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Mechanisms of nickel carcinogenesis¹

by F William Sunderman Jr, MD²

SUNDERMAN FW Jr. Mechanisms of nickel carcinogenesis. *Scand J Work Environ Health* 1989;15:1–12. Recent investigations on possible mechanisms of nickel carcinogenesis are reviewed, emphasizing cellular uptake and intracellular translocation of nickel, morphological transformation of cells by nickel compounds, chromosomal damage, DNA strandbreaks and DNA-protein complexes produced by nickel compounds, mutagenic effects of nickel, influence of nickel on the helical transition of B-DNA to Z-DNA, nickel-induced infidelity of DNA synthesis, free radicals and lipid peroxidation induced by nickel exposures, nickel inhibition of DNA repair, nickel as a tumor promotor, nickel inhibition of natural-killer (NK) cell activity, manganese and magnesium antagonism of nickel carcinogenesis, and speculation that Ni^{2+} might replace Zn^{2+} in finger-loop domains of transforming proteins. The weight of evidence supports the following tentative conclusions: differences in the carcinogenic activities of nickel compounds may reflect variations in their capacities to provide nickel ions (eg, Ni^{2+}) at critical sites within target cells; Ni^{2+} can initiate carcinogenesis, possibly by mutagenesis, chromosome damage, formation of Z-DNA, inhibition of DNA excision-repair or epigenetic mechanisms; Ni^{2+} can function as a tumor promotor; Ni^{2+} can enhance tumor progression by inhibiting NK cell activity; and nickel carcinogenesis can be suppressed or modified by certain other metals (eg, manganese and magnesium).

Key terms: tumor promotion.

This paper summarizes recent advances in knowledge pertaining to cellular and molecular mechanisms of nickel carcinogenesis on the basis of publications that have appeared during the decade since my previous survey of the subject (1). For information on related topics, such as the epidemiology of respiratory tract cancers among nickel-exposed workers, carcinogenesis bioassays of nickel compounds in experimental animals, and biological monitoring of nickel exposure, readers are referred to recent monographs and reviews (2–7).

Cellular uptake and translocation of nickel

The cellular bioavailability of nickel (Ni), ie, the ability of nickel to enter target cells and to release nickel ions, appears to be a major determinant of the carcinogenic and toxic effects of nickel compounds (8–10). The mechanisms of the cellular uptake of nickel include (i) endocytosis of particulate nickel compounds and alloys, (ii) transport of Ni^{2+} ions via Ca^{2+} channels, and (iii) transmembrane diffusion of lipophilic nickel compounds (eg, nickel carbonyl). Cos-

ta and his co-workers (11, 12) demonstrated that the endocytosis of nickel-containing particles is influenced by particle size, surface properties (eg, zeta potential), and chemical composition. Particles of crystalline nickel sulfides, which are potent carcinogens, are avidly phagocytized by cultured fibroblasts and slowly dissolve within lysosomal vesicles to release nickel ions. Some of the partially solubilized particles appear to be translocated to the perinuclear zone. A portion of the nickel enters the nucleus, either in ionic or particulate form, and some nickel (presumably Ni^{2+}) becomes bound to nucleoproteins (especially to heterochromatin) (13, 14).

Several investigators (15–18) have shown that Ni^{2+} crosses cell membranes via Ca^{2+} channels and evidently competes with Ca^{2+} for specific receptors. The intracellular nickel concentrations that are achieved by the Ca^{2+} transport route are probably lower than those reached by endocytosis, and this possibility could be the explanation for the greater carcinogenic activities of certain particulate nickel compounds when they are compared to soluble nickel salts (19). Nickel carbonyl [$\text{Ni}(\text{CO})_4$], a volatile, lipophilic carcinogen, readily diffuses across cell membranes and undergoes intracellular oxidation to release carbon monoxide, which is eventually exhaled, and Ni^{2+} , a portion of which binds to nucleoproteins (20–22). The pronounced differences in the carcinogenic activities of nickel compounds that have been observed in carcinogenesis bioassays (23–25) hypothetically reflect variations in the rates of (i) dissolution of the compounds in extracellular fluids, (ii) uptake of the compounds by target cells and subsequent intracellular re-

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lease of Ni^{2+} , and (iii) cellular efflux of Ni^{2+} versus translocation of Ni^{2+} to nuclear binding sites.

Morphological transformation of mammalian cells

Costa et al (26, 27) and DiPaolo & Casto (28) reported that in vitro exposures of Syrian hamster embryo (SHE) cells to alpha nickel subsulfide ($\alpha\text{Ni}_3\text{S}_2$) or nickel sulfate (NiSO_4) produce high incidences of transformed foci, whereas exposure to amorphous nickel sulfide (NiS), which is noncarcinogenic in animal bioassays, yields negative results in cell transformation assays. Moreover, when NiSO_4 was administered to pregnant Syrian hamsters on day 11 of gestation, morphological transformation was observed in cell cultures derived from progeny excised on day 13 of gestation (28). Costa et al (26, 27) showed that morphological transformation of SHE cells by $\alpha\text{Ni}_3\text{S}_2$ is dose-dependent and can be prevented by the addition of manganese dust to the medium. Several clones of $\alpha\text{Ni}_3\text{S}_2$ -transformed cells produced fibrosarcomas following subcutaneous transplantation to nude mice (27). Costa & Mollenhauer (12) demonstrated that morphological transformation of SHE cells by various particulate nickel compounds is proportional to phagocytic uptake of the particles by Chinese hamster ovary (CHO) cells.

Costa & Heck (29) obtained positive results for nickel dust, nickel selenide (Ni_3Se_2), nickelic oxide (Ni_2O_3), and nickel chloride (NiCl_2) in the SHE cell transformation assay, whereas a sample of nickel monoxide (NiO) yielded negative results under the same experimental conditions. Tang et al (30) noted that nickel dust, crystalline NiS, and NiCl_2 yield positive results for morphological transformation in SHE cells. My colleagues and I (10) tested several preparations of NiO and nickel-copper oxide for the induction of morphological transformation of SHE cells and observed strong correlations between the results of transformation assays and certain biological attributes of the compounds, including (i) in vitro dissolution half-times in serum and cytosol, (ii) in vitro phagocytosis indices, and (iii) in vivo stimulation of erythropoiesis following intrarenal administration to rats. Costa et al (31) noted that the potency of crystalline NiS for the induction of morphological transformation in SHE cells averaged 2.5-fold that of soluble NiCl_2 , a probable reflection of avid cellular uptake of crystalline NiS particles by phagocytosis, in comparison to the moderate uptake of Ni^{2+} by ion channels. Hansen & Stern (32) compared the activities of five nickel compounds [nickel dust, $\alpha\text{Ni}_3\text{S}_2$, Ni_2O_3 , NiO, and nickel acetate [$\text{Ni}(\text{C}_2\text{H}_3\text{O}_2)_2$]] for the in vitro transformation of body hamster kidney (BHK-21) cells. Although the nickel compounds varied substantially in their transforming potencies, the compounds produced the same proportions of transformed colonies when tested at equitoxic dosages (eg, 50 % survival). Hansen & Stern

(32) concluded that the intracellular bioavailability of Ni^{2+} is the property that determines the transforming potency of nickel compounds.

Haugen et al (33) showed that human fetal kidney explants, exposed in vitro to NiSO_4 , develop signs of morphological transformation and anchorage independence, but the apparently transformed colonies fail to develop tumors after implantation in nude mice. Lechner et al (34) reported a negative outcome of attempts to transform human bronchial epithelial cells by exposure to NiSO_4 for 40 d; none of the nickel-treated cultures developed anchorage independence or produced tumors upon injection into nude mice. Biedermann & Landolph (35) found that NiO, $\alpha\text{Ni}_3\text{S}_2$, NiSO_4 , and $\text{Ni}(\text{C}_2\text{H}_3\text{O}_2)_2$ cause a stable anchorage independence of human diploid foreskin fibroblasts, but do not induce other in vitro markers of morphological transformation, such as focus formation or immortality. Thus, in contrast to the strongly positive results obtained with SHE cells, human cell cultures seem only partially susceptible to morphological transformation by nickel compounds (33–35).

Costa & Mollenhauer (12), Rivedal & Sanner (36, 37), and Uziel & Butler (38) observed synergistic effects of benzo(a)pyrene (BaP) and nickel compounds on the morphological transformation of SHE cells. For example, Rivedal & Sanner (36) showed that exposing SHE cells to BaP followed by exposure to NiSO_4 results in a transformation frequency of 10.7 %, compared to 0.6 % and 0.5 % for SHE cells exposed to the respective individual substances. These in vitro findings are consistent with those of an earlier report by Maenza et al (39) of carcinogenic synergism between $\alpha\text{Ni}_3\text{S}_2$ and BaP following intramuscular administration to rats.

Mitotic aberrations and sister chromatid exchanges

The chromosomal damage induced by nickel compounds is illustrated by the studies listed in table 1. Swierenga & Basrur (40) showed that crystalline nickel subsulfide arrests the mitosis of rat myocytes in telophase or posttelophase and therefore suggests that Ni^{2+} may impair the dissolution of mitotic spindles by binding to sulfhydryl groups on the spindle fibers. Chromosome gaps, breaks, and/or sister chromatid exchanges have been demonstrated in various cell populations exposed in vitro to nickel compounds (19, 41–49), in bone marrow cells NiCl_2 -treated hamsters and mice (50, 51), and in peripheral blood lymphocytes from nickel-exposed workers (52–54). Sen & his co-workers (19, 47, 48) noted that chromosome aberrations induced by in vitro exposures of SHE cells to NiCl_2 or crystalline NiS frequently occur in the protein-rich heterochromatic regions of chromosomes, and especially include decondensation or fragmentation of the heterochromatic long arm of the X-chromosome of CHO cells.

DNA strandbreaks and DNA-protein cross-links

Using the alkaline elution technique, Ciccarelli and his associates (55–57) noted deoxyribonucleic acid (DNA) strandbreaks and DNA-protein complexes in renal cell nuclei from rats killed 2 to 20 h after an intraperitoneal injection of nickel carbonate (NiCO_3). Robison et al (58) and Robison & Costa (59) observed dose-related increases of DNA strandbreaks, detected by alkaline elution, in cultured CHO cells exposed in vitro to NiCl_2 , $\alpha\text{Ni}_3\text{S}_2$, or crystalline NiS , but *not* in cells exposed to amorphous NiS under similar conditions. Swierenga & McLean (60) assessed the ability of nickel compounds to cause DNA strandbreaks in cultured rat hepatocytes by fluorescent analysis of DNA unwinding, which is more sensitive than the alkaline elution technique. Strandbreaks were not observed after exposures of cells to subtoxic concentrations of particulate $\alpha\text{Ni}_3\text{S}_2$, dissolved $\alpha\text{Ni}_3\text{S}_2$, or NiCl_2 ; however, strandbreaks were found at higher nickel concentrations that reduced cell survival and therefore suggested that strandbreaks may reflect cell demise rather than mutagenic events. Patierno and his co-workers (61–63) showed that the DNA-protein complexes induced by NiCl_2 treatment of CHO cells involve nuclear proteins that normally reside in close association with DNA. The DNA-protein complexes detected by the alkaline elution analysis of NiCl_2 -treated CHO cells are dissociated by the addition of sodium dodecyl

sulfate; therefore Ni^{2+} is probably not a bifunctional cross-linking agent (63). Patierno et al (63) speculated that Ni^{2+} exposure induces conformational changes of chromatin that establish stable DNA-protein complexes. Conway et al (64) found that the addition of magnesium chloride (MgCl_2) to culture media reduces Ni^{2+} uptake and inhibits NiCl_2 -induced DNA strandbreaks and DNA-protein complexes in CHO cells and human osteosarcoma cells, as detected by alkaline elution profiles.

Mutagenesis in mammalian cells

As reviewed by Hansen & Stern (65), nickel compounds practically always yield negative results for bacterial mutagenesis in the *Salmonella typhimurium* and *Escherichia coli* tester strains. Mutagenesis tests of nickel compounds in mammalian cells have been conducted by several groups of investigators (35, 60, 66–72). Miyaki et al (72) investigated the mutagenicity of NiCl_2 for cultured Chinese hamster V-79 cells at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus by selecting for resistance to 8-azaguanine. At a Ni^{2+} concentration of 0.4 mmol/l, the cell survival averaged 55 %, and the mutation frequency was insignificantly increased. At a Ni^{2+} concentration of 0.8 mmol/l, the cell survival averaged only 0.4 %, and the mutation frequency was increased 2.7-fold over the control values. Miyaki et al (72) con-

Table 1. Studies of chromosome damage induced by nickel compounds in vivo and in vitro.

Authors	System	Observations
Swierenga & Basrur (40)	Rat embryo myocytes in vitro	Exposure to alpha nickel subsulfide caused aberrations of mitotic spindles and telophase arrest
Nishimura & Umeda (44)	Mouse FM3A carcinoma cells in vitro	Nickel chloride, nickel acetate, and nickel sulfide caused sister chromatid exchanges and increased chromosome breakage
Wulf (49)	Human lymphocytes in vitro	Incubation with nickel sulfate increased the number of sister chromatid exchanges
Larramendy et al (42)	Syrian hamster embryo cells & human lymphocytes	Nickel sulfate exposure in vitro caused dose-related increases in the number of sister chromatid exchanges
Saxholm et al (46)	Human lymphocytes	Alpha nickel subsulfide caused a mild increase in the number of sister chromatid exchanges
Newman et al (43)	Human lymphocytes in vitro	Nickel chloride caused dose-related sister chromatid exchanges, especially in chromosomes #1, #2, and B group
Ohno et al (45)	Don Chinese hamster cells	Nickel sulfate and nickel chloride exposure in vitro caused increased numbers of sister chromatid exchanges
Waksvik & Boysen (53), Waksvik et al (54)	Human lymphocytes in vivo	Active and retired nickel refinery workers had increased chromosome breaks and gaps (not sister chromatid exchanges)
Chorvatovicova (50)	Hamster bone marrow in vivo	Nickel chloride injection intraperitoneally caused dose-related chromosome gaps and breaks
Andersen (41)	Murine P338D ₁ macrophages	Nickel sulfate exposure in vitro caused a slight increase in the number of sister chromatid exchanges, not dose-related
Sen & Costa (1, 48), Sen et al (47)	Chinese hamster ovary & C3H10T 1/2 cells in vitro	Nickel chloride and crystalline nickel sulfide caused sister chromatid exchanges, especially in heterochromatic areas, eg, hamster X chromosome long arm
Mohanty (51)	Murine bone marrow in vivo	Nickel chloride injections intraperitoneally caused dose-related increases in the numbers of gaps and breaks
Deng et al (52)	Human lymphocytes in vivo	Nickel platers' chromosomes contained increased numbers of breaks and sister chromatid exchanges

cluded that NiCl_2 exhibits weak mutagenicity but that detecting mutations is difficult because of cell toxicity. Hsie et al (71) used a similar technique to test the mutagenicity of NiCl_2 in cultured Chinese hamster ovary $\text{K}_1\text{-BH}_4$ cells with 6-thioguanine as the purine analogue. Preliminary results suggested that Ni^{2+} is mutagenic, but the authors cautioned that the finding needed confirmation, since the assay was influenced by the ionic composition of the medium and by the physiological state of the cells. Mutagenesis evidently required Ni^{2+} concentrations sufficient to inhibit growth and kill most cells (71).

Selecting for trifluorothymidine-resistant mutants at the thymidine kinase locus, Amacher & Paillet (66) tested the mutagenicity of NiCl_2 in murine lymphoma L51784Y cells. Ni^{2+} exposures yielded small but statistically significant increases in mutation frequencies. For example, at a Ni^{2+} concentration of 0.4 mmol/l, cell survival averaged 27 %, and mutation frequency was increased to 4.3-fold of the control level. At a Ni^{2+} concentration of 0.7 mmol/l, cell survival averaged 5 %, and mutation frequency was increased to 7.6-fold of the control value. Gurley et al (70) did not detect any increase in mutations at the HGPRT locus in CHO cells that were exposed to nickel arsenide (Ni_3As_2), even at high concentrations that caused >99 % cell death. Swierenga & McClean (60) reported that rat hepatocytes developed 8-azaguanine-resistant colonies after exposures to toxic concentrations of particulate $\alpha\text{Ni}_3\text{S}_2$, dissolved $\alpha\text{Ni}_3\text{S}_2$, or NiCl_2 . However, mutations at the HGPRT locus were not significantly increased at concentrations of the nickel compounds that allowed >20 % survival (60). Biedermann & Landolph (35) tested the mutagenicity of $\alpha\text{Ni}_3\text{S}_2$ in human diploid foreskin fibroblasts, but did not find any increase of ouabain-resistant or 6-thioguanine-resistant colonies, even at an $\alpha\text{Ni}_3\text{S}_2$ concentration that increased the frequency of anchorage-independence 200-fold.

Biggart & his co-workers (67, 68) studied the mutagenic effect of NiCl_2 on rat kidney cells infected with murine sarcoma virus (MuSV) mutant "ts 110," which has a defective expression of the *v-mos* transforming gene. Brief exposure of the cell cultures to NiCl_2 at virtually nontoxic concentrations produced a sevenfold increase in the reversion to the transformed phenotype in comparison with the spontaneous reversion frequency in control cultures. Christie & Tummolo (69) observed a strong mutagenic response in Chinese hamster g12-V-79 cells exposed in vitro to crystalline NiS . This stable cell line, developed by transfection of pSV2gpt into *hprt*⁻ Chinese hamster V-79 cells (73), enables mutational loss of *gpt* expression to be detected by the addition of 6-thioguanine. Subtoxic exposure to crystalline NiS caused a >50-fold increase of such mutations over the background level, whereas similar NiSO_4 caused only a twofold increase (39). The experimental systems described by Biggart and his co-workers (67, 68) and Christie & Tummolo (69) evidently

provide the first practical and sensitive assays for nickel-induced mutagenesis in eukaryotic cells.

Helical transition of B-DNA to Z-DNA

Double-stranded DNA, which is normally found as a right-handed double-helix (B-DNA), can, under certain conditions, adopt a left-handed double helical form (Z-DNA) (74, 75). The Z-DNA conformation is favored by nucleotide sequences with alternating purines and pyrimidines, especially alternating guanine and cytosine residues. Nordheim et al (76) speculated that certain carcinogens may act at regulatory regions of DNA to induce transition between the B and Z forms and thereby distort transcriptional activity. van de Sande et al (77) showed that Ni^{2+} , Co^{2+} , and Mn^{2+} induce in vitro transition of double-stranded poly-d(G-C) from B to Z conformation and that removal of the various divalent cations with ethylenediaminetetraacetate (EDTA) produces instantaneous Z to B reversal. Liquier et al (78) reported that B to Z helical transition of double-stranded poly-d(G-C) is induced by submillimolar concentrations of NiCl_2 , NiSO_4 , NiCO_3 , and $\alpha\text{Ni}_3\text{S}_2$ and therefore suggests that stabilization of Z-DNA sequences could be involved in nickel carcinogenesis.

Taillandier et al (79) and Taboury & Taillandier (80) demonstrated, by circular dichroism and infrared spectroscopy, that Ni^{2+} induces poly-d(A-C)·poly-d(G-T) to adopt the Z conformation, even at 20°C. By studying the infrared spectra of deuterated double-stranded polynucleotides, Adam et al (81) deduced that the binding of Ni^{2+} to the N7 site of deoxyadenosine stabilizes the purine in *syn*-conformation and favors formation of the left-handed double helix. Hacques & Marion (82) showed that methylation of cytosine residues of double-stranded poly-d(G-C) at the C5 position, a DNA modification associated with decreased levels of transcription, reduces the stoichiometry required for Z conformation of double-stranded poly-d(G-m⁵C) to 1 Ni^{2+} /4 nucleotide phosphates. Such findings suggest that the control of chromatin structure and the gene transcriptional activity may be affected by low concentrations of Ni^{2+} in cell nuclei. Z-DNA is strongly immunogenic, and antibodies to Z-DNA are available to detect Z-DNA in cell nuclei during cellular differentiation and malignant transformation (76, 83). Therefore, it should be technically feasible to determine whether or not Z-DNA stabilizes during nickel carcinogenesis.

Infidelity of DNA synthesis and depurination of DNA

Sirover & Loeb (84) reported close correspondence between (i) the capacities of various metal ions to impair the fidelity of the replication of synthetic polynucleotide templates by avian myeloblastosis virus

DNA polymerase in vitro and (ii) the carcinogenic activities of the respective metals, on the basis of human epidemiologic studies, animal bioassays, and mutagenesis tests. They proposed that testing for an impaired fidelity of DNA replication might serve as a screening assay for carcinogenic or mutagenic metal compounds (84). Miyaki et al (85) also found that the metals that are commonly regarded as "carcinogenic" cause the misincorporation of nucleotides by *E coli* DNA polymerase I in vitro, whereas the putatively "noncarcinogenic" metals do not impair the fidelity of DNA synthesis. Zakour et al (86) proposed three possible explanations for the metal-induced infidelity of DNA replication as follows: (i) altered substrate conformation at the substrate-binding site of DNA polymerase, (ii) altered enzyme conformation at the catalytic site of DNA polymerase, or (iii) altered template-base specificity, owing to reactions of metals with DNA that affect the complementarity of base-pairing. Schaaper et al (87) provided data that support the third hypothesis by showing that Ni^{2+} interacts with DNA in vitro and promotes deoxyadenosine hydrolysis by cleaving bonds between the purine base and deoxyribose, a phenomenon which leads to the release of adenine. The resulting apurinic sites of DNA are believed to induce mutations during subsequent replication by DNA polymerase, mediated by the misincorporation of purines, particularly deoxyadenosine, in the daughter strand (88). The chemical mechanism whereby Ni^{2+} induces DNA depurination has not been established, but it may involve free-radical reactions akin to Ni^{2+} -mediated lipid peroxidation.

Free radicals and lipid peroxidation

Exposures of humans and experimental animals to various chemical toxins and ionizing radiation induce the

formation of free radicals of oxygen in tissues. Free radicals of oxygen, especially the powerful hydroxyl radical ($HO^{\bullet}$), attack nucleic acids, proteins, and lipids and result in genotoxicity, membrane damage, cellular influx of Ca^{2+} , and, ultimately, cell death (89, 90). Lipid peroxidation is a major focus of current toxicologic research, since peroxidative damage contributes to many forms of xenobiotic injury and the molecular products of lipid peroxidation can readily be detected in tissues, body fluids, and excreta (91, 92). Peroxidation of polyunsaturated fatty acids (eg, linolenic and arachidonic acids) produces (i) conjugated dienes, which can be identified by their ultraviolet absorption spectra, (ii) malondialdehyde, which can be measured by the thiobarbituric acid (TBA) color reaction, (iii) alkanes and alkenes (eg, ethane, ethylene), which can be determined in exhaled breath by gas chromatography, (iv) protein adducts, which can be detected by electrophoresis, (v) lipofuscin pigments, which can be identified by staining reactions in histological sections, (vi) unstable lipid hydroperoxides, which can be determined by chemiluminescence, and (vii) hydroxy- and epoxy- derivatives (eg, 4-hydroxy-nonenal, a potent mutagen), which can be assayed by high-performance liquid chromatography, gas chromatography, and mass spectrometry (93, 94). Several of these techniques have been used to study nickel toxicity in rodents (table 2) and have shown that lipid peroxidation participates in the pathogenesis of acute nickel poisoning.

The chemical reactions whereby Ni^{2+} induces lipid peroxidation in vivo have not been established, but several hypotheses have been proposed (103). One hypothesis is an indirect mechanism, mediated by the Ni^{2+} displacement of Fe^{2+} or Cu^{2+} from their intracellular binding sites (eg, ferritin, ceruloplasmin) and resulting in lipid peroxidation by the Fenton and

Table 2. Studies of lipid peroxidation induced by nickel compounds in vivo and in vitro. (TBA = thiobarbituric acid, DNA = deoxyribonucleic acid, MDA = malondialdehyde)

Authors	System	Observations
Yonaha et al (95)	TBA assay of rat liver microsomes	Incubation of microsomes with nickel ions yielded MDA
Hasan & Ali (96)	TBA assay of rat brain homogenate	Lipid peroxidation was increased in the brain of rats treated with nickel chloride
Fawade & Pawar (97)	TBA assay of rat liver microsomes	Lipid peroxidation was increased in the liver of rats treated with nickel chloride
Ueda et al (98)	Strandbreaking of Φ X174 DNA	Autoxidized methyl linolenate caused DNA damage upon incubation with nickel ions
Sunderman et al (99)	TBA assay of rat tissues	Increased MDA-like chromogens in the liver, kidney, and lungs of rats treated with nickel chloride
Donskoy et al (100)	Microsomal lipids from rat liver	High levels of conjugated dienes in lipid extracts 24 h after an injection of nickel chloride
Knight et al (101)	Exhalation of ethane in rat breath	Increased ethane exhalation 13–23 h after an injection of nickel chloride
Kasprzak et al (102)	TBA assay of rat tissues	Increased MDA levels in the liver and kidney 24 h after nickel acetate injection
Sunderman (103)	Plasma MDA and red blood cell deformability	Elevated plasma lipoperoxides and reduced filtrability of red blood cells in rats treated with nickel chloride
Athar et al (104)	Iron and TBA assays of rat liver	Increased liver iron in rats treated with nickel ions could be the mechanism of lipid peroxidation

Haber-Weiss reactions, catalyzed by the $\text{Fe}^{2+}/\text{Fe}^{3+}$ or $\text{Cu}^{1+}/\text{Cu}^{2+}$ redox couples. A second hypothesis is that the $\text{Ni}^{2+}/\text{Ni}^{3+}$ redox couple directly generates free radicals of oxygen by one-electron transfer reactions. A third hypothesis is that Ni^{2+} accelerates degradation of lipid hydroperoxides to form lipid- $\text{O}^{\bullet}$ radicals and therefore propagates (rather than initiates) autocatalytic peroxidative reactions. A fourth hypothesis is an indirect mechanism whereby Ni^{2+} impairs cellular defenses against peroxidation by depleting free-radical scavengers (eg, glutathione) or inhibiting catalase, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase, or other enzymes that protect against free-radical injury (100).

That lipid peroxidation may be involved in nickel carcinogenesis was indicated by a study of Ueda et al (98) in which ΦX174 double-stranded supercoiled DNA was incubated in the presence of autoxidized methyl linolenate and various transition metal ions, followed by agarose gel electrophoresis to determine the extent of DNA strand cleavage. No DNA cleavage occurred solely in the presence of the autoxidized methyl linolenate, but DNA cleavage was $>50\%$ after additions of submillimolar concentrations of Cu^{2+} , Fe^{2+} , Fe^{3+} , Ni^{2+} , or Zn^{2+} , listed in order of diminishing activity. The DNA cleavage was blocked by EDTA (suggesting direct involvement of the metal ions) and by free-radical scavengers (98). This experiment indicates that Ni^{2+} -activated lipid peroxidation can damage DNA in vitro. Lipid peroxidation could also hypothetically contribute indirectly to nickel carcinogenesis, since certain lipid peroxidation products (eg, malondialdehyde, 4-hydroxynonenal) are mutagenic and other products (eg, hydroperoxy fatty acids) may be tumor promoters (105–107).

Inhibition of DNA excision-repair

Robison et al (108, 109) studied the influence of various nickel compounds on the repair of DNA strandbreaks in CHO and SHE cells. Their assay system involved (i) labeling parental DNA with [^{32}P]-orthophosphate, (ii) exposing the cells to nickel compounds, (iii) labeling repaired DNA with [^3H]-deoxythymidine, (iv) fractionating the DNA by equilibrium density gradient centrifugation, and (v) calculating, as an index of DNA repair, the ^3H to ^{32}P ratio of the DNA fraction that retained the equilibrium sedimentation density of parental DNA. Cells exposed in vitro to NiCl_2 , $\alpha\text{Ni}_3\text{S}_2$, and crystalline NiS (but not amorphous NiS) appeared to have enhanced DNA repair activity, even at concentrations of the nickel compounds that did not cause detectable DNA strandbreaks. Hamilton-Koch et al (110) used a nick-translation assay to detect DNA damage in cultured human fibroblasts exposed to a subcytotoxic concentration of NiCl_2 ; they reported that the DNA strandbreaks induced by Ni^{2+} were repaired within 2 h postexposure.

The studies of Robison et al (108, 109) and Hamilton-Koch et al (110) indicate that cells exposed to nickel compounds retain the ability to repair DNA strandbreaks, and this ability primarily involves the action of DNA ligases.

Other studies indicate that cells exposed to nickel compounds lose the ability to repair damaged DNA via the excision-repair pathway, which involves the coordinated actions of endonucleases and DNA polymerases. Swierenga and his co-workers (40, 111) assayed DNA repair in freshly isolated rat hepatocytes by measuring unscheduled DNA synthesis autoradiographically after exposures to NiCl_2 and $\alpha\text{Ni}_3\text{S}_2$. They found that NiCl_2 and $\alpha\text{Ni}_3\text{S}_2$ inhibit, in a concentration-dependent manner, the excision repair of DNA damage induced in rat hepatocytes by exposure to methylmethanesulfonate, a potent alkylating agent. Likewise, Hartwig & Beyersmann (112) showed that NiCl_2 inhibits the excision repair of mutations induced at the HGPRT locus in V-79 Chinese hamster cells by exposure to ultraviolet light. Snyder & Davis (113) demonstrated that Ni^{2+} inhibits, in a dose-related fashion, the excision repair of pyrimidine dimers induced in HeLa cells by exposure to X-ray or ultraviolet irradiation. These studies suggest that inhibition of the excision-repair pathway may play a role in nickel carcinogenesis.

Tumor promotion and blockage of cell-to-cell communication

To test whether nickel acts as a tumor promotor for nasopharyngeal carcinogenesis by dinitrosopiperazine (DNP), Ou et al (114) gave rats a single subthreshold injection of DNP (9 mg), followed by sustained exposure to NiSO_4 , either in drinking water for six weeks (3.7 mg Ni/d) or by seven weekly local applications of NiSO_4 in gelatin solution in the nasopharynx (37 μg Ni/week). No neoplasms developed in five control groups (12 rats/group) that received DNP alone, NiSO_4 alone by either route, or the vehicles, whereas two nasopharyngeal tumors developed in both groups of 12 rats that received DNP in combination with NiSO_4 . Although the tumor incidences of these groups were too low for statistical significance, the authors inferred that NiSO_4 may act as a tumor promotor. This suspicion was strengthened by similar results in a repetition of the experiment (115). Kurokawa et al (116) tested the tumor-promoting effect of NiCl_2 on renal carcinogenesis by N-ethyl-N-hydroxyethylnitrosamine (EHEN). Rats were given EHEN in drinking water (0.5 g/l) for two weeks to initiate carcinogenesis and were treated for 25 weeks thereafter with NiCl_2 in drinking water (0.6 g/l); control rats received EHEN for two weeks, followed by distilled water for 25 weeks. Renal neoplasms developed in 12 of the 15 rats that received EHEN plus NiCl_2 , compared to corresponding development in 3 of the 15 rats that received EHEN alone ($P < 0.05$).

As discussed in the section on morphological transformation, Rivedal & Sanner (36, 37) described a promotion-like effect of NiSO_4 on in vitro transformation of SHE cells that had been exposed to BaP. These findings suggest that nickel salts may, under certain circumstances, function as tumor promoters. It is interesting that Ling-Wei et al (117) found a highly positive correlation between nickel concentrations in drinking water and nasopharyngeal cancer morbidity among residents of the Xiangxi region of Hunan, China, and Isacson et al (118) noted an association between nickel concentrations in drinking water and morbidity from lung and bladder cancers among residents of towns in Iowa in the United States.

Ruch & Klaunig (119) reviewed the evidence that tumor promoters share the ability to inhibit cell-to-cell communication via gap junctions. Intracellular communication, which involves the passage of low molecular-weight molecules between adjacent cells via gap junctions, is an important mechanism for controlling cell growth and replication; disruption of gap junctional communication occurs during the neoplastic process and may be a prerequisite for tumor development (119). Studies of Loch-Caruso et al (120) and Miki et al (121) suggest that Ni^{2+} resembles classical tumor promoters [eg, 12-*O*-tetradecanoylphorbol-13-acetate (TPA)] in the ability to block cell-to-cell communication. Loch-Caruso et al (120) investigated the effects of NiCl_2 on cocultures of (i) wild-type Chinese hamster V-79 cells (proficient in 6-thioguanine metabolism) with (ii) mutant V-79 cells (unable to metabolize 6-thioguanine to its toxic metabolite). At NiCl_2 concentrations that did not affect cell viability, the recovery of mutant cells was significantly increased and indicated an inhibition of junctional communication. Miki et al (121) found that the intercellular transfer of [^3H]-uridine was 98 % inhibited by exposure of National Institutes of Health 3T3 cells to NiSO_4 , under conditions that did not significantly impair cell viability. Miki et al (121) concluded that tumor promoting effects of Ni^{2+} may contribute to nickel carcinogenesis, but stressed that the tumor-promoting activity of Ni^{2+} does not exclude tumor-initiating capabilities of nickel compounds.

Inhibition of natural-killer cell activity

Smialowicz et al (122) reported that natural-killer (NK) cell activity and, to a less extent, T-cell-mediated immune responses are inhibited in spleens of NiCl_2 -treated mice, whereas immune responses mediated by B-cells are unaffected by NiCl_2 treatment. Profound inhibition of splenic NK cell activity was also observed in NiCl_2 -treated rats, although NiCl_2 did not affect the immune responses of either T-cells or B-cells in rats (123). Consistent with the established role of NK cells in the immune defense against spontaneous and transplanted tumors, Smialowicz et al (122, 123) showed that NiCl_2 inhibition of NK cell activity en-

hanced the susceptibility of mice and rats to develop lung tumors following the injection of syngeneic tumor cells. Daniels et al (124) infected mice with a sublethal dose of murine cytomegalovirus and then administered NiCl_2 by intramuscular injection or inhalation. Parenteral doses of NiCl_2 inhibited the spontaneous and virus-augmented NK cell activity in mouse spleen and enhanced the mortality of mice from cytomegalovirus infection, whereas the administration of NiCl_2 by inhalation had no effect on NK cell activity or mortality. Daniels et al (124) did not detect any influence of the NiCl_2 treatments on interferon levels in mouse serum or splenic homogenates. This finding is significant, since Treagan and her associates (125, 126) had previously reported that NiCl_2 depressed interferon production in vitro.

Kasprzak et al (127) did not find any significant effect of $\alpha\text{Ni}_3\text{S}_2$ on NK cell activity in mononuclear cells from the peripheral blood or spleen of rats sacrificed 1, 3, 7, or 14 d postinjection (5 mg/rat; 2.5 mg in each hind leg, intramuscularly). Judde et al (128) measured the NK cell activity of mononuclear cells in blood specimens obtained from rats during eight months after an injection of $\alpha\text{Ni}_3\text{S}_2$ or nickel dust. A weakly tumorigenic dose of $\alpha\text{Ni}_3\text{S}_2$ (5 mg, intramuscularly) caused a transient decrease in NK cell activity from 17 to 23 weeks postinjection; the original level could be restored by the administration of interferon. A strongly tumorigenic dose of nickel dust (20 mg, intramuscularly) caused a sustained decrease of NK cell activity from 8 to 23 weeks postinjection; this decrease did not respond to interferon treatment. Prospective analysis of NK cell responses in individual rats showed that a persistent depression of NK cell activity was restricted to the rats that subsequently developed sarcomas at the injection site of nickel dust; in these rats the time from the injection of nickel dust to tumor detection was positively correlated with the mean level of NK cell activity. Judde and his co-workers (128) concluded that the inhibition of basal NK cell activity and an unresponsiveness to interferon may play a significant role in the development of nickel-induced sarcomas in rats.

Shinkai et al (129) and Lichtenheld et al (130) have isolated and characterized "perforin," a cytolytic factor from cytoplasmic granules of murine NK cells. The amino acid sequence of perforin is rich in cysteine and histidine residues, including two domains that meet the sequence criteria for Zn^{2+} binding (vide infra). I speculate that the inhibitory effect of nickel on NK cell activity might reflect Ni^{2+} inactivation of perforin by substitution for Zn^{2+} at the active site of the enzyme.

Manganese and magnesium antagonism of nickel carcinogenesis

The carcinogenic effects of nickel compounds in experimental animals are antagonized by the simultane-

ous administration of manganese and magnesium compounds. The anticarcinogenic effect of *manganese* was reported by my colleagues and I (131) on the basis of the finding that an admixture of manganese dust with $\alpha\text{Ni}_3\text{S}_2$ suppressed the carcinogenicity of $\alpha\text{Ni}_3\text{S}_2$ after intramuscular administration to rats. This observation was confirmed in several subsequent studies (128, 132–134). Copper, chromium, and aluminum dusts had no significant effect on nickel carcinogenesis when tested under the same conditions (132–134). Manganese dust did not alter (i) the mobilization rate of nickel from the injection site, (ii) the distribution of nickel in body fluid compartments, or (iii) the cumulative elimination of nickel in urine and feces, but it did reduce the concentration of ultrafiltrable nickel in the supernatant fraction of muscle homogenates from injection sites of rats at five to six months postinjection, compared to the findings with rats that received only $\alpha\text{Ni}_3\text{S}_2$ (133). The inhibitory effect of manganese dust on muscle tumorigenesis by $\alpha\text{Ni}_3\text{S}_2$ is dose-dependent; the effect appears to be local rather than systemic, since tumor incidence was reduced when manganese dust and $\alpha\text{Ni}_3\text{S}_2$ were mixed and injected into one hind leg but not when the manganese dust was injected into one hind leg and $\alpha\text{Ni}_3\text{S}_2$ into the other (132, 133). Manganese dust also reduced the induction of renal cancers in rats after the intrarenal injection of $\alpha\text{Ni}_3\text{S}_2$ (135) and blocked in vitro morphological transformation of SHE cells by $\alpha\text{Ni}_3\text{S}_2$ (26). Judde et al (128) showed that an admixture of manganese dust and nickel dust prevented the depression of NK cell activity produced by nickel dust alone and inhibited the induction of sarcomas by nickel dust.

The anticarcinogenic effect of *magnesium* was reported by Kasprzak et al (136) on the basis of the finding that a mixture of magnesium carbonate (MgCO_3) and $\alpha\text{Ni}_3\text{S}_2$ reduced the incidence of injection-site sarcomas to 25 % of that of treated rats, compared to a sarcoma incidence of 70 % for rats that received an intramuscular injection of $\alpha\text{Ni}_3\text{S}_2$ alone. Calcium carbonate had no significant effect when tested under the same conditions. The inhibitory influence of MgCO_3 on $\alpha\text{Ni}_3\text{S}_2$ tumorigenesis was confirmed in a further study that demonstrated a dose-dependent and strictly local character of the effect (127). Miki et al (121) found that NiSO_4 inhibition of cell-to-cell communication in NIH 3T3 cells was partially prevented by the addition of magnesium sulfate (MgSO_4) to the medium. Conway et al (64) observed that raising the extracellular level of magnesium ions inhibited NiCl_2 -induced DNA strandbreaks, DNA-protein complexes, and sister chromatid exchanges in CHO cells, as detected by alkaline elution, and also inhibited in vitro morphological transformation of SHE cells.

Kasprzak et al (137) showed that certain essential divalent metal ions compete with Ni^{2+} for high-affinity binding sites in DNA from calf thymus, ranking $\text{Mg}^{2+} \geq \text{Mn}^{2+} > \text{Ca}^{2+} \geq \text{Cu}^{2+} = \text{Zn}^{2+}$. Thus, the strongest inhibitors of the in vitro binding of Ni^{2+} to DNA

are Mg^{2+} and Mn^{2+} , which are ions of the same metals that attenuate nickel carcinogenesis in vivo.

Finger-loop domains of transforming proteins

“Zn-finger proteins” are a newly discovered class of DNA-binding proteins that regulate gene expression in prokaryotic and eukaryotic organisms (138, 139). The so-called “finger-loop domain” of such proteins comprises a segment, approximately 18 to 28 amino acids in length, with pairs of histidine and/or cysteine residues separated by two to five amino acids at each extremity. The four histidine and cysteine residues provide a tetrahedral coordination site for Zn^{2+} , which stabilizes the intervening sequence of amino acids in a loop that fancifully resembles a “finger” or a “hairpin” (140, 141). Tandem repeats of finger-loop domains have been identified in several regulatory proteins (eg, human transcription factor “Sp 1”), but many such proteins contain only a single finger-loop domain (142, 143). Site-specific binding of the finger-loop domain(s) to double-stranded DNA constitutes a fundamental mechanism whereby proteins can control gene expression (144, 145).

My colleagues and I have proposed that substituting Ni^{2+} for Zn^{2+} in finger-loop domains of transforming proteins might constitute a molecular mechanism of nickel carcinogenesis (146). As a first step towards testing this hypothesis, a computer search was made of the National Biomedical Research Foundation (NBRF) protein sequence data-base for possible finger-loop motifs in transforming proteins encoded by oncogenes. The putative finger-loop domains in protein sequences were identified through the application of the following search criteria:

$$[a]_{-x_{2,5}}-[a]_{-x_{4-15}}-[a]_{-x_{2-5}}-[a],$$

where “a” is either cysteine or histidine and “x” denotes any amino acid. The search identified possible finger-loop domains in the *myc*, *fms*, *fps*, *raf-1*, *rfp*, *src*, *syn*, *yes*, *erbA*, *int-1*, and *TGF- α* gene products (141). Zn^{2+} has an ionic radius of 0.72 Å, compared to the 0.69 Å radius of Ni^{2+} ; both metals readily form coordination complexes with thiol-sulfur and imidazole-nitrogen atoms. Therefore, it was suggested (141) that substituting Ni^{2+} for Zn^{2+} in DNA-binding finger-loop domains of transforming proteins might affect their functions as regulators of gene expression and could conceivably induce site-specific free-radical reactions (eg, depurination of DNA) at loci critical for oncogenesis.

Concluding remarks

Carcinogenesis is a multistage process involving initiation, promotion, and progression; the process is influenced by genetic, chemical, and immunologic defenses (eg, tumor suppressor genes, antioxidant

nutrients, NK cell activity) (147). The investigations of nickel carcinogenesis that have been reviewed in this article support the following conclusions: (i) differences in the carcinogenic activities of nickel compounds may be attributable to variations in their capacities to provide Ni^{2+} at critical sites within target cells; (ii) Ni^{2+} can initiate carcinogenesis, possibly by direct genotoxicity (eg, by free-radical reactions), by inducing a conformational transition of B-DNA to Z-DNA, by the transposition or modulation of regulatory genes, by the inhibition of DNA excision-repair, or by various epigenetic mechanisms; (iii) Ni^{2+} can serve as a tumor promoter (eg, by blocking cell-to-cell communication or, possibly, by stimulating lipid peroxidation), (iv) Ni^{2+} can enhance tumor progression (eg, by inhibiting NK cell activity), and (v) manganese and magnesium compounds can furnish potential pharmacological approaches to prevent or modify the process of nickel carcinogenesis.

In light of the recent rapid advances in knowledge of the multistage process of carcinogenesis and ongoing progress in elucidating the biochemistry and toxicology of nickel compounds, I envision that a clear delineation of the cellular and molecular mechanisms of nickel carcinogenesis will be achieved by the year 2000.

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