. Lead has been used extensively since antiquity, total annual world production now being of the order of 5 million tons. The major consumer is the automobile industry, with its lead battery and alkyllead gasoline additive. Other major uses are in alloys, paints, printing, cables, pipes, and glazes. Lead levels in soil may vary widely between 2 and 200 mg/kg. Lead levels in drinking water are of the order of 0.01 mg/l., but soft water in lead pipes or cisterns may have high levels of lead, values of 3 mg/l having been recorded. In rural areas the lead level in air is usually below 0.1 $\mu g/m^3$, but this may increase to 10 $\mu g/m^3$ and even higher levels in urban areas with heavy traffic.

Lead is a contaminant in food and water. Total diet studies in industrial countries indicate a daily intake of lead of the order of 200-300 μg. Intake from drinking water provides about 20 μg and inhalation of city air about another 20 μg per day. However, the total intake may be considerably increased in soft water areas, and where food and drink are contaminated, as from lead glazes on ceramic tableware. Baby foods in tins may contain lead and children may ingest lead from paint flakes and toys. Street dust may be heavily contaminated with lead in urban areas and very high levels have been recorded in the vicinity of lead smelters and mines.

Occupational exposure has been heavy in the past in lead smelting and refining, in lead battery manufacture and in many other industrial processes, lead poisoning still being one of the commonest industrial intoxications. The forms of lead most commonly encountered are lead fume in refining operations, lead oxide in battery manufacture and tetraethyllead as a gasoline additive.

The TLV-TWA for inorganic lead, fume, and dust and for lead arsenate (as Pb) is 0.15 mg/m^3. The WHO International Standard for drinking water sets a limit of 0.1 mg lead/l., and the WHO Provisional Tolerable Weekly Intake from food and water is 3 mg for adults, equivalent to 0.05 mg/kg body weight.

Mutagenic Effects

In common with a number of other metals, lead has been shown to be capable of inhibiting the mitotic spindle, organic compounds being particularly

potent in this respect (*1*). Chromosomes in human lymphocytes from lead workers and from children exposed to lead have been examined by a number of investigators (*50-54*), and these studies have been recently reviewed (*55*). The results have been conflicting, for some studies have shown evidence of chromosome damage and others have not done so, even in the presence of a toxic effect. Possible reasons for the lack of agreement could be the mixed exposure to lead, zinc, and cadmium in some of the groups investigated or lower exposure levels in the groups with negative results. However, there is also lack of agreement on the effects of lead acetate applied *in vitro* to human lymphocytes in what appear to have been carefully carried out experiments (*56, 57*). The frequency of morphological abnormalities of sperm in men with occupational lead exposure has been observed to be positively related to blood lead levels ranging from a mean of 23 to a mean of 75 μg/100 ml (*58*). This latter maximally exposed group consisted of 23 workers with evidence of lead poisoning. While such morphological abnormalities may be related to a reduction in fertility, they are not necessarily indicative of genetic damage. Furthermore, the observations have yet to be confirmed. Lead chloride decreased the fidelity of DNA synthesis in a system using viral DNA polymerase (*5*). Lead oxide enhanced viral transformation in hamster embryo cells, showing intermediate activity in relation to other metals tested (*17*). However, lead acetate has not been shown to be mutagenic in the Salmonella (Ames test) assay for point mutations (*59*) nor in the intraperitoneal host-mediated assay in mice (*60*).

Carcinogenic Effects

Experimental. Renal tumors have been produced in rats following the subcutaneous injection (*61*) and the subcutaneous plus intraperitoneal injection (*62*) of lead phosphate. Adenoma and carcinoma of the kidney has been observed by several investigators (*63-67*) in both rats and mice following the administration in the diet of lead subacetate and in rats of lead acetate. Large doses of lead were used in these experiments, which gave rise to cystic nephritis with tubular cell damage, eosinophilic inclusion bodies and foci of regenerating tubular epithelium. In some instances, bilateral tumors were observed (*63*). Interstitial cell tumors of the testis have been observed in rats following prolonged feeding with lead acetate, but the frequency of their occurrence in control rats was not stated (*64*). In a further experiment (*66*), adenomas of kidney, pituitary, and prostate gland were observed. A small number of cerebral gliomas as well as renal tumors have also been recorded (*67*). Five malignant lymphomas developed in 41 female Swiss mice injected subcutaneously with 0.6 mg tetraethyllead dissolved in tricaprylin (*68*). However, tumors in organs other than the kidney have not been confirmed by other investigators, and it should be borne in mind that lymphoma in the mouse can be of viral origin. A cocarcinogenic effect has been postulated for lead oxide when administered intratracheally in hamsters together with benzo(a)pyrene (*69*). The lead oxide may have acted as a carrier in this experiment. The doses of lead salts used in the animal experiments described above were high, interfering with haem synthesis. Boyland et al. (*63*) reasoned that carcinogenesis might have been related to the associated porphyrinuria rather than to the ingested lead. However, the production of porphyrinuria by other means did not give rise to an excess of renal tumors, neither did the concomitant administration of lead with other prophyrinuric agents give rise to the excess of tumors seen with lead acetate alone.

Syrian hamster embryo cell cultures treated with graded doses of lead acetate showed neoplastic transformation with a dose response relationship, and these cells when injected into hamsters produced fibrosarcomas (*70*).

Epidemiological. In a mortality study of lead accumulator and other lead workers in Britain, the men were divided into three exposure categories on their previous urinary lead excretion. A comparison of observed with expected deaths from all malignant neoplasms showed no excess of cancer deaths in the group with the highest lead exposure (*71*). However, in the group with what was termed negligible exposure (lead in urine values within the normal range), there was a significant excess of observed deaths from malignant neoplasms at all sites when pensioners and employed men were taken together. A mortality study followed over a 23-year period was performed in the USA on a group of over 7000 workers in battery factories and smelters who were exposed to lead for a minimum period of one year (*72, 73*). Lead absorption in many of these workers was greatly in excess of currently accepted standards. The corrected standardized mortality ratio for all causes was 99 for battery workers and 107 for smelter workers, with an excess cancer mortality from all malignant neoplasms, but seen only in smelter workers. An excessive, but not statistically significant mortality from cancer of the respiratory system and of the digestive organs was seen in both smelter and battery plant workers. In a 5-year follow-up study of over 5000 workers from the above cohort, this mortality pattern was not maintained, with a small deficit in malignant

neoplasms in smelters and a small, but significant, excess in battery plants, largely accounted for by malignancies of unknown primary site. The earlier excess mortality from cancer of the digestive organs was not confirmed, although a small excess mortality from lung cancer was again seen. Only one renal tumor was recorded. No internal trends with exposure levels could be demonstrated, and this, together with the absence of smoking histories, where an excess of heavy smokers in the lead exposed group could account for the relatively small excess of lung cancer, did not support a carcinogenic role for lead (*74*).

A case control study of children with Wilms tumors of the kidney reported to the Connecticut tumor registry explored the possibility of perinatal exposure to carcinogenic agents. The paternal occupation as recorded on the birth certificate was taken as an indicator of potential exposure (*75*). An association was claimed between paternal occupations related to lead in the group developing Wilms tumor compared with the control group. However, the study could provide no evidence that the fathers with "occupations related to lead" had actually been exposed to lead.

Comment and Evaluation

The IARC (*76*) accepted the evidence for the carcinogenicity of lead acetate when given orally to rats and mice and of lead subacetate and lead phosphate given orally to rats, producing benign and malignant tumours of the kidney, but commented that the increased frequency of tumours observed at other sites required confirmation. The IARC (*77*) were unable to evaluate the significance of lymphoma developing in Swiss mice following the injection of tetraethyllead because of the propensity of lymphoma to develop spontaneously in this strain.

The IARC in the original evaluation concluded that there was no evidence to suggest that exposure to lead salts causes cancer of any site in man. However, only one epidemiological study (*71*) was available at that time on workers exposed to inorganic lead compounds and no studies to assess cancer mortality following exposure to tetraethyllead.

Since the IARC evaluation, the results of one further epidemiological study have become available (*72*, *74*) but although a small excess of cancer deaths was found, this cannot be attributed to lead exposure.

In any interpretation of the significance of the observations on renal cancer in rats and mice fed with lead salts it should be borne in mind that the doses used were very high in relation to human exposure. Rats and mice appear to be relatively insensitive to the toxic effects of lead and so were able to survive the large doses given.

Public Health Implications and Research Needs

The long history of exposure to lead, both in occupational and general population groups with the lack of any clinical evidence suggesting a carcinogenic effect makes it unlikely that a high cancer risk exists. Human sensitivity to its toxic effects is likely to exert a protective action against the exposure that might be necessary to give rise to cancer, if in fact a carcinogenic potential exists for lead.

If cancer incidence is marginally increased as a result of lead exposure, retrospective epidemiological studies are unlikely to be sensitive enough to show this. As the kidney is the only organ clearly implicated in experimental cancer, it would be worthwhile performing case-control studies assessing lead exposure in patients with renal carcinomas. Follow-up studies on cancer incidence or mortality in people with a history of childhood lead poisoning may also be of value in determining whether lead or its compounds can give rise to cancer in man.

The conflicting observations on the mutagenic effects of lead should be resolved with further carefully controlled experiments and with refinements in techniques for assessing point mutations and more direct observations on the capacity of lead to damage nucleic acids.

Manganese

Sources of Exposure

Manganese is widely distributed as the twelfth most abundant element in the earth's crust. It is present in a number of ores, the commonest being pyrolusite, containing the black dioxide. World production, of the order of 7 million tons in the 1950's trebled in the 1970's. Manganese is a valuable metallurgical constituent, its most important alloy being ferromanganese. The dioxide is used in certain batteries; the permanganate has oxidising properties and is used in disinfection.

The average manganese content of soil is between 600 and 900 mg/kg. Most drinking waters contain less than 100 μg/l.

Cereals may contain between 10 and 100 mg/kg and are the main source of the element. The daily intake in the diet has been estimated at between 2 and 4 mg but values three times as high have been reported.

Heavy occupational exposure has been recorded in mines, processing and ferromanganese plants, with air concentrations exceeding 100 mg/m^3. However, concentrations above 2 mg/m^3 are associated with an increasing risk of toxic effect. The TLV (ceiling value) for manganese and compounds is 5 mg/m^3 and the TLV-TWA for manganese fume 1.0 mg/m^3.

Manganese is an essential trace metal in a great variety of organisms, from bacteria to plants and mammals, and by analogy it is likely to be essential in human metabolism although a health hazard from manganese deficiency in man has not been confirmed. Pyruvate decarboxylase is a manganese metalloenzyme and several other important enzymes are manganese dependent. Manganese appears to have a role in carbohydrate and lipid metabolism, in embryonic development, growth and brain function.

Mutagenic Effects

Manganese was first considered to be a bacterial mutagen as far back as 1951 (*78*).

There is evidence that manganese is able to react with DNA. Antibiotic resistant mutants (*79*) and petite mutations have been observed in yeast cells treated with manganese, indicative of a mutation effect on mitochondrial DNA (*80*). These authors suggested that manganese acts as an error producing factor on replicating mitochondrial DNA, by a direct action on mitochondrial DNA polymerase. Manganese has been shown to reduce the fidelity of DNA synthesis *in vitro*. Substitution of manganese for the magnesium ion resulted in an increase in misincorporation by bacteriophage T4 DNA polymerase (*81*) and by avian virus DNA polymerase (*82*). Manganese chloride in a system using avian virus DNA polymerase decreased fidelity of DNA by at least 30% with increased error frequency and diminished synthesis (*5*). Enhanced viral transformation of hamster embryo cells was demonstrated with manganese chloride, which showed intermediate activity compared with other metals which were tested (*17*). Mutations induced by the manganous ion in bacteriophage T4 have been shown to be reversible by 2-aminopurine and thought to be of the transition type (*83*).

Carcinogenic Effects

Experimental. Lymphosarcoma developed after 18 months in 67% of a group of DBA mice treated with manganese chloride compared with 24% in a control group (*84*). Rats and Swiss albino mice were dosed with pure manganese powder, manganese dioxide or manganous acetylacetonate suspended in trioctanoin (*85*). The rats were given intramuscularly total doses of 90 mg, 90 mg and 300 mg, respectively, and by gavage 240 mg in multiple treatments. The mice were given total doses of 10 mg, 15 mg and 30 mg respectively, intramuscularly only. No difference in tumor incidence was noted between treated and control animals with regard to manganese powder and manganese dioxide. In contrast, a statistically significant number of fibrosarcomas (19 tumors in 50 rats) developed at the injection site in the rats given manganous acetylacetonate, with a mean latent interval of 17 months. The author commented that manganous acetylacetonate suspended well in the vehicle, its reaction, therefore, not fitting the hypothesis of Brand et al. (*86*) for foreign body carcinogenesis.

There is some evidence that in certain situations manganese may be able to suppress the response to an administered carcinogen. Thus, manganous acetate decreased the yield of hepatomas following the administration of dimethylaminoazo benzene (*87*). The incidence in injection site sarcoma in rats receiving nickel subsulfide either alone or in combination with equimolar amounts of aluminium, copper or chromium dusts was 96-100%. However, in a group of rats given an equimolar amount of manganese dust, the incidence of sarcoma fell to 63%. In a further experiment, 5 μmole nickel subsulfide given intramuscularly with 20 μmole manganese powder reduced the tumor incidence from 12 out of 15 rats given nickel subsulfide with chromium dust, to 1 out of 15 (*88*). The hypothesis was propounded that manganese may antagonize nickel inhibition of RNA polymerase activity.

Clinical and Epidemiological. There are no clinical reports implicating manganese as a human carcinogen. Epidemiological studies have been performed on manganese miners and other occupational groups with the aim of eliciting information on the neurotoxic and pulmonary effects of manganese exposure, and these have been summarised (*89*). There are as yet no epidemiological studies which have attempted to relate manganese exposure to cancer mortality or incidence.

Evaluation

There is accumulating evidence for the mutagenicity of manganese. There is some evidence that one organo manganese compound, manganous acetylacetonate, can give rise to injection site sarcoma in rats. There is no evidence at present for a carcinogenic effect of manganese in man.

Research Needs

Further work is required to determine the potential of manganese in giving rise to point mutations and in inducing transformation in cell culture. Work on experimental carcinogenesis with manganese compounds is lacking. In epidemiological studies on occupational and other groups with manganese exposure attention should be directed to estimation of the cancer risk.

Mercury

Sources of Exposure

Mercury is found in nature mainly as a sulfide, in low concentration in the earth's crust except for rich focal deposits where it may also be present in metallic form. Mercury is a fairly volatile element which is released into the atmosphere and deposited again to form a natural global cycle estimated as at least 30,000 tons a year. Annual world production is of the order of 10,000 tons and industrial activities involving mercury, together with the combustion of fossil fuels, adds a man made cycle to the above. The chloralkali industry is the largest consumer, followed by the electrical and paint industries, measuring instruments, agriculture, dentistry and the chemical industry. Organo mercurials have been widely used as fungicides in the wood pulp and paper industries, in paints and in seed dressings.

Soil levels of mercury are low, of the order of 50 μg/kg and uptake by plants is low, even where seed dressings have been applied. Uncontaminated water also has a low level of mercury, below 1 μg/l., and except for the vicinity of mines and industrial emissions, air levels in urban areas are of the order of 50 ng/m^3.

Mercury is a food contaminant which has been extensively studied, with an average daily intake in the UK of 5 to 10 μg total mercury. However, mercury in the aquatic environment, from natural sources or resulting from human activity, can be methylated by microbial action and concentrated in food chains. Fish may, therefore, have high levels most of which is methyl mercury. The average intake of methyl mercury from fish is of the order of 2 μg/day, but fish eaters may ingest 20 μg/day and the consumption of fish from contaminated waters has given intakes of up to 5000 μg/day.

Occupational exposure occurs most commonly to metallic mercury vapor, but also to a variety of inorganic mercury compounds as aerosols and to alkyl mercurials.

Poisoning following exposure to metallic mercury vapour has been common in the past. The TLV-TWA for mercury vapor and all mercurial compounds except alkyl mercurials (as Hg) is 0.05 mg/m^3. For alkyl mercurials this figure is 0.01 mg/m^3. The WHO upper limit for mercury in drinking water is 1 μg/l. The WHO Provisional Tolerable Weekly Intake is 0.3 mg total mercury, of which not more than 0.2 mg should be present as methylmercury.

Mutagenic Effects

Mercury compounds produce a variety of effects on the genetic material, the organic being more active than inorganic compounds. These mutagenic effects have been reviewed by Ramel (*90*). Mercury in common with a number of other metals can damage the mitotic spindle giving rise to C-mitosis. Alkylmercury compounds have been shown to be particularly potent in this respect, methylmercury being even more potent than colchicine. All mercury compounds tested on root tip cells of *Allium cepa* induce polyploidy and other deviating chromosome numbers in the cell. Nondisjunction and sex-linked recessive lethals have been produced in *Drosophila melanogaster* following feeding of the larvae with Ceresan-M (*91*). Chromosome abnormalities have been produced in animal and human cell cultures and these have been briefly reviewed by Leonard (*92*).

Skerfving (*93*) observed dose-related chromosome aberrations in lymphocytes of consumers of methylmercury-contaminated fish. They found a significant increase at blood methylmercury levels of around 100 μg/l., of aneuploidy, unstable chromosome type aberrations and of cells with chromatid type aberrations. An increase in human lymphocyte chromosome aberrations following both *in vivo* and *in vitro* exposure to methyl mercury has also been demonstrated by Kato and Nakamura (*94*). In the Iraq epidemic, lymphocyte cultures showed no significant difference in chromosomal aberrations between exposed and control subjects. However, in Skerfving's study the duration of exposure was long, from 3 to 20 years, while in Iraq the total exposure was limited to a few months (*95*). Verschaeve et al. (*96*) found chromosome aberrations and increased aneuploidy in methylmercury-exposed workers but did not report exposure levels.

These results provide support for the hypothesis that exposure to organomercury compounds may result in genetic damage to human somatic cells. However, while mercury is distributed to mammalian gonads, there is no good evidence that damage to germ cells has been produced. Ramel's (*97*) experiments provide evidence that organic mercurials can cause genetic alterations by two different mechanisms, one resulting in chromosome aberrations

and one in chromosome loss following spindle inhibition.

Carcinogenic Effects

Experimental. In spite of its undoubted mutagenic potential on eukaryocytes, mercury and its compounds do not appear to be oncogenic. Druckrey (*98*) reported spindle-shaped sarcomas containing fine droplets of mercury in the abdominal muscles of rats two years after intraperitoneal injection of metallic mercury.

Mercuric chloride was able to enhance viral transformation in hamster embryo cell cultures at a concentration of 0.05*mM*, thus showing intermediate activity together with cobalt, lead and manganese also considered here (*17*).

Clinical and Epidemiological. There are no convincing clinical reports or any epidemiological studies which suggest that any form of cancer in man may be related to exposure to either inorganic or organic mercury compounds.

Comment and Evaluation

Inorganic and especially organic mercurials have been demonstrated to give rise to chromosome damage in experimental systems, and long-term exposure to methylmercury has been shown to give rise to somatic chromosome abnormalities in man. There is no indication that mercury or its compounds are human carcinogens. It may be that mercury compounds are too toxic to permit sufficient exposure in either animals or man to reveal a carcinogenic effect.

Research Needs

There is little known concerning the interaction of mercurial compounds with nucleic acids. The mutagenic activity of mercurial compounds needs further testing in mammalian systems. Should the chromosome abnormalities seen in human somatic cells following exposure to methylmercury also occur in germ cells, individuals with inherited chromosomal defects would be more likely to develop malignant disease. Such inherited chromosome aberrations should be looked for.

Platinum

Sources of Exposure

Platinum is present in the earth's crust in very low concentrations, alloyed with other metals in Group VIII of the periodic table. The richest source of platinum containing minerals is found in South Africa, but even here the concentration of platinum is no greater than 10 ppm. Total world production of the platinum group metals in 1975 was of the order of 170,000 kg. Platinum has many uses related to its catalytic properties and its resistance to corrosion and oxidation; and some derivatives have recently been found to have a limited use in cancer chemotherapy.

Only a few measurements of platinum concentrations in the environment have been reported. No measurable amounts of platinum have been found in soil, water or ambient air samples in the USA except in precious metal refineries. Here air concentrations between 0.16 and 0.38 $\mu g/m^3$ were recorded. Soluble salts of platinum (as Pt) have been assigned a TLV-TWA of 0.002 mg/m^3. The introduction of catalytic converters containing the platinum group metals to remove pollutants from automobile exhausts has provided a new source of environmental contamination, in particular in the vicinity of highways. The maximum accumulation of small particles of platinum group metals in the atmosphere in a "worst case" situation would not, according to a U.S. study (*99*) exceed 0.06 $\mu g/m^3$. Similarly, the concentration of platinum in the top soil adjacent to a highway with heavy traffic was not expected to exceed 0.008 ppm after a period of ten years.

Biochemical and Toxicological Considerations

The six metals in the platinum group are nontoxic and nonallergenic in their metallic states. The complex salts of platinum, but not of the other metals in the group, act as powerful sensitizers, ammonium hexachloroplatinate and hexachloroplatinic acid being particularly potent in this respect (*99*). Allergenicity appears to be related to the number of chlorine atoms present in the molecule, but other soluble platinum compounds are also active.

Some ionic derivatives can react selectively with specific chemical sites in proteins such as disulfide bonds and terminal-NH_2 groups, with functional groups in amino acids, and in particular with receptor sites in nucleic acids. These compounds exhibit neuromuscular toxicity and nephrotoxicity. Neutral complexes of platinum, such as *cis*-dichlorodiammine platinum (II) and analogs have the property of inhibiting cell division and have antibacterial activity. Some of these have antitumor activity: *cis*-dichlorodiammine platinum (II); *cis*-tetrachlorodiammine platinum (IV); dichloroethylene-diammine platinum (II); oxalodiammine platinum (II); malonatodiammine platinum (II); *cis*-dichloro-

bis(ethyleneimine) platinum (II); *cis*-dichlorobis(cyclohexylamine) platinum (II); and 1,2-dinitratodi-(a)mminecyclohexane platinum (II).

To have antitumor activity, the complexes should be neutral and should have a pair of cis leaving groups. Other metals in the group give complexes which are inactive or less active than the platinum analog. The antitumor activity of these square-planar complexes is stereospecific, for whereas the *cis* complexes are active, the corresponding *trans* forms are inactive. This is believed to be due to stereoselectivity of the biochemical reaction within the cell, and not to differences in metabolism or availability. Two *cis*-monodentate or one bidentate leaving group is required, the rate of exchange of the leaving groups should be neither too low nor too high and should fall into a restricted "window of lability" centered roughly on that of the chlorides, and the ligands trans- to the leaving groups are preferentially strongly bonded, relatively inert amine systems (*100, 101*).

At therapeutic dosages, these complexes produce severe and persistent inhibition of DNA synthesis with little inhibition of RNA and protein synthesis. The transport of DNA precursors through the plasma membrane is not inhibited and neither is DNA polymerase activity. It is believed that the platinum complexes react directly with DNA (*102*). They react both monofunctionally and bifunctionally with active sites on the bases, being mainly localized in regions of the DNA that are rich in guanosine and cytosine. Some 80% of an injected platinum complex is rapidly excreted in the urine and there does not appear to be selective uptake in tumor tissue. The mechanism of action of these complexes has been further reviewed (*99, 103*).

Mutagenic Effects

The fortuitous discovery that cetin coordination complexes of platinum had the property of inhibiting cell division in *E. coli* but not cell growth (*104*) led to intensive investigation of the group of compounds in view of their potential as antitumor agents. Most observations have been performed with *cis*-dichlorodiammine platinum (II) (*cis*-DDP). A strong mutagenic effect of *cis*-DDP has been demonstrated in bacterial test systems, both with *E. coli* (*105*) and Salmonella (*106*). The strain of Salmonella used is known to be specifically reverted by base-pair substitution mutagens. *Cis*-DDP has been shown to form both intra- and interstrand crosslinks with human DNA in cultures of HeLa cells (*107*), this crosslinking effect being probably due to the structure of the whole molecule rather than to its metal component. Two analogs of *cis*-DDP, *cis*-dichlorobiscyclopentylamine platinum (II) and cis-dichlorobispyrollidine platinum (II), have also been shown to be mutagenic without microsomal activation in the Ames test but less active than *cis*-DDP. *Cis*-DDP has been shown to induce the growth of bacteriophage from lysogenic strains of *E. coli* (*108*). From these observations Reslova was able to show a correlation between the antitumor activity of *cis*-DDP and its ability to bind DNA and induce phage from bacterial cells.

Cis-DDP has been shown to cause chromosome aberrations in cultured hamster cells (*109, 110*) and also a significant, dose-dependent increase in sister chromatid exchanges, the increase in exchange frequency being more than 3-fold at a concentration of 1.0 μg Pt/ml (*110*). These authors were able to demonstrate a point mutation effect with the induction of 6-thioguanine-resistant mutants in a dose-dependent manner, the potency of *cis*-DDP being comparable to that of benzo(a)pyrene. In addition, morphological transformation of hamster embryo cells was obtained with concentrations of 0.1 to 0.25 μg Pt/ml. Enhancement of viral transformation of hamster embryo cells was produced at a concentration of less than 0.05*mM* platinum together with antimony, arsenic, cadmium, and chromium showing the highest activity in this respect (*17*).

Attention should be drawn to the technique of performing certain mutagenicity assays using dimethyl sulfoxide as the vehicle, *cis*-DDP reacts with dimethyl sulfoxide so that not more than 20% of the original complex is present after 2 hr. The solvolysis of *cis*-DDP in dimethyl sulfoxide has been monitored by nuclear magnetic resonance (*111*).

Antitumor and Carcinogenic Effects

Experimental. *Cis*-DDP is active in mice and rats against a variety of tumors induced by chemical or viral agents. Administration has resulted in cures or significant regression in animals with sarcoma, leukemia, and other neoplasms, and many results have been reported (*99*). In contrast, there have been few observations published on the possible carcinogenic activity of the platinum coordination complexes.

In a well controlled study, *cis*-DDP has been shown to significantly increase lung adenoma frequency and to give rise to skin papillomas and carcinomas in mice (*112*). *Cis*-DDP administered intraperitoneally weekly over 10 to 19 weeks in a total dose of 32.5 mg/kg increased the adenoma frequency from 0.5 to 0.8 adenoma/mouse to 10 to 16 adenomas/mouse after 8 months. Administered in the same way, in a total dose of 25.9 mg/kg,

cis-DDP together with topical applications of croton oil produced skin papillomas in half the survivors at 41 weeks, with epithelioma in three mice by the end of the year. Groups of 20 rats were given six weekly subcutaneous injections of *cis*-dichlorobiscyclopentylamine platinum (II) (DCP) or *cis*-dichlorobispyrrolidine platinum (II) (DPP) in trioctanoin. Six and three rats, respectively, developed sarcomata at the injection site, a further two (DPP) rats and one (DCP) rat developed metastasizing sarcoma in the abdominal cavity without sarcoma at the injection site, and one (DPP) rat developed an epidermal carcinoma of Zymbal's gland. There were no skin papillomas, epitheliomas, or sarcomas in the control animals. *Cis*-PDD being moderately water soluble would be unlikely to be retained at the injection site and so was not tested for sarcoma induction. Its two derivatives are much less water soluble which would result in poorer delivery to peripheral tissues. *Cis*-DCP also increased adenoma frequency in the mouse, but at a much lower rate than *cis*-DDP. The authors considered *cis*-DDP to be a moderately active carcinogen and rather more active than ethyl carbamate, with which they made an experimental comparison.

Clinical and Epidemiological. Chemotherapy with *cis*-DDP usually in combination with other drugs has produced significant regression in testicular and ovarian tumors, in some cancers of the head and neck and in certain lymphomas. Cancers of the gastrointestinal tract and of the breast appear to be refractory to treatment with *cis*-DDP. There are no reports of cancer related to occupational exposure to platinum compounds. No epidemiological study of cancer mortality or incidence in platinum workers has been reported. However, because of the high risk of intractable sensitization to soluble salts of platinum, workers have been monitored for health effects for many years and exposure to platinum has been strictly controlled at very low levels.

Comment and Evaluation

The experimental evidence indicates that certain platinum coordination complexes are electrophilic reactants and direct acting mutagens. On theoretical grounds these complexes would be expected to act as initiators of the carcinogenic process. In a single series of reported experiments the results support the hypothesis (*112*), for the adenoma frequency in the strain of mouse employed is known to be a good indicator of carcinogenic activity. Skin papilloma and epithelioma production following the application of croton oil as promoting agent again supports the hypothesis

The use of platinum coordination complexes in cancer therapy may expose the recipient to a further risk of cancer should a sufficient prolongation of life span be attained. This pattern of activity would be consistent with that of other electrophilic reactants, such as the alkylating agents used in cancer chemotherapy.

There is no evidence to suggest an increased cancer risk following occupational exposure to platinum compounds. The low exposure levels consistently maintained in the occupational environment make such a possibility unlikely.

Research Needs

The carcinogenic activity reported for the platinum coordination complexes requires confirmation and further study in appropriate animal models. There is currently considerable research activity in progress on these complexes in view of both their practical chemotherapeutic and theoretical importance.

Selenium

Sources of Exposure

Selenium is found mainly in the form of various metallic selenides usually associated with sulfide ores from which it is extracted as a by-product. In soil it is also present as basic ferric selenite and calcium selenate. World production is of the order of 1500 tons per annum, its principal uses being in electronics, in the glass, pigment, rubber, and chemical industries. Soil concentrations vary from 0.1 to over 1000 mg/kg, from deficient to seliniferous areas, with commonly found values between 1 and 10 mg/kg. Some plants, including cereals and grasses concentrate selenium from the soil and give rise to poisoning in grazing animals. Selenium levels in drinking water rarely exceed 10 μg/l., but high values have been found in some alkaline waters. The average daily intake of selenium in food and water varies widely in different countries, from 60 μg/day to 300 μg/day. A low selenium intake in a broad selenium deficient belt in China has been associated with Keshan disease, an endemic juvenile cardiomyopathy (*113*).

Occupational exposure to selenium may occur in copper refineries, where the first case of poisoning was recognized, in rectifier production plant and in various industries using selenium. Levels between 40 and 400 %gmg/m^3 have been encountered, but few environmental measurements have been reported. The TLV-TWA for selenium compounds (as Se) is 0.2 mg/m^3.

Selenium is an essential trace element in several animal species and is added to foods in selenium-

deficient areas. Its role in human metabolism is obscure. It is associated with -SH groups in proteins and has a metabolic relationship with the tocopherols. Selenium compounds and the tocopherols are antioxidants which may help to maintain cellular stability by inhibiting oxidation of lipids in cell membranes. The element is an essential component of glutathione peroxidase and has a role in ubiquinone biosynthesis. The toxicology of selenium has been recently reviewed (*114*, *115*).

Mutagenic Effects

Nakamuro et al. (*116*) observed dose related chromosome aberrations in cultured human leukocytes treated *in vitro* with a number of selenium compounds, selenites being more active than selenates. Most of the aberrations were chromatid gaps, but an increase in chromatid breaks and exchanges was also observed. Rec assay using *B. subtilis* with different recombination capacities, and observation of a loss of transforming activity of *B. subtilis* DNA, indicated that substantial damage to DNA had been produced. Other authors (*4*) failed to obtain changes in human leukocytes, but the reason for this may have been the low doses employed. However, Shamberger et al. (*117*) reported a significant reduction in chromosome breakage induced by dimethylbenzanthracene in cultured human lymphocytes treated with selenite.

Carcinogenic Effects

Experimental. Long-term feeding experiments with both selenites and selenates have been performed in rats and mice. The initial study performed in rats showed an increased incidence of nonmetastasizing liver tumors which occurred only in association with cirrhosis (*118*) and a later study showed a small number of hepatomas and adenomas (*119*). In a detailed study (*120*) in which the dietary pattern of selenium was varied as well as the selenium content, a similar number of tumors was found in both experimental and control groups, but with no liver tumors, and it was concluded that selenium was not related to tumor occurrence. In a study by Schroeder and Mitchener (*121*) a significant increase in a variety of tumors was found in the treated group, but again none involved the liver. The treated rats lived longer than the controls and the results are thus difficult to evaluate. In a second study by the same investigators (*122*), on mice, no increase in tumor incidence was found. Hepatomas were also induced by feeding mice with the fungicide selenium diethyl dithiocarbamate, initially by gavage and subsequently by addition to the diet (*123*). While a significant increase in the incidence of tumors was found in the exposed mice, similar results were obtained in mice given a selenium free dithiocarbamate.

To complicate the assessment of the role of selenium as a possible carcinogenic agent, a number of studies have demonstrated an inhibitory effect of selenium on tumor yield in rats and mice following the administration of standard carcinogens. This effect has been observed following both local application and selenium enrichment of diets. Thus sodium selenide applied with croton oil or sodium selenite given in the diet decreased the skin tumor incidence following painting with dimethylbenzanthracene (*124*) and a similar effect was seen following skin application of benzo(a)pyrene after feeding with sodium selenite (*125*). Rats fed 2-acetylaminofluorene showed mammary carcinoma and hepatoma inhibition which appeared to be related to selenium concentration in the diet (*126*). A 50% reduction in the yield of liver tumors in rats given dimethylaminobenzene with 5 ppm sodium selenite added to the diet had been observed much earlier (*127*). Furthermore, the addition of selenium oxide to drinking water at a rate of 2 mg/l appeared to lower the incidence of spontaneous mammary tumors in a group of virgin mice (*128*). Further details of these and other experiments including dose rates, are given in reviews by Fishbein (*114*) and IARC (*129*).

Epidemiological. In a study of 300 workers exposed to selenium in a rectifier plant over a period of up to 26 years, no increase in cancer mortality was found when compared with expected rates (*130*). There were six cancer deaths from different sites where 5.1 were expected, but the completeness of ascertainment of the mortality data is not known. There are no other epidemiological data on cancer mortality in occupationally exposed groups known to the author.

Shamberger and his colleagues have carried out epidemiological studies in general population groups in the U.S., comparing total cancer mortality and mortality from cancer at various sites in high and low seleniferous areas, based on selenium content of forage crops and of human blood (*131*). A high negative correlation was found between blood selenium levels and age specific cancer death rates. In a further study in which high and low selenium urban areas were matched (*132*) mortality was lower, in high selenium areas, from cancer at a number of sites, including lymphoma, gastrointestinal, respiratory, and, in the female, breast and reproductive organs. Similar inverse correlations were observed between both dietary selenium intake and selenium levels in whole blood and cancer mortality patterns in different countries

(*133*). Blood selenium levels in patients with certain cancers, in particular of the gastrointestinal tract and with Hodgkin's disease were found to be significantly lower than in control patients with other diseases (*134*), but this lower blood selenium level may of course have followed and not preceded the cancer.

Comment and Evaluation

The IARC (*129*) considered the available data in animals insufficient to allow an evaluation of the carcinogenicity of selenium compounds. They considered the data in man to provide no suggestion that selenium is carcinogenic. The IARC commented further that the evidence for a negative correlation between regional cancer death rates and environmental selenium levels is not convincing. With regard to the early animal experimental work, hepatic tumors were obtained only in rats with pre-existing cirrhosis resulting from selenium toxicity, they did not metastasize and the observation has not been repeated in the same or other species. There are also difficulties in interpretation of the data from the other experiments on carcinogenesis quoted above. There is some consistency in the observations on an apparently antagonistic effect of selenium towards the induction of tumors in different organs by a number of carcinogens.

In man there are no epidemiological or clinical data to suggest that any selenium compound may be carcinogenic, but there is one small study only, with negative results. The observations on a negative correlation between cancer mortality and selenium levels in the natural environment have to be interpreted with caution. Blood selenium levels did not correlate well with environmental assessments of high and low selenium areas (*134*). The lack of congruence between these areas and regional cancer statistics meant that arbitrary classifications had to be made which were not always supported by the data on selenium level in the environment, as a result of which the association has been judged to lack strength and consistency (*135*).

Research Needs

More information is required on the mutagenic activity of selenium compounds, both with regard to their ability to produce chromosome abnormalities and to produce mutations in bacterial systems. The activity of selenium compounds in inducing transformation in cell culture requires investigation. The status of selenium as a possible electrophilic reactant requires investigation. As one possibility, abnormal methylation of nucleic acids by a compound such as adenosylselenomethionine may occur (*136*). Carefully controlled work is required on experimental carcinogenesis with selenium compounds.

The paucity of epidemiological data on cancer mortality and incidence in groups with occupational exposure to selenium compounds is striking.

The possibility that selenium may prevent some forms of human cancer from developing, perhaps by inactivating a more potent carcinogen, requires further investigation.

Titanium

Sources of Exposure

Titanium is widely distributed in the earth's crust, where it is the eighth commonest element. It has many uses, with an annual production of over 1-1/2 million tons. Titanium alloys include surgical implants which resist corrosion by body fluids and ferro titanium used in the steel industry. The dioxide is extensively used as a white pigment in paint, paper and plastics. It is also used in food as a colouring agent, in cosmetics and in pharmaceuticals. Other titanium compounds are used as catalysts. Titanium concentrations in drinking water range between 0.5 and 15 μg/l. Many vegetables and cereals contain high levels of titanium. The daily intake from dietary sources has been estimated at between 0.3 and 2 mg of the element. Occupational exposure may may be heavy and concentrations in air up to 50 mg/m^3 have been recorded. Titanium dioxide has been classified as a nuisance particulate, with a TLV of 10 mg/m^3. Titanium is poorly absorbed from the gut, and no essential metabolic role has yet been ascribed to this element.

Mutagenic Effects

Titanium nitrate, while not giving rise to C-mitosis in root cells of *Allium cepa*, did however induce sticky chromosomes manifested mainly by the formation of anaphase bridges (*1*). Abnormal staining of the chromosomes at metaphase-anaphase was also seen.

Carcinogenic Effects

Titanium dioxide, together with ferric oxide considered in this paper, did not produce transformation of Syrian hamster embryo cells in culture, even though concentrations as high as 20 μg/ml of medium were used (*137*).

Experimental. Titanium oxalate or acetate given in drinking water at a rate of 5 mg (Ti)/l to 150 mice of both sexes for their whole life span from weaning,

produced no increase in tumor frequency or other adverse effect compared with control animals (*138*). In a long-term feeding study performed by the National Cancer Institute (*139*), rats and mice given titanium dioxide under their standard bioassay protocol experienced no increase in cancer. Inbred Fischer rats were injected intramuscularly at monthly intervals with 200 mesh fine titanium metal powder suspended in 0.2 ml trioctanoin to a total dose of 39 mg in male rats and 23 mg in female rats, and observed over a period of 820 days (*140*). 2/50 rats developed fibrosarcoma and 3/50 rats developed lymphosarcoma. There were no such tumors in an equal number of control rats injected with the vehicle or in groups of rats injected similarly with powdered copper or iron, but tumors were obtained with powdered nickel and a few tumors with powdered chromium. The organic compound titanocene, dichlorodicyclopentadienyl titanium suspended in trioctanoin and injected intramuscularly in rats and mice has also given rise to injection site fibrosarcomas and some animals developed hepatoma and malignant lymphoma of the spleen but details were not given (*141*).

Clinical and Epidemiological. Titanium-containing alloys used as surgical implants have not been associated with cancer or other adverse effects following long term contact with tissues. Heavy long-term occupational exposure to titanium dioxide dusts has not given rise to ill effects or been associated with cancer. There are no epidemiological studies in workers with heavy past exposure to titanium containing dusts.

Comment and Evaluation

The data are insufficient for an evaluation of the carcinogenic activity of titanium. Titanium compounds appear to be biologically inert. Mutagenic activity has not been investigated except for a minimal effect on chromosomes in a single experiment. Cell transformation has not been observed. The tumor yield was small in Furst's experiment (*141*), but the results cannot be dismissed, as copper and iron injected in the same way produced no tumors at all. There is no evidence to suggest that titanium compounds have acted as human carcinogens.

Conclusion

The eight metals considered above have little in common, except for some evidence that they, in one form or another, can give rise to genetic damage or to experimental cancer. Of the great variety of occupations where exposure to one of these metals may occur, only haematite mining has been shown to involve an increased human cancer risk, raising the possibility that haematite might in some way act as a carcinogen or potentiate the activity of another carcinogen. The stimulus of therapeutic application in anticancer therapy led to intensive investigation of platinum coordination complexes, and as a result, although observations on experimental carcinogenesis are at present scanty, these complexes fit the model of compounds that bind to cellular nucleophiles which are direct acting mutagens and also capable of cancer initiation. Cobalt has given rise to injection site cancer and lead to renal cancer in animal studies, following ingestion in large doses, and both metals show evidence of mutagenic activity. One organotitanium and one organomanganese compound, again in large doses have produced a small yield of injection site tumors, but only the latter appears to be mutagenic. Some mercury compounds produce genetic damage, but have not given rise to cancer. The role of selenium remains enigmatic. Metals as mutagenic initiators of cancer have been further considered by Flessel (*142*).

A carcinogenic potential for these metals does not in itself imply an increase in human cancer risk. Length, intensity and route of exposure together with the physical and chemical form of the metal are some of the factors which act as determinants of outcome. Epidemiological observations are essential for the assessment of human risk. While considerable progress has been made in developing laboratory tests for the prediction of carcinogenic activity, there is as yet no systematic approach to the recording and collection of epidemiological data in the occupational environment. Past employment records are, in practice, often destroyed after a minimal period and exposure data, or even a record of jobs done may be nonexistent. At the least, it should be obligatory for employment records to be retained, if possible in a standard form. Occupational exposures are often multiple, with more than one possible carcinogen being involved, and both the nature of the industrial process and exposure levels may change over the years. For research purposes, a cumulative occupational record compiled for a sample of a selected workforce and linked to mortality records and cancer registration would be of inestimable value.

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Effects of Metals in *in Vitro* Bioassays

by Michael A. Sirover*

The capacity of *in vitro* bioassays to detect the potential carcinogenicity of metal compounds is reviewed. The *in vitro* bioassays discussed include: bacterial reversion analysis to determine the capacity of metal salts to revert *Salmonella typhimurium* histidine auxotrophs or to revert *Escherichia coli* WP 2 tryp⁻ to tryptophan prototrophy; examination of the ability of metal salts to preferentially inhibit cell growth in *Bacillus subtilis* cells deficient in DNA repair pathways; determination of the ability of metal salts to induce resistance to base analogs in mammalian cells; the capacity of metal salts to enhance viral transformation of mammalian cells or to transform cells in the absence of virus; and the ability of metal salts to induce chromosomal aberrations in mammalian cells. Using each of these *in vitro* bioassays, diverse metal compounds have been identified as potential carcinogens. Furthermore, the use of different compounds of a specific metal may allow a determination of the valence which may be required for carcinogenesis.

Introduction

Repeated observations have suggested that environmental agents are causative factors in the development of human neoplasia (*1-5*). As epidemiological studies as well as animal experimentation can be performed on only a few compounds at great expense and over extended intervals, recent attention has focused on the development of short-term *in vitro* bioassays through which suspected carcinogens may be detected (*6-10*). The most widely used bioassay is that developed by Ames which uses reversion to histidine prototrophy in *S. typhimurium* as a means to detect potential human mutagens and carcinogens (*11, 12*). This bioassay, as well as most others which have been developed, ultimately quantitate the capacity of environmental agents to induce an alteration in the nucleotide sequences which comprise the genetic information in the cells DNA.

Metals have been shown to be carcinogenic in laboratory animals (*13-15*) and have been implicated as human carcinogens (*16, 17*). In this review the main *in vitro* bioassays which have been used to screen a large number of metal compounds for their mutagenicity and for their potential carcinogenicity will be discussed. In particular, this review will examine the capacity of metal compounds to induce mutations in procaryotes and in eucaryotes; to enhance the viral transformation of mammalian cells in culture; to induce transformation of hamster embryo cells *in vitro*; and to induce sister-chromatid exchanges and other chromosomal aberrations in eucaryotic and in human cells. The other main bioassay used to detect potential metal mutagens and carcinogens, the infidelity of DNA synthesis *in vitro* (*18-20*), is discussed in a companion paper.

In this review I shall also consider the hypothesis that short-term *in vitro* bioassays may be used to determine which compounds of specific metal carcinogens pose the greatest risk with regard to the induction of human neoplasia.

Bacterial Mutagenesis as an *in Vitro* Bioassay for Metal Compounds

The observation that many chemical carcinogens are also mutagens has led to the development of several bacterial systems as *in vitro* bioassays. The basis of these assays in the quantitation of the capacity of environmental agents to revert previously induced mutations or to inhibit their capacity for cell growth. A brief description of these assays will be provided for the purpose of this

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review. A more extensive review of each assay can be consulted for more detailed analysis of the systems *per se* or for a further consideration of metal mutagenesis (*12*, *21*, *22*).

Foremost among *in vitro* bioassays is that developed by Ames in which the reverse mutation of histidine requiring strains of *S. typhimurium* to histidine prototrophy is quantitated. The tester strains used contain additional defects to facilitate mutagenesis. In the initial experiments defining the Salmonella assay approximately 250 chemicals were examined (*23*, *24*). Of the 175 chemical carcinogens tested, 157 or 90% were mutagenic. In contrast, of the 108 non-carcinogenic chemicals tested, 94 or 87% did not produce detectable his^+ revertants above background levels. Subsequent analysis using this assay has been extended to a wide variety of individual chemicals as well as to complex mixtures of environmental agents (*11*).

The second bacterial system which has been used to screen metal compounds measures reverse mutations in *E. coli* strain WP 2 $tryp^-$ (*22*). Analogous to the Salmonella assay this system quantitates the capacity of exogenous agents to revert the bacteria to tryptophan prototrophy. As DNA is considered the critical target for chemical carcinogens, the *E. coli* WP 2 strains employed are usually deficient in specific DNA repair pathways. This deficiency enhances the potential for detecting environmental mutagens as DNA lesions will tend to persist for greater intervals due to the cells inability to excise the DNA adducts.

The third bacterial system utilized as an *in vitro* bioassay for metal compounds measures the capacity of exogenous agents to inhibit cell growth (*25*). Recombination proficient (rec^+) and recombination deficient (rec^-) strains are used. The hypothesis which forms the basis of this bioassay is that the agent tested will inhibit cell growth to a greater degree in the rec^- strains which is deficient in DNA repair. One presumes that the persistent lesions in DNA will prevent the expression of specific genes whose products are essential to cell growth or that the DNA lesions will prevent DNA replication and thus inhibit cell growth. Thus, in parallel cultures, one can measure an inhibition zone of cell growth for rec^+ cells as well as for rec^- cells. The expectation is that the greater the difference in inhibition zones, the greater the potential mutagenicity of the compound tested.

Effects of Metals in Bacterial Bioassays

Chromium has been implicated as a human carcinogen inducing the formation of lung tumors after industrial exposure (*15*). Venitt and Levy (*26*) in 1974 reported that chromates were mutagenic in the *E. coli* B WP 2 system. Using hexavalent chromium compounds they observed a 3- to 4-fold increase in the number of revertants per plate as compared to untreated controls. Similarly, Nishioka (*27*) using the rec assay demonstrated that hexavalent chromium compounds strongly inhibited cell growth and thus had a demonstrative rec effect. In parallel studies, he also showed that potassium dichromate was mutagenic in *E. coli* B WP 2 $tryp^-$ $uvr\ A^-$ which is deficient in nucleotide excision repair (*28*).

In spite of the usefulness of the Ames assay in analyzing diverse types of chemicals, initial studies using this *in vitro* assay failed to detect the mutagenicity of several metal carcinogens (*23*, *24*). This might have reflected technical problems which related to the chelation of free metal cations by components of the bacterial medium. However, later studies by several groups, including that of Ames, have been successful in determining metal mutagenesis using this *in vitro* assay. Lofroth and Ames (*29*) reported that chromate and dichromate induced frameshift mutations in three different strains of *S. typhimurium*. Further studies by Petrilli and de Flora (*30*) confirmed that hexavalent chromium compounds (sodium dichromate, chromic acid and calcium chromate) produced significant increases in histidine reversion using four tester strains. The nature of the reverse mutations required to regain histidine prototrophy suggested that the chromium compounds produced both frameshift and base pair mutations. Furthermore, the mutagenic activity of hexavalent chromium was independent of microsomal metabolism. Using lead chromate, Nestman et al. (*31*) reported increased mutagenicity in both the Ames Salmonella assay and the *E. coli* WP 2 tryp-fluctuation test. As both chromium and lead are potential human carcinogens, it was of interest to observe that the substitution of lead chloride for lead chromate resulted in an elimination of the mutagenic effect of the metal compound.

In contrast to the mutagenic capacity of hexavalent chromium, parallel studies demonstrated that trivalent chromium was inactivated as a mutagen (*29*, *30*). With the use of two trivalent chromium compounds (chromium potassium sulfate and chromic chloride), no increase in the reversion frequency was observed in the presence or absence of a microsomal fraction from rat liver or from human erythrocytes. Although hexavalent chromium could be deactivated by enzymatic metabolism (*32*), trivalent chromium could not be converted to the hexavalent form. Of interest was the observation that microsomes from rat lung had no effect on the

mutagenicity of hexavalent chromium. These results may be of importance as chromium may localize and accumulate in human lung during occupational or environmental exposure. Thus the inability of human lung to deactivate hexavalent chromium compounds may play a role in chromium carcinogenesis in humans. However, trivalent chromium could be chemically oxidized *in vitro* by using potassium permangenate (*23*). Similarly, Nishioka had reported that the rec effect of potassium dichromate was lost after treatment with Na_2SO_3, which is the reducing agent (*27*). Thus, it would appear that although chromium *per se* is a metal carcinogen, the valence state of the metal within a specific compound may be of prime importance (Table 1). Further study is required to determine whether trivalent chromium compounds can be considered relatively innocuous with regard to carcinogenicity and whether the focus should remain on hexavalent compounds.

Arsenic compounds have been examined in each of these three bacterial *in vitro* assays (Table 1). Trivalent arsenic (arsenite) and pentavalent arsenic (arsenate) were inactive in the Ames assay (*29*). In contrast, Nishioka reported that rec effects were observed with both trivalent ($AsCl_3$ or $NaAsO_2$) and pentavalent (Na_2HAsO_4) arsenic (*27*). The result with $AsCl_3$ in the rec assay is the initial report that a free metal cation (As^{3+}) was a potential mutagen. Furthermore, trivalent arsenic as sodium arsenite induced $tryp^+$ reversions in *E. coli* WP2. However, recent results suggest that arsenite may not induce mutations in *E. coli* WP2 cells (T. Rossman, M. Molina, D. Stone, and W. Troll, Mutat. Res. Submitted). It was suggested that the results observed by Nishioka might have been due to the intrinsic variability of background mutation frequencies. As arsenic is implicated as a human carcinogen, the absence of arsenic induced mutation would predicate another mechanism. One intriguing possibility is that arsenic may inhibit the enzymes which comprise the excision repair pathways (*28*, *34*).

Selenium has been investigated in both the rec assay and in the Ames Salmonella test. Using the rec assay, Nakamuro et al. (*23*) tested five selenium compounds (*35*). They reported that selenate failed to produce any difference in inhibition zones. However, selenite did show a small but significant rec effect. In contrast, Lofroth and Ames reported that using the Salmonella test system, selenite was not a mutagen (<< 0.01 revertants/mole) but that selenate produced a small number of mutations (0.03 revertants/mole) (*29*). Recently, Noda et al. reexamined this question (*36*). They reported that selenate and selenite were weak mutagens in both the rec assay and in the Salmonella test. In their experiments which did not utilize a microsomal activating system, selenite induced 0.2 revertants/mole while selenate induced 0.05 revertants/mol in the Ames assay. The basis for these conflicting results is unknown but may spring from the marginal effects of selenium as a bacterial mutagen.

The role of platinum compounds as cancer chemotherapeutic agents has resulted in several investigations on the capacity of these compounds to act as bacterial mutagens (*37-40*). In particular, the stereospecificity implicit in the usefulness of *cis*-platinum (II) diamminedichloride (cis-PDD) as compared to its *trans*isomer in chemotherapy has also been examined in *in vitro* bioassays. Beck and Brubaker initially reported that cis-PDD was

Table 1. Metal mutagenesis in bacterial *in vitro* bioassays.[a]

Metal	Valence or charge	Reversion of *S. typhimurium* his−, revertants/nmole	Reversion of *E. coli* WP 2 tryp−, colonies/plate	Rec effect in *B. subtilis* (difference in inhibition zone)	Reference
Arsenic	3	0.01	N.D.	+	(*27*, *29*)
	5	0.01	37.7 (8.0)	+	
Chromium	3	172 (194)[b]	N.D.	−	(*27*, *32*, *33*)
	6	925 (238)[b]	41.7 (8.0)	+	
Platinum	−2	2	N.D.	N.D.	(*42*)
	0	100	N.D.	N.D.	
	+2	0.2	N.D.	N.D.	
Selenium	4	0.20	N.D.	±	(*29*, *35*, *36*)
	6	0.05	N.D.	±	

[a]Determination of the capacity of metal compounds to induce mutations or to preferentially inhibit cell growth are described in the respective references. The numbers in parentheses refer to the number of mutations observed in simultaneous untreated controls. Charge refers to the net charge on the metal compound while valence defines the state of a metal within a compound. The convention of Nishioka has been used as a determination of the rec effect (*27*).

[b]In revertants per plate.

cytotoxic to several *E. coli* strains deficient in DNA repair (*41*). They concluded that this cytotoxicity was due to the production of intrastrand crosslinks in DNA.

Mutagenicity studies in the Salmonella assay demonstrated the capacity of *cis*-PDD to induce histidine reversions. In a study of 12 platinum compounds, Le Cointe et al. investigated the stereospecificity of platinum-induced mutagenesis (*42*). They observed that *cis*-PDD was the most potent mutagen tested. In the Ames assay cis-PDD produced 100 reversions/moles as compared to a reversion frequency of between 0.2-2.0 reversions/mole observed for the other eleven platinum compounds. Le Cointe et al. also reported that PDD produces base-pair mutations. Of interest was the observation that the charge of the platinum compound appeared to directly affect the reversion frequency (Table 1).

Although chromium, arsenic, selenium, and platinum have been examined in some detail, rigorous analysis of other metals using all three *in vitro* bioassays remains undetermined. Manganese induced mutations in bacteriophage T_4 (*43*) and in yeast (*44*). Copper caused mutations in *E. coli* (*45*) and in *B. subtilis* (*46*). Nishioka has screened a large number of metal salts in the rec assay (*27*). In addition to the metals previously discussed, rec effects were observed for cadmium, mercury, manganese, and for molybdenum. However, silver, beryllium, cobalt, copper, iron, nickel, lead, and zinc were negative in this assay. However, a systematic analysis of metal mutagenesis has not yet been performed using either the Ames Salmonella assay or the *E. coli* WP 2 $tryp^-$ *in vitro* bioassays.

Metal Mutagenesis in Mammalian Cells

The decreased sensitivity of mammalian cells to base analogues has been used to detect metal compounds as mutagens in mammalian cells (*47, 48*). In particular, mutants in the HGPRT locus can be identified by the acquired capacity to grow in media containing thioguanine or 8-azaguanine. The HGPRT locus specifies a purine salvage enzyme, the hypoxanthine-guanine phosphoribosyl transferase. A deficiency in this enzyme renders the cell insensitive to the base analogues. Thus, in contrast to bacterial systems which quantitate the reversion of a previously induced mutation, this system measures the induction of the initial mutation. In general, hamster cells have been used as model systems to approximate the effects of metals on the HGPRT locus in mammalian cells.

O'Neill et al. reported that platinum as *cis*-dichlorodiammine platinum (II) induced thioguanine resistance in Chinese hamster ovary cells (*49*). Using 3 μM *cis*-PDD they observed an increase of approximately 30-fold in induced mutation as compared to spontaneous mutations in parallel cultures. Similar results were reported by Turnbull et al. using V79 Chinese hamster cells (*50*). A comparison of six platinum compounds by Taylor et al. (*51*) demonstrated that a variety of platinum compounds induced 8-azaguanine resistance in CHO cells. In this study, both *cis*-PDD and $Pt(SO_4)_2$ were equally effective in inducing mutations. In contrast, similar treatment with varying concentrations of K_2PtCl_4 or K_2PtCl_6 did not induce any alteration in sensitivity to 8-azaguanine. However, repeated subculture of CHO cells in 10 μM K_2PtCl_6 resulted in a 2- to 3-fold increase in the induction of 8-azaguanine-resistant colonies. This induction required exposure of the cells for 10 population doublings and was maintained after 20 population doublings. Further analysis by Zwelling et al. in V79 Chinese hamster lung cells demonstrated that *cis*-PDD was more mutagenic than *trans*-PDD at equitoxic doses (*52*). This confirms the previous results observed using bacterial *in vitro* assays. Furthermore, Zwelling et al. reported that the number of interstrand crosslinks produced by both compounds were identical. These results would indicate that other lesion(s) were the causative DNA lesions for mutagenesis.

Chromium has also been examined in the Chinese hamster V79 system for the induction of 8-azaguanine resistance. In agreement with those results obtained in bacterial assays, Newbold et al. observed that hexavalent chromium compounds induced mutations while trivalent chromium compounds were inactive (*53*). Three hexavalent compounds were used, each of differing solubility in water; potassium dichromate which is highly water-soluble, zinc chromate which is slightly water-soluble, and lead chromate which has a very low solubility. Both potassium dichromate and zinc chromate induced significant numbers of colonies resistant to 8-azaguanine. In contrast, lead chromate was inactive yielding results comparable to those observed with trivalent chromium compounds. These results are of interest in that lead chromate was mutagenic in the Ames assay. The reason for this discrepancy remains unknown. Other metals have been analyzed in mammalian mutagenesis assays (*54*). These results should be available in the near future (J. P. O'Neill, personal communication).

Enhancement of Viral Induced Transformation by Metal Compounds

Exposure of hamster embryo cells to the simian adenovirus SA7 results in viral transformation of the cells (*55*). Subsequent analysis after transformation demonstrated that the viral genome was integrated into the hamster DNA as defined by nucleic acid hybridization studies. In a series of publications, Casto and DiPaolo reported that treatment of the cells with diverse chemical carcinogens resulted in an enhancement of the frequency of viral induced transformation (*56, 57*). In particular, treatment with polycyclic aromatic hydrocarbons or with alkylating agents significantly enhanced viral transformation as compared to that observed with the virus alone. They hypothesized that this increase resulted from additional sites in DNA which were now accessible to the virus for integration. The production of these extra sites were due presumably to DNA alterations induced by carcinogen-DNA interactions.

Using this system as an *in vitro* bioassay, they then examined whether specific metal compounds could similarly enhance viral transformation (*58*). In these experiments they investigated 25 metals in a total of 38 metal salts. Of the 38 metal salts which were examined, 24 significantly enhanced viral transformation. These metal compounds were divided into three categories according to the metal concentration required for enhancement. The first group which showed highest activity at the lowest concentrations included antimony, arsenic, cadmium, chromiun and platinum. The second group contained beryllium, cobalt, copper, lead, manganese, mercury, nickel, silver, thallium, and zinc. The third group contained only iron. There was a direct relationship between the capacity of metal salts to enhance viral transformation and their reported mutagenicity or carcinogenicity. Of the metals tested, only the results with zinc were inconclusive. A partial summary of the data is presented in Table 2.

It is interesting to note that treatment of the cells with soluble metal salts resulted in increases in viral transformation. This is in contrast to the results observed using bacterial assays in which most metal cations did not induce mutations. Of interest also is the agreement of the results in this bioassay as compared to the results obtained using the fidelity of DNA synthesis *in vitro* as a test system (*18-20*). Those metals which enhanced viral transformation altered the accuracy of DNA synthesis; metals which did not increase transformation did not affect fidelity. The only exception was iron which enhanced transformation but did not alter fidelity.

Transformation of Syrian Hamster Cells by Metal Compounds

Recent studies by DiPaolo and Casto have demonstrated that morphological transformation of Syrian hamster cells could itself by used as an *in vitro* bioassay to detect potentially carcinogenic metal compounds (*59*). In these experiments, cells were exposed to varying concentrations of metal salts for one day. After 7-8 days of culture, the cells were analyzed for transformation. The cells were classified

Table 2. Effect of metals on the transformation of mammalian cells.[a]

Metal	Valence	Enhancement of viral transformation (enhancement ratio; concentration) mM[b]	Transformation of Chinese hamster cells (transformed colonies/total colonies), %[c]
Arsenic	3	2.4 (0.1)	4.13 (5)
Beryllium	2	2.6 (0.56)	6.40 (5)
Cadmium	2	2.2 (0.001)	4.02 (1)
Chromium	6	2.4 (0.01)	3.48 (1)
Nickel	2	3.4 (0.38)	5.45 (10)
Barium	2	0.9 (4.8)	—
Calcium	2	0.8 (6.8)	—
Lithium	1	0.5 (23.6)	—
Strontium	2	0.9 (3.8)	—
Titanium	4	0.8 (12.5)	—

[a]The capacity of metals to enhance viral transformation or to transform mammalian cells in the absence of exogenous virus was determined as described (*59, 60*). The enhancement ratio was determined by calculating the ratio of transformed foci observed in the presence of metals to that observed in untreated cells.

[b]The numbers in parentheses refer to the lowest concentration required to observe enhancement or the highest concentration used for compounds scored as negative.

[c]The numbers in parentheses refer to the concentration (μg/ml) required to observe that percentage of transformation.

as transformed if the colony formation was altered due to uncontrolled cell growth and due to irregular cell arrangement.

In this system 12 metals were examined (Table 2). Six metal compounds induced morphological transformation. Positive results were achieved with nickel sulfate, cadmium acetate, sodium chromate, nickel subsulfide, beryllium sulfate, and sodium arsenate. In each instance increasing concentrations of the metal salt resulted in an increase in the number of transformed colonies observed. No transformation was observed in untreated cultures. Furthermore, each metal in the compounds used has been implicated as a carcinogen in animals or in humans.

In contrast to the positive results observed with those metal salts, no transformation was observed using ferric oxide, titanium dioxide, sodium tungstate, zinc chloride, aluminum chloride, or with amorphous nickel sulfide. Of these metals tested *in vivo*, the determination of the carcinogenicity of iron and of zinc have been inconclusive. Iron in the form of iron-dextran and intratesticular injection of zinc have produced tumors (*60, 61*). However, administration of the metals by all other routes tested or in other forms has not resulted in tumor formation. Thus the negative results obtained in this bioassay as well as other results obtained in other *in vitro* tests could serve potentially as supportive data for a determination of the hazards of iron and of zinc compounds.

Induction of Chromosomal Aberrations by Metal Compounds

The capacity of exogeneous agents to perturb normal chromosome structure has been used quite successfully as an *in vitro* bioassay (*62-65*). Cytogenic analysis provides a rapid and reproducible test to determine whether exposure to specific chemicals results in the production of diverse chromosomal lesions. This type of analysis also permits the simultaneous determination of various types of chromosomal perturbations. The facilitation by exogeneous agents of genetic disturbances at the chromosomal level would presumably be due to DNA damage. In particular, strand breaks or the induction of DNA crosslinks would provide attractive sites for the transfer of nucleotide sequences to sites on other chromosomes.

The *in vitro* determination of chromosomal aberrations produced *in vivo* in cells from individuals exposed to specific chemicals may provide the most relevant bioassay to monitor environmental hazards. In particular, human lymphocytes provide a readily accessible source with which to assay large populations of exposed individuals. Using populations of industrial workers, recent studies on the capacity of metals to produce chromosomal aberrations have been equivocal. Bauchinger et al. determined the extent of chromosomal perturbations in lymphyocytes from workers in a zinc smelter plant (*66*). These individuals had increased blood levels of lead and of cadmium. These workers had a 2- to 3-fold increase in the number of aberrations/cell with respect to comparable controls. Furthermore, the increase in aberrations was not selective but instead reflected an overall increase for each type of perturbation. However, other studies by Evans and O'Riordan (*67*) and by O'Riordan et al. (*68*) failed to observe any increase in chromosomal aberrations in industrial workers exposed to lead or to cadmium.

Analysis of the capacity of chromium compounds to induce chromosomal aberrations substantiates the importance of hexavalent chromium in metal carcinogenesis. Using hamster embryo cells, Tsuda and Kato reported that increasing concentrations of potassium chromate induced increasing numbers of chromosome gaps, chromatid or chromosome breaks, or chromosome exchanges (*69*). However, prior treatment of potassium dichromate with Na_2SO_3 eliminated the induction of chromosomal aberrations. This is in agreement with previous results in bacterial systems demonstrating that metabolism of hexavalent chromium eliminated chromium induced mutagenesis.

Similar studies in other mammalian cells demonstrated the capacity of hexavalent chromium to induce chromosomal perturbations. In human lymphocytes, Nakamuro et al. examined the chromosome breaking capacity of hexavalent chromium (*70*). At low levels of exposure, the yield of aberrations was linear with dose. In Chinese hamster ovary cells, hexavalent chromium induced a 10 fold increase in the number of chromosomal perturbations as compared to the untreated control (*71*). With sodium dichromate, the number of chromatid breaks or gaps was dose-dependent. In mouse cells, both $K_2Cr_2O_7$ and CrO_3 induced large numbers of aberrations (*72*). Sister chromatid exchanges were observed in human fibroblasts exposed to either K_2CrO_4 or to $K_2Cr_2O_7$ (*73*).

In contrast to the results observed with hexavalent chromium, exposure of mammalian cells to trivalent chromium did not result in the production of similar numbers of chromosomal perturbation (*70*). Although these results support those observed in bacterial assays, trivalent chromium did affect chromosomal structure in some studies. In human

leukocytes, hexavalent chromium compounds were significantly more effective in inducing chromosomal rearrangements than were trivalent chromium compounds. However, all of the compounds tested were comparably effective inducing chromatid gaps. The significant differences were observed in the number of chromosome breaks or exchanges. These results would suggest that the latter type of perturbation is of more importance with regard to potential chromium carcinogenesis.

Exposure to cadmium salts has resulted in the induction of chromosomal aberrations. Using Chinese hamster cells, Rohr and Bauchinger reported that exposure to $10^{-4}M$ $CdSO_4$ resulted in a significant production of chromosomal perturbations (*74*). Determination of the mitotic index suggested a pronounced effect of cadmium. As unsynchronized cells were used in this experiment, it was not possible to ascertain whether a specific part of the cell cycle was affected or whether cadmium exhibited a nonspecific inhibitory effect. Using a system comparable to that of Casto and DiPaolo, Zasukhina et al. (*75*) examined the ability of $CdCl_2$ to affect the induction of chromosomal aberrations caused by Kilham virus. Using primary rat embryo cells, they reported that $CdCl_2$ by itself failed to produce any detectable number of aberrations above background levels. In contrast, administration of $CdCl_2$ to virus-containing cultures increased by 2-fold the number of perturbations which were observed. These two studies are of interest in that they demonstrated the effect of free metal cations on the induction of chromosomal aberrations. In contrast to many bacterial studies reporting the lack of activity of free metal cations, this study suggests that the interaction of free metal cations with chromosomal structures may result in specific perturbations.

Several systems have been used to determine that platinum as *cis*-dichlorodiammine platinum (II) induced chromosomal aberrations. In human lymphocytes, Wiencke et al. (*76*) demonstrated that exposure to increasing concentrations of *cis*-PDD resulted in a proportional increase in the number of sister chromatid exchanges (SCE). At a *cis*-PDD concentration of 1.0 μg/ml, they reported an approximately 5-fold increase in the number of SCE. As a function of sequential cell division, there was an inverse relationship between the number of SCE and the number of cell divisions after exposure. Further analysis in Chinese hamster cells demonstrated that caffeine produced a synergistic effect on the induction of chromosomal aberrations by *cis*-PDD (*77*).

Conclusion

In this review I have attempted to briefly summarize recent studies using the major *in vitro* bioassays to evaluate the potential carcinogenicity of metal compounds. In particular, most investigations have focused on those metals for which carcinogenicity data are available. As shown in Table 3, some of these metals have been analyzed in several of the bioassays. However, as is evident from this compilation, wide gaps remain with

Table 3. Effect of metals in *in vitro* bioassays.[a]

Metal	Carcinogenicity *in vivo*	Bacterial mutagenesis	Mammalian mutagenesis	Enhancement of viral transformation	Transformation of mammalian cells	Chromosomal aberrations
Arsenic	+	−?	N.D.	+	+	N.D.
Beryllium	+	N.D.	N.D.	+	+	N.D.
Cadmium	+	−?	N.D.	+	+	+
Chromium	+	−(3)	−(3)	N.D.(3)	N.D.(3)	−?(3)
		+(6)	+(6)	+(6)	+(6)	+(6)
Cobalt	?	N.D.	N.D.	+	N.D.	N.D.
Copper	?	+*	N.D.	+	N.D.	N.D.
Iron	?	?	N.D.	+	−	N.D.
Lead	+	−?	N.D.	+	N.D.	N.D.
Manganese	?	+*	N.D.	+	N.D.	N.D.
Mercury	?	−(1)	N.D.(1)	N.D.(1)	N.D.	N.D.
		+(2)	N.D.(2)	+(2)	N.D.	N.D.
Nickel	+	N.D.	N.D.	+	±	N.D.
Platinum	?	+(2)	+(2)	+(2)	N.D.	+(2)
Selenium	?	+	N.D.	N.D.	N.D.	N.D.
Zinc	?	−	N.D.	+	−	N.D.

[a]The determinations of the interactions of metal compounds with *in vitro* bioassays are taken from the text and from the appropriate references. Determinations of carcinogenicity are taken from the references (*13-17*). The designation of −? refers to compounds tested in only one of the available assays. The number in parenthesis refers to the valence or charge of the metal. The asterisk (*) refers to results obtained in systems other than the major *in vitro* bioassays.

respect to our knowledge of the effects of many of these metals.

The greatest use of *in vitro* bioassays with respect to metals may be to determine which forms of a specific metal may pose the greatest risk. It may be argued that *in vivo* data have indicated which metals are potential human carcinogens. However, it may also be a reasonable expectation that not all forms of a given metal pose this hazard. The valence of the particular metal within the compound, the net charge on the metal compound, the orientation of the metal within the compound as well as the solubility of the metal salt may be determining factors. Thus, several investigations performed with chromium compounds suggest that, although *in vivo* data demonstrate the carcinogenicity of chromium in animals or in man, *in vitro* experiments would indicate that exposure to hexavalent chromium which crosses cell membranes is biologically more active than comparable exposure to trivalent chromium which does not cross cell membranes. Unfortunately, similar studies have not been performed with most of the other metals listed. Furthermore, most metals have not been systematically analyzed with respect to their capacity to act as bacterial mutagens or for their capacity to induce chromosomal aberrations in mammalian cells.

Although many metals have been tested for *in vivo* carcinogenicity, other metals have not been adequately tested. Thus, *in vitro* bioassays may also serve to identify other metals which are potential human carcinogens. *In vitro* bioassays may thus function to screen a large number of metal compounds as a first step to focus attention on specific metal salts. These salts may then be examined *in vivo*. For example *cis*-dichlorodiammine platinum (II) induces bacterial mutations as well as inducing chromosomal aberrations. It is of interest that this and related compounds produced sarcomas after s.c. injections in rats, and that it was an initiator for mouse skin after IP injections (*78*). In this respect, it behaves like most cancer chemotherapeutic agents which are carcinogenic. However, if *in vitro* bioassays are used as a "first tier" test for metal compounds, the unique solubility problems of metal salts and their potential interactions with media components have to be considered with regard to negative results.

Recent evidence suggests that diverse metals may act synergistically to potentiate the effects of other mutagens or carcinogens. Stich et al. reported that the mutagenicity of ascorbic acid in the Ames Salmonella assay was increased 80-fold by the addition of copper (*79*). Similarly, copper, cobalt, manganese, iron and zinc increased the mutagenic effect of *cis*-5,6-dihydro-6-hydroperoxy-5-hydroxythymine on transforming DNA of *H. influenzae* (*80*). Further studies demonstrated that copper could increase the induction of sister chromatid exchanges in Chinese hamster cells caused by exposure to ascorbic acid or to sodium bisulfite (*81*). Furthermore, manganese, copper, or iron enhanced unscheduled DNA synthesis induced by isoniazid or other hydrazines (*82*). Thus, the synergistic effects of transition metals suggest the possibility that *in vitro* bioassays may serve as useful tools to further investigate the capacity of metals to enhance the mutagenicity or carcinogenicity of other agents.

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