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MOLECULAR MECHANISMS OF NICKEL CARCINOGENESIS

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INTRODUCTION

The molecular mechanisms involved in metal carcinogenesis are not well understood (1), even though a number of metal compounds (e.g. As, Ni, Cr, Cd) have been identified as potentially carcinogenic to humans based upon epidemiological studies, or are known to induce tumors in experimental animals (e.g. Ni, Cr, Cd, Be, Pb). This situation has been largely due to the problems involved in studying metals. During the carcinogenic process, metal ions can affect DNA indirectly through oxidative damage, or by effects on DNA polymerase. In cases where the metals interact directly with DNA, it is difficult to determine the extent of DNA-metal binding because, with few exceptions, the coordinate-covalent bonding of metals to the DNA bases is generally unstable to isolation. Additionally, ligand exchange reactions and metal redistribution can occur during the isolation of metal-cellular ligand complexes. This is particularly true with metals such as nickel. Therefore, research has focused on the effects of metals on DNA in cells, rather than studying the distribution or binding of the metal itself. On the positive side, metals are relatively simple chemicals that produce cancer without extensive metabolism. In principle, they represent a useful tool in obtaining general insights about chemical carcinogenic processes.

While other studies have examined nickel carcinogenesis, this review focuses largely on the work from my laboratory, which studies the mech-

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anisms of nickel carcinogenesis through the use of cultured cells. Other investigators have contributed to our knowledge of metal carcinogenesis, and many of these findings are summarized more comprehensively in another recent review (2).

NICKEL

Introduction

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Epidemiological studies of refinery workers exposed to nickel by inhalation have demonstrated a high incidence of lung, nasal, and pharyngeal cancers (3, 4). Evidence points to particulate nickel compounds as among the most dangerous human carcinogens (3–5). More recent epidemiological evidence suggests, however, that water-soluble nickel salts, which are present in the electrolysis component of nickel refinery plants, may also participate in inducing human cancers (6). It is difficult to elucidate the mechanisms of carcinogenesis from epidemiological reports alone, but animal and cell culture model systems have been utilized to investigate these mechanisms in more detail.

One problem with studying respiratory carcinogens is the lack of an adequate animal model for addressing the problem of human inhalation carcinogenesis. The rat is not the best model because it is a "nose only" breather, and inhalation studies in rats do not always result in cancers even when well-established human carcinogens such as chromate are tested (6). Another problem that may have relevance to comparing cancer data from the rat to the human is that rat hemoglobin is structurally different from that of humans in that the cys-125 of the rat hemoglobin is 40 times more reactive toward agents that react as sulfhydryl groups (7). This and many other differences could affect metal distribution to tissues and yield a different response in the rat compared with the human. Despite these problems, crystalline nickel subsulfide has been shown to induce respiratory cancers in rats following inhalation exposure (8). More studies are in progress to assess carcinogenic activity of other nickel compounds by inhalation exposure.

The expense and difficulty of performing inhalation carcinogenesis studies has led to the administration of nickel compounds to animals by other routes. A large body of evidence demonstrates the carcinogenic potency of nickel compounds in animals at the injection site. These studies represent a model for the potential of a chemical to induce cancer in vivo. For example, intramuscular injection of crystalline nickel subsulfide and crystalline nickel sulfide in Fischer rats produces essentially 90–100% incidence of cancers at the site of injection (3, 4). Nickel compounds can be carcinogenic at other injection sites, including intrarenal sites (3, 4). However, not all particulate nickel compounds exhibit equivalent carcinogenic potencies. Amorphous

nickel sulfide, for example, produces a low tumor incidence at the site of injection (9).

Water-soluble nickel salts such as nickel chloride or nickel sulfate have generally not produced cancers in experimental animals even after repeated administration (10). Recent studies, however, have demonstrated that kidney tumors can be produced if a tumor promoter is administered in combination with these salts (11).

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MOLECULAR MECHANISMS OF NICKEL CARCINOGENESIS

Bioavailability of Nickel

The carcinogenic potencies of different compounds appear to be related to the bioavailability of nickel (presumably Ni^{2+}) to critical intracellular sites (12). One interesting example of bioavailability is noted in the comparison of the carcinogenic potency of crystalline nickel sulfide and the weak carcinogen amorphous nickel sulfide. We have found that the crystalline nickel sulfide compounds, which were potent in inducing tumors in animals, also transformed cells in tissue culture. In contrast, the amorphous nickel sulfide particles, which did not induce tumors in animals, were not active in transforming cultured cells (13). It is interesting to note that the exposure time required to transform cells in vitro is short, with little extracellular dissolution of soluble nickel from these relatively insoluble nickel particles occurring during that time. Additionally, the dissolution half-time of amorphous nickel sulfide in serum is very similar to that of crystalline nickel subsulfide, 24 and 34 days respectively (14). Based upon these published data, it is unlikely that differences in dissolution half-time could explain the strikingly different carcinogenic potencies of these two compounds. However, one cannot exclude the possibility that under certain biological conditions, the dissolution rates might be more dissimilar than those previously described (14).

In tissue culture studies, we have observed what might be a plausible explanation for the differences in carcinogenic potencies of different nickel compounds (13). Crystalline nickel subsulfide compounds were actively phagocytized by cultured fibroblasts undergoing transformation; noncarcinogenic amorphous nickel sulfide particles were not (13). Based upon these studies, we proposed that cells phagocytizing these particles in vivo included not only professional phagocytes such as macrophages (which do not go on to form cancer cells) but also nonmacrophage cancer-forming target cells, via the process referred to as *facultative phagocytosis* (12). Phagocytosis of a nickel particle would deliver large quantities of metal into the cell and may explain why certain less water-soluble nickel compounds are more carcinogenic than freely soluble compounds.

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When the basis for the difference in phagocytosis between amorphous or crystalline nickel sulfides was investigated, it was found that the surface properties of these particles were important to their ability to gain entry into cells (15, 16). By zeta potential measurements, amorphous nickel sulfide particles were found to have a positive surface charge, whereas crystalline nickel sulfide compounds had a more negative surface charge (15–17). Zeta potential is a measure of the surface charge of a particle. It is obtained by comparing the movement of particles in an electrical field. These charge differences were also confirmed in culture media by the binding of particles to filter paper discs offering different surface charges. This study was undertaken because normally the zeta potential can only be determined in H_2O; our particles would contact cells in tissue culture media (16). The importance of a negative surface charge for enhanced phagocytosis was further supported by studies where the surface charge of the particles was changed (18). Treatment of amorphous nickel sulfide particles with lithium aluminum hydride ($LiAlH_4$) increased the particles' negative charge. $LiAlH_4$ is a potent reducing agent that adds negatively charged hydride atoms to a particle surface. The enhanced phagocytosis of these particles led to a cell-transforming activity for amorphous NiS that became equivalent to that of the crystalline nickel sulfide particles (18).

Tables 1 and 2 show the striking differences in the phagocytosis of and transformation by amorphous and crystalline nickel sulfide, and demonstrate how these parameters were greatly altered by surface charge reduction on the particles (18). As shown in Table 2, $LiAlH_4$ treatment of the noncarcinogenic amorphous nickel sulfide particles caused these particles to exhibit a greater incidence of transformation than that of the untreated crystalline nickel sulfide particles alone. As observed in many other systems, the transforming activity of these nickel-containing particles was proportional to their uptake by cells. Even $LiAlH_4$-treated crystalline NiS particles were phagocytized in greater numbers and were more active in cell transformation as compared with untreated particles (19).

These results clearly illustrate the importance of several variables including the surface charge and the surface properties of these particles, and the phagocytic process itself, in the transformation and carcinogenic mechanisms of the nickel sulfide compounds. Another situation that results in differential uptake of particles is the release of nickel ions from parent particles and their effects on phagocytic activity (17). Soluble nickel ions have been shown to inhibit phagocytosis. Consequently, weakly carcinogenic amorphous nickel sulfide particles, which were dissolved at a slightly greater rate than the crystalline nickel sulfide particles, might release more biologically available nickel ions that inhibit the phagocytic process (17).

The availability of ionic nickel during dissolution seems to play an impor-

Table 1 Phagocytosis of solvent washed and $LiAlH_4$ reduced crystalline and amorphous NiS particles in CHO cells

Treatment compound	Phagocytosis[a] (%, 10 μg/ml)
Amorphous NiS	
Untreated	4.8 ± 0.16
Pyridine washed	9.9 ± 2.05[b]
$LiAlH_4$ reduced in pyridine	27.7 ± 0.90[b]
Crystalline αNiS	
Untreated	29.2 ± 2.52
Pyridine washed	33.3 ± 9.8
$LiAlH_4$ reduced	53.1 ± 4.59[b]

CHO cells grown in monolayer culture were exposed in 60-mm diameter tissue culture dishes to 10 μg/ml (1.78 $\mu g/cm^3$ surface growth area) of the particle preparations shown in the table for 1 day. Following treatment, cells were fixed, stained, and the percentage of cells containing intracellular particles was determined by light microscopy. Particle size of the preparations ranged from 1 μm to 3.6 μm and within this range there is little effect of particle size on phagocytosis. Each number shown in the table is the mean of 4 plates where at least 500 cells were examined in each plate.

[a] No. of cells with phagocytized NiS particles/total no. of cells examined, mean ± S.D. for 4 plates.

[b] Uptake differs from untreated particles. $P < 0.05$ Student t-test.

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tant role in the phagocytic uptake of the different nickel particles. Just a few hours storage of freshly ground particles in an aqueous medium reduced their uptake (17). By aging newly ground amorphous nickel sulfide particles for just 24 hours, the phagocytic uptake decreased substantially and was reduced to half the original activity in 7 days (17). Crystalline nickel sulfide particles were less affected by aging in aqueous suspensions, probably due to their ordered and stable structure. These studies illustrate that in addition to the surface structure and charge, the methods used to grind and store nickel particles are equally as important as particle size and contact with aqueous systems in governing the potential phagocytosis of nickel particles (12).

The ingestion of crystalline nickel sulfide particles was visualized by electron microscopy and by video-intensification microscopy (20, 21). These studies provided important insight into the uptake mechanisms and the intracellular events following phagocytosis of nickel particles. Crystalline nickel sulfide particles were phagocytized in regions of active membrane ruffling

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Table 2 Enhancement of cellular transformation following lithium aluminum hydride reduction o crystalline and amorphous nickel sulfide particles

Treatment compound	Exposure concentration μg/ml	Exposure concentration μg/cm² growth area	No. of plates	Transformed colonies/tota surviving colonies[a](%)
Amorphous NiS particles				
1.0 μm, untreated	1	0.13	7	2/510 (0.39)
	5	0.63	8	8/1949 (0.41)
	10	1.27	9	10/2054 (0.49)
2.66 μm, pyridine washed	1	0.13	5	1/636 (0.15)[b]
	5	0.63	8	1/614 (0.16)[b]
	10	1.27	6	3/248 (1.21)[b]
$LiAlH_4$ reduced	■	0.13	3	5/1088 (0.46)[b]
2.26 μm, reduced in pyridine	5	0.63	6	20/826 (2.42)[c]
	10	1.27	5	6/183 (3.27)[c]
Crystalline αNiS particles				
2.37 μm, untreated	5	0.63	6	17/1155 (1.47)
	10	1.27	6	26/972 (2.67)
2.07 μm, pyridine washed	■	0.13	6	10/1784 (0.56)[d]
	5	0.63	5	27/1026 (2.63)[c]
	10	1.27	4	12/268 (4.47)[b]
$LiAlH_4$ reduced	1	0.13	3	4/264 (1.51)[d]
3.76 μm, reduced in pyridine	5	0.63	9	24/972 (2.47)[c]
	10	1.27	7	40/534 (7.49)[c]

The cell transformation assay was conducted as described (13). The particle size of each preparation is shown with th treatment compound.

[a] No. of transformed colonies/total no. of surviving colonies.

[b] Not statistically significant from exposure to corresponding untreated particles.

[c] Differs significantly from corresponding untreated particles; $P < 0.001$ $\times$ ^{2} test.

[d] Corresponding concentration not available for statistical analysis.

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(21). Following endocytosis of the crystalline nickel sulfide particles, the particles exhibited saltatory motion in the cell, and lysosomes were observed to interact repeatedly with the particles in a manner similar to that observed during the digestion of micropinosomes (21). The particles were not observed to be exocytized. With time, the particles tended to aggregate around the nucleus. This aggregation was coincident with the vacuolization of the particles, with the vacuoles become increasingly acidified as revealed by acridine orange fluorescence (21). Crystalline nickel sulfide particles exhibit a greatly accelerated rate of dissolution at acid pH as compared with physiological pH, and these experiments describe key intracellular events that catalyzed the dissolution of the particles.

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Figure 1 illustrates the impact of phagocytosis on the dissolution of nickel sulfide particles (22). Figure 1a shows that the number of particles associated with cells decreases as a function of time, suggesting particle dissolution. Using radioactive particles (^{63}Ni), even though visible particles were lost from the cells, the loss of total nickel from the cell during the same period was not substantial. This implied that the particles were being solubilized but the nickel was remaining in the cell (22). Figure 1b shows the decrease in the diameter of the particles outside the cell as compared with those inside. As depicted, the diameter of the particles in the culture media remain much the same for 8 days, whereas their diameter decreases within the cell.

Several studies examining the intracellular distribution of nickel ions following treatment of cells with nickel sulfide particles showed that soluble nickel derived from phagocytized nickel sulfide particles entered the nucleus (21, 22). Electron microscopy studies indicated that nickel particles per se did not enter the nucleus (13, 20).

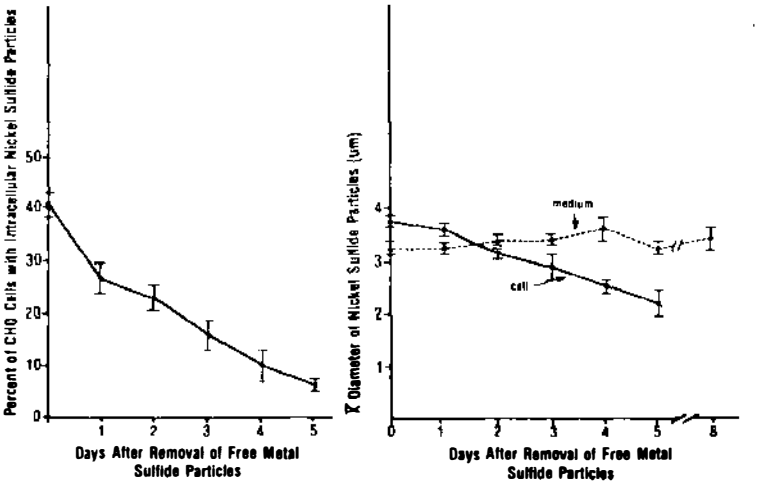

Figure 1 Intracellular and extracellular dissolution of crystalline NiS particles. CHO cells grown in 100-mm tissue culture dishes were treated with 10 μg/ml (1.2 μg/cm^2 growth areas (NiS). After a 24 h treatment, cells were washed three times with saline, fresh metal-free medium was added, and cells were incubated again for different periods. (*a*) Intracellular dissolution of crystalline NiS particles with time. At the end of each time point, cells were fixed with 95% ethanol and stained with crystal violet. The percentage of cells with intracellular particles was determined by examining at least 1000 cells per plate for internalized NiS particles using a light microscope. (*b*) Comparison of intracellular and extracellular dissolution of crystalline NiS particles. In (*a*), each point represents the mean ± SD of at least 150 cells in each of 8 random sections of a plate. In (*b*), each point is the mean ± SD of at least 20 particles sized at random. The mean diameter of crystalline NiS particles was determined with an eyepiece micrometer calibrated at each magnification against a stage micrometer. (Reproduced from ref. 22, with permission.)

The acidification of vacuoles containing nickel sulfide particles probably represented a carcinogenic activation step, assuming that particle dissolution and Ni(II) entry into the nucleus are critical factors in nickel sulfide-induced carcinogenesis. Recent studies have shown that some forms of DNA damage induced by NiS particles, but not by $NiCl_2$, can be suppressed by pretreatment of cells with Vitamin E (X. H. Lin, M. Costa, manuscript in preparation). These experiments suggest that an oxidative mechanism, possibly the result of phagocytosis-induced respiratory burst oxygen metabolism, may be operative in particulate NiS but not NiCl-induced DNA damage. There is also the possibility that the Ni^{2+} ions solubilized from the particles promote oxidative damage to a greater extent than if nickel ions are presented to the cell. In general, however, Ni^{2+} does not readily undergo oxidation-reduction reactions under biological conditions, and would not be expected to generate as many oxygen radicals as, for example, Cu and Fe ions. These results again emphasize that the mode of nickel delivery to the cell, i.e. soluble ions vs phagocytized nickel particles greatly affect resultant injury.

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Chromatin Damage

Complete chemical carcinogens, including the carcinogenic nickel compounds, which directly induce transformation in cell culture and tumors at virtually any site of administration in animals, should also exhibit some DNA damaging activity. Direct DNA damage has been analyzed in vitro by the alkaline sucrose gradient technique (24), nucleoid sedimentation analysis (25), and alkaline elution (26–28). These methods measure DNA single-strand breaks primarily, but the alkaline elution technique can also detect DNA-DNA and DNA-protein crosslinking. Nickel-induced DNA damage in vivo has been studied following nickel carbonate administration to male Sprague-Dawley rats (28, 29). Nickel carbonate was found to induce single-strand breaks in kidney and lung but not liver (28, 29). It was also found to induce the formation of DNA-protein crosslinks in kidney.

Using mammalian cultured cells, both water-soluble nickel salts, such as nickel chloride, and relatively water-insoluble salts, including crystalline nickel sulfide, which were phagocytized by these cells, induced single-strand breaks as determined by alkaline sucrose gradients (24). Both soluble and the phagocytized insoluble nickel salts induced DNA single-strand breaks and also DNA-protein crosslinks in cultured mammalian cells when studied by alkaline elution (26, 27). The formation of DNA-protein crosslinks in cultured cells by nickel compounds exhibits a very narrow dose-response range (26, 27). In several studies, DNA-protein crosslinking was not detectable in nonproliferating cells, whereas proliferating cells exhibited protein-DNA crosslinking and strand breaks (26, 27). However, when cells were synchronized and treated with nickel during various phases of the cell cycle, cells

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in the late S-phase of the cell cycle exhibited substantially higher amounts of strand breaks and DNA-protein crosslinks compared with cells in other phases of the cell cycle (27).

Figure 2 illustrates the alkaline elution curves for DNA strand breaks and DNA-protein crosslinks following treatment of cells with nickel chloride at various phases of their cell cycle. To examine crosslinking, untreated or nickel-treated cells were given a preliminary dose of radiation to induce DNA strand breaks. Following this test dose of radiation, crosslinking was indicated by the slower rate of elution in the nickel-treated cells as compared with the untreated cells. This slowed rate of elution was reversed by treatment of the lysate with protease K, confirming that it was DNA-protein crosslinks rather than DNA-DNA linkages that reduced the rate of DNA elution (Figure 2). As shown in Figure 2, the elution rate of the DNA during late S-phase in nickel-treated irradiated cells was not linear when examined for crosslinking and, in fact, appeared to have a bi-phasic component. These results suggest that nickel was inducing DNA-protein crosslinks in a nonrandom manner. The late S-phase of the cell cycle was also the time period when cells were most sensitive to the cytotoxic effects of nickel (27). Figure 2 also shows that most of the DNA strand breaks occurred during this phase of the cell cycle. It is interesting to note that the late S phase is the time period when heterochromatic DNA is replicated. These results correlate well with the heterochromatin-specific effects of nickel described below.

Biochemical fractionation of chromatin also indicated that nickel was bound and produced complexing of proteins to DNA in a very small fraction of chromatin (27, 30, 31). This chromatin fraction, characterized as a magnesium-insoluble heterochromatin fraction, is where most of the nickel-induced protein-DNA complexes were found and is also where more stable ^{63}Ni binding occurred (27, 30, 31). The proteins that were complexed to the DNA by nickel chloride were studied further.

While protein complexing to DNA was stable to high salt and non-ionic detergents, it was disrupted by SDS (30). According to chemical principles, DNA-protein complexes involving Ni(II) are kinetically active. Although nickel can form coordinate-covalent bonds, the DNA-protein complexes were not as stable as, for example, the kinetically inert complexes formed by trivalent chromium (32, 33). Due to this lack of stability, it was more difficult to quantitatively study the proteins complexed to the DNA by nickel than it was for chromate (32, 33). Following extensive extractions of the DNA isolated from nickel-treated cells with high salt, urea, and nonionic detergents, a substantial amount of protein remained bound (30, 31). These proteins, complexed to DNA by nickel, could, however, be released by treatment with an ionic detergent (SDS). The proteins were separated on polyacrylamide gel electrophoresis and characterized according to their molecular weight (30, 31).

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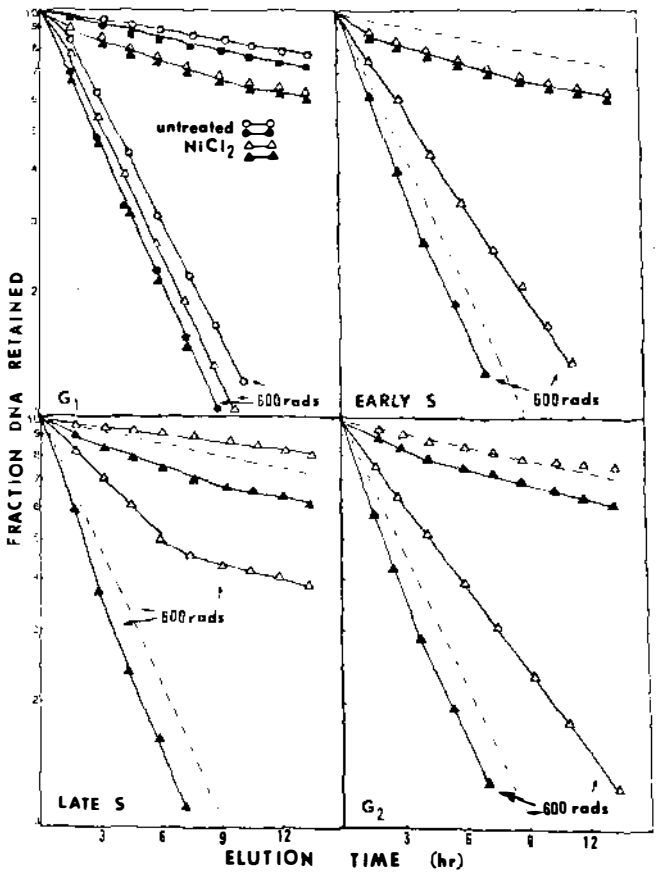

Figure 2 Cell cyclc-specific DNA-protein crosslinking in CHO cells treated with $NiCl_2$. CHO cells were synchronized in metaphase. The mitotic block was released by washing and replating the cultures in fresh medium at 37°C. Cells were treated with 2.5 mM $NiCl_2$ in complete medium for a 3 h time interval beginning at 1 (G_1, 1–4 h), 4 (early S, 4–7 h), 7 (late S, 7–10 h), or 12 h (G_2, 12–15 h) after replating. Both treated (triangles) and untreated (circles) cultures were lysed in the presence (closed symbols) or absence (open symbols) of proteinase K, and the DNA was analyzed by alkaline elution. In some instances, to detect crosslinking, a test dose of X-rays was given as indicated in the figure. (Reproduced from ref. 27, with permission).

Chromosome Damage by Nickel Compounds

Considering the carcinogenic potencies of nickel compounds, the extent of DNA damage, as assessed by alkaline sucrose gradients, alkaline elution or DNA-repair synthesis, was not striking. In contrast, the chromosome damage induced by nickel appeared to be a more sensitive barometer of the genotoxic-

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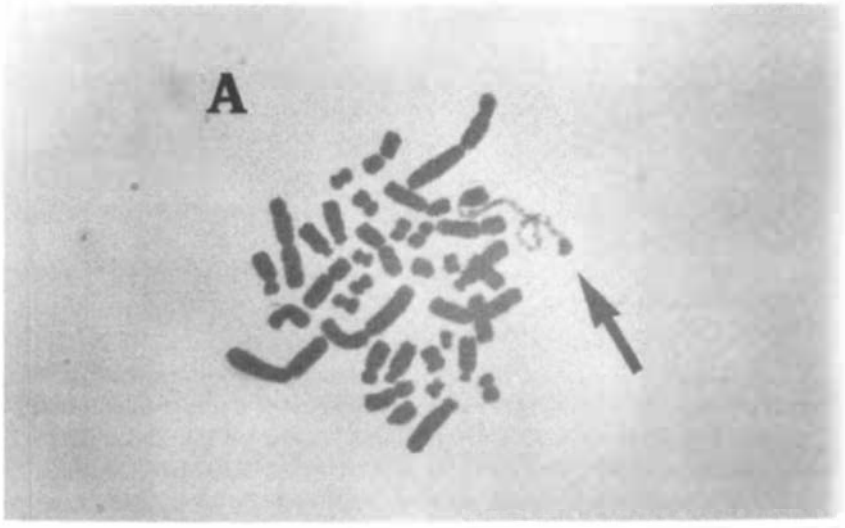
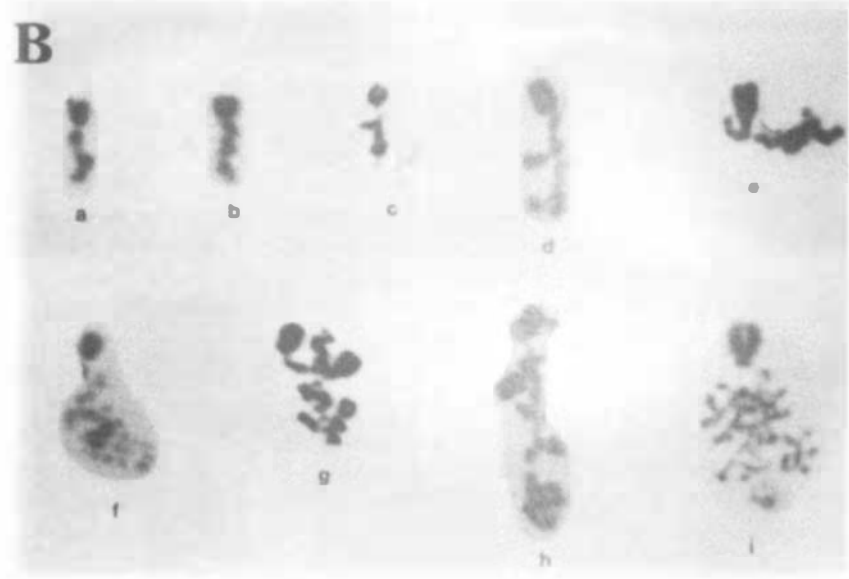
ity of nickel (34). Figure 3 demonstrates the selectivity of nickel in producing chromosome damage in heterochromatic regions. The arrow in Figure 3a shows the preferential decondensation produced by nickel in the heterochromatic long arm (q arm) of the X chromosome of Chinese hamster cells. In this species, the long arm of the X chromosome is the longest contiguous region of heterochromatin. Nickel also produces other chromosomal damage including gaps, breaks, and exchanges in both heterochromatin and euchromatin; however, a higher incidence of damage is produced by nickel in heterochromatic regions.

Figure 3b shows the progressive and selective damage produced in the X chromosome by nickel when the concentration and/or exposure times were increased (35). Note the lack of decondensation of the euchromatic short arm of the X chromosome in contrast to the dramatic decondensation in the heterochromatic long arm. Heterochromatin represents a highly condensed structure consisting of DNA and protein. It is believed that this region of chormatin, which is maintained in its condensed state by magnesium, represents a reactive site for nickel binding (36). Nickel is very similar to magnesium and, in fact, studies have shown that magnesium can antagonize the genotoxicity, cell transformation, and animal tumor induction by nickel compounds (36–39). Nickel also inhibits the function of and decreases the fidelity of DNA polymerase, a Mg^{2+}-requiring enzyme (for review, see ref. 2).

MUTATIONS INDUCED BY NICKEL

Nickel compounds are not generally mutagenic in prokaryotic assays, but can be weakly positive in mammalian cells, especially in mutation assays that score mutagenesis in the *hpgrt* or thymidine kinase genes. Prokaryotic assays including *Salmonella his* reversion, *Bacillus subtilis rec*$^+$, and *Escherichia coli trp*$^-$ assays (for review, see ref. 40) show that nickel is not readily mutagenic. Particulate nickel compounds are not expected to be active in prokaryotic assays since bacteria are not capable of phagocytosis. Unless the particle concentration of nickel is high enough to yield extracellular soluble mutagenic species, nickel should not mutate bacteria. Soluble nickel chloride has also been examined for mutagenic activity in a number of bacterial tester strains (41). In these studies, conditions were established that resulted in substantial uptake of the metal ion into bacterial cells; however, even when the metal uptake could be detected in the cells, there was still an absence of mutagenicity (41). The apparent lack of mutagenic activity in bacterial systems cannot be attributed to insufficient entry of the nickel ions into bacterial cells (41). Rossman et al, using a forward mutation assay involving the induction of λ-prophage in *E. coli* showed that long-term exposure to nickel





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acetate (3×10^{-4} M) gave a positive response with an eightfold increase in prophage induction over control values (42). This assay, however, is primarily detecting DNA damage rather than mutations. The response of nickel in this assay was similar to that obtained by manganese chloride, but much less than that of potassium chromate (76-fold over background) or MNNG (127-fold above background).

Nickel chloride was shown to be negative at the *hpgrt* locus in C3H cells (43), but positive at the same locus in V79 cells (44). It was also positive at the thymidine kinase locus of mouse lymphoma L5178Y-TK$^{-/+}$ cells (45). It is interesting that nickel exhibited little mutagenic activity in bacterial systems but produced a stronger reponse in mammalian cells. This may be because mammalian cells contain more DNA-associated proteins than do bacterial cells. Since nickel has a higher affinity for ligands on proteins than for those of DNA and would tend to interact more with these proteins, it may not be surprising that nickel has a greater genotoxic affect in mammalian cells than in bacteria. A similar increase in mutagenic activity from bacteria to mammalian cells is also seen with Cd and Pb.

Additionally, some mammalian mutation assays such as the mouse lymphoma assay are able to detect deletion mutations and nickel may be producing deletions as one mutagenic mechanism (2). This is supported by recent work where a mammalian cell line containing a transfected bacterial *gpt* gene (46), presumably located in an autosome, yielded a strong mutagenic response with crystalline nickel sulfide (47). The *hpgrt* gene, a frequently examined target gene for mutagenesis, is located on the X chromosome. Since most cells only have one active X chromosome, a mutation involving a large deletion will not survive to become a mutant cell if there is a concomitant loss of essential genes adjacent to the *hprt* gene. In contrast, if the mutagenesis target gene is located on an autosome, the allelic chromosome can complement the loss of adjacent essential gene sequences and cells with large deletions around the target gene may survive. It is possible, therefore, that nickel will produce a high mutagenic response in assays that appropriately

Figure 3 Chromosome damage induced in Chinese hamster ovary cells by $NiCl_2$. Cells were treated for 16 h with 1 mM $NiCl_2$ and mitotic cells were collected following colcemid exposure. Figure 3A shows a cell where the heterochromatic long arm of the X chromosome was not properly condensed during mitosis. Figure 3B is a photograph of a Chinese hamster ovary cell X chromosome after the cells had been treated with crystalline NiS particles. Cells were treated with crystalline NiS particles ranging in concentration from 10 to 20 μg/ml for 24 to 48 h. The figure shows an increasing degree of fragmentation of the long arm of the X chromosome with higher dosages and longer exposure times (a-i). Note the absence of fragmentation of the euchromatic short arm. (Reproduced from ref. 36, with permission.)

detect deletions. Since nickel interferes with DNA replication and affects protein-DNA interactions, it may cause deletions rather than point mutations (for review, see ref. 2).

An interesting feature of nickel is that it often gives a synergistic response when combined with a second genotoxic agent (for review, see ref. 2). Nickel is synergistic with other agents, including alkylating agents, UV-light, aminoacridine, and benzopyrene at a number of mutagenic, genotoxic, and carcinogenic endpoints. This suggests that a mechanism or component of its genotoxicity is different from that of other agents.

GENETIC CHANGES AND OVERALL MODEL OF NICKEL CARCINOGENESIS

The selective damage that nickel produced in the heterochromatin of Chinese hamster cells might have significance for nickel carcinogenesis. When the effects of increasing extracellular magnesium concentration on chromosomal damage induced by nickel chloride were investigated, it was found that magnesium selectively suppressed the nickel-induced damage in heterochromatin more than in euchromatin (36). Raising the extracellular levels of magnesium could completely suppress nickel chloride-induced transformation, suggesting that damage produced in heterochromatin might have significance in nickel-induced carcinogenesis in Chinese hamster cells (36). Other studies have shown that magnesium is capable of suppressing NiS-induced muscle tumors in vivo if it is administered within the first few days at the same injection site as the crystalline NiS (37–39).

To investigate this heterochromatic damage further, the incidence of transformation of freshly isolated male and female Chinese hamster embryo cells was studied (48). Since a substantial region of the X chromosome in these cells contains heterochromatin, it was hypothesized that if nickel interaction with heterochromatin was important in its carcinogenesis, then a difference in the incidence of transformation in male and female cells might be found.

The data obtained thus far demonstrated a higher incidence of transformation of male Chinese hamster embryo cells compared to female cells (48). It is difficult to explain these data based on the common knowledge that female cells generally have only one active X chromosome. However, a number of recent studies show that individual X-linked genes may escape general X chromosome inactivation (49), If transformation-related genes escape X-inactivation and are expressed from both homologs of the X chromosome, then female cells could be less susceptible to transformation if both of these genes must be inactivated. This discussion assumes that inactivation or deletion of a tumor suppressor gene is important for nickel carcinogenesis.

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Over half of the recovered male nickel-transformed cell lines had a complete deletion of the heterochromatic long arm of the X chromosome as the major chromosome aberration occurring in these cells (48). Recent experiments are addressing the possible significance of this deletion in the heterochromatic long arm of the X chromosome during nickel carcinogenesis (50). In collaboration with J. C. Barrett, an Xq-deleted nickel-transformed cell line was restored with a normal Chinese hamster X chromosome (50, 51). Restoration of the normal X chromosome caused a large percentage of the previously immortal nickel-transformed cells to senesce. Some clones that did not senesce but received an intact normal X chromosome did, however, exhibit a reduction in their ability to grow in soft agar. Based upon these and other studies, it is believed that nickel carcinogenesis may involve a deletion or an inactivation of a gene that is important in normal cellular senescence. This event would certainly be only part of the complicated process by which nickel induces cell transformation and carcinogenesis. There is currently no evidence that nickel carcinogenesis involves oncogene activation and more work is required to establish whether there is a role for oncogene and tumor suppressor genes in nickel carcinogenesis.

In summary, the bioavailability of nickel to the DNA, combined perhaps with some indirect oxidative damage derived from the irritant effect of particle phagocytosis, is probably instrumental in initiating nickel carcinogenesis. Nickel ions by interacting selectively with heterochromatin cause deletions of DNA sequences either by altering the DNA template (e.g. by distortion of heterochromatin structure) and/or by inhibiting the DNA polymerase when it copies these regions of DNA. These deletions may in fact result in the loss of a senescence/tumor suppresor gene involved in nickel carcinogenesis.

Literature Cited

1. Costa, M., Kraker, A. J., Patierno, S. R. 1984. Toxicity and carcinogenicity of essential and non-essential metals. In *Progress in Clinical Biochemistry and Medicine* 1:1–49. Berlin: Springer-Verlag
2. Coogan, T. P., Latta, D. M., Snow, E. T., Costa, M. 1989. Toxicity and carcinogenicity of nickel compounds. *CRC Crit. Rev. Toxicol.* 19(4):341–84
3. Sunderman, F. W. Jr. 1984. Carcinogenicity of nickel compounds in the animals. In *Nickel in the Human Environment*, IARC Monogr. Ser. ed. F. W. Sunderman, Jr., 53:127–42. Lyon: IARC Sci. Publ.
4. *Health Assessment Document for Nickel and Nickel Compounds, EPA/600/8-83/012FF*. 1986. US Environ. Prot. Agency
5. Kaldor, J., Peto, J., Easton, D., Doll, R., Hermon, L., Morgan, L. 1986. Models for respiratory cancer in nickel refinery workers. *J. Natl. Cancer Inst.* 77(4):841–48
6. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans: 1990. *Chromium, Nickel and Welding*. Vol. 49. In press
7. Hughes, G. J., De Jong, C., Fischer, R. W., Winterhalter, K. H., Wilson, K. J. 1981. Modification by simetryn sulphoxide of a specific thiol group in rat haemoglobin. *Biochem. J.* 199:61–67
8. Ottolenghi, A. D., Haseman, J. K., Payne, W. W., Falk, H. L., MacFar-

Annu. Rev. Pharmacol. Toxicol. 1991.31:321-337. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

land, H. N. 1974. Inhalation studies of nickel sulfide in pulmonary carcinogenesis of rats. *J. Natl. Cancer Inst.* 54: 1165–72
9. Sunderman, F. W. Jr., Maenza, R. M. 1976. Comparisons of carcinogenicities of nickel compounds in rats. *Res. Commun. Chem. Pathol. Pharmacol.* 14: 319–30
10. Kasprzak, K. S., Gabryel, P., Jarczewska, K. 1983. Carcinogenicity of nickel(II)hydroxides and nickel(II)sulfate in Wistar rats and its relation to the *in vitro* dissolution rates. *Carcinogenesis* 4(3): 275–79
11. Kasprzak, K. S., Diwan, B. A., Konishi, N., Misra, M., Rice, J. M. 1990. Initiation by nickel acetate and promotion by sodium barbital of renal cortical epithelial tumors in male F344 rats. *Carcinogenesis* 11:647–52
12. Costa, M., Heck, J. D. 1984. Perspectives on the mechanism of nickel carcinogenesis. In *Advances in Inorganic Biochemistry*, ed. G. L. Eichhorn, L. Marzilli, 6:285–310. New York: Elsevier
13. Costa, M., Mollenhauer, H. H. 1980. Carcinogenic activity of particulate metal compounds is proportional to their cellular uptake. *Science* 209:515–17
14. Kuehn, K., Sunderman, F. W. Jr. 1982. Dissolution half-times of nickel compounds in water, rat serum and renal cytosol. *J. Inorg. Biochem.* 17:29–39
15. Abbracchio, M. P., Heck, J. D., Caprioli, R. M., Costa, M. 1981. Differences in surface properties of amorphous and crystalline metal sulfides may explain their toxicological potency. *Chemosphere* 10:897–908
16. Abbracchio, M. P., Heck, J. D., Costa, M. 1982. The phagocytosis and transforming activity of crystalline metal sulfide particles are related to their negative surface charge. *Carcinogenesis* 3:175–80
17. Costa, M., Heck, J. D. 1983. Influence of surface charge and dissolution on the selective phagocytosis of potentially carcinogenic particulate metal compounds. *Cancer Res.* 43:5652–56
18. Heck, J. D., Costa, M. 1982. Surface reduction of amorphous NiS particles potentiates their phagocytosis and subsequent induction of morphological transformation in Syrian hamster embryo cells. *Cancer Lett.* 15:19–26
19. Costa, M., Heck, J. D. 1982. Specific nickel compounds as carcinogens. *Trends Pharmacol. Sci.* 3:408–10
20. Costa, M., Simmons-Hansen, J., Bedrossian, C. W. M., Bonura, J., Caprioli, R. M. 1981. Phagocytosis cellular distribution and carcinogenic activity of particulate nickel compounds in tissue culture. *Cancer Res.* 41:2868–78
21. Evans, R. M., Davies, P. J. A., Costa, M. 1982. Video time-lapse microscopy study of phagocytosis and intracellular fate of crystalline nickel sulfide particles in cultured mammalian cells. *Cancer Cells* 42:2729–35
22. Abbracchio, M. P., Simmons-Hansen, J. S., Costa, M. 1982. Cytoplasmic dissolution of phagocytized crystalline nickel sulfide particles: A prerequisite for nuclear uptake of nickel. *J. Toxicol. Environ. Health* 9:663–76
23. Deleted in proof
24. Robison, S. H., Cantoni, O., Costa, M. 1982. Strand breakage and decreased molecular weight of DNA induced by specific metal compounds. *Carcinogenesis* 3:657–62
25. Robison, S. H., Cantoni, O., Costa, M. 1984. Analysis of metal-induced DNA lesions and DNA-repair replication in mammalian cells. *Mutat. Res.* 131:173–81
26. Patierno, S. R., Costa, M. 1985. DNA-protein crosslinks induced by nickel compounds in intact cultured mammalian cells. *Chem.-Biol. Interact.* 55:75–91
27. Patierno, S. R., Sugiyama, M., Basilion, J. B., Costa, M. 1985. Preferential DNA-protein cross-linking by $NiCl_2$ magnesium-insoluble regions of fractionated Chinese hamster ovary cell chromatin. *Cancer Res.* 45:5787–94
28. Ciccarelli, R.B., Wetterhahn, K. E. 1984. Molecular basis for the activity of nickel. See Ref. 3, pp. 201–13
29. Ciccarelli, R. B., Wetterhahn, K. E. 1984. Nickel-bound chromatin, nucleic acids, and nuclear proteins from kidney and liver of rats treated with nickel carbonate in vitro. *Cancer Res.* 44:3892–97
30. Patierno, S. R., Sugiyama, M., Costa, M. 1987. Effect of nickel(II) on DNA-protein binding, thymidine incorporation, and sedimentation pattern of chromatin fractions from intact mammalian cells. *J. Biochem. Toxicol.* 2:13–23
31. Patierno, S.R., Costa, M. 1987. Effects of nickel(II) on nuclear protein binding to DNA in intact mammalian cells. *Cancer Biochem. Biophys.* 9(2):113–26
32. Miller, C. A. III, Costa, M. 1988. Characterization of DNA-protein complexes induced in intact cells by the carcinogen chromate. *Mol. Carcinogen.* 1(2):125–33
33. Miller, C. A., III, Costa, M. 1989. Analysis of proteins crosslinked to DNA

Annu. Rev. Pharmacol. Toxicol. 1991.31:321-337. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

after treatment of cells with formaldehyde, chromate, and cisdiamminedichloroplatinum(II). *Mol. Toxicol.* 2:11–26

34. Sen, P., Costa, M. 1985. Induction of chromosomal damage in Chinese hamster ovary cells by soluble and particulate nickel compounds: Preferential fragmentation of the heterochromatic long arm of the X-chromosome by carcinogenic crystalline NiS particles. *Cancer Res.* 45:2320–25
35. Sen, P., Costa, M. 1986. Pathway of nickel uptake influences its interaction with heterochromatic DNA. *Toxicol. Appl. Pharmacol.* 29(6):606–13
36. Conway, K., Wang, X.-W., Xu, L. S., Costa, M. 1987. Effect of magnesium on nickel-induced genotoxicity and cell transformation. *Carcinogenesis* 8(8): 1115–21
37. Kasprazak, K. S., Waalkes, M. P., Poirier, L. A. 1986. Effects of magnesium acetate on the toxicity of nickelous acetate in rats. *Toxicology* 42:57–68
38. Kasprzak, K. S., Waalkes, M. P., Poirier, L. A. 1986. Antagonism by essential divalent metals and amino acids of nickel(II)—DNA binding *in vitro*. *Toxicol. Appl. Pharmacol.* 82:336–43
39. Kasprzak, K. S., Waalkes, M. P., Poirier, L. A. 1987. Effects of essential divalent metals on carcinogenicity and metabolism of nickel and cadmium. *Biol. Trace Elem. Res.* 13:253–73
40. Heck, J. D., Costa, M. 1983. *In vitro* assessment of the toxicity of metal compounds. II. Mutagenesis. *Biol. Trace Elem. Res.* 4:319–30
41. Biggart, N. W., Costa, M. 1986. Assessment of the uptake and mutagenicity of nickel chloride in *Salmonella* tester strains. *Mutat. Res.* 175:209–15
42. Rossman, T. G., Molina, M., Meyer, L. W. 1984. The genetic toxicology of metal compounds. I. Induction of λ prophage in *E. coli* $WP2_s(\lambda)$. *Environ. Mutagen.* 6:59–69
43. Nishimura, M., Umeda, M. 1978. Mutagenic effect of some metal compounds on cultured mammalian cells. *Mutat. Res.* 54:246–47 (Abstr.)
44. Miyaki, M., Akamatsu, M., Ono, T., Koyama, H. 1979. Mutagenicity of metal cations in cultured cells from Chinese hamster. *Mutat. Res.* 68:259–63
45. Amacher, D. A., Paillet, S. C. 1980. Induction of trifluorothymidine-resistant mutants by metal ions in L5178Y/TK$^{+/-}$ cells. *Mutat. Res.* 78:279–88
46. Klein, C. B., Rossman, T. G. 1990. Transgenic Chinese hamster V79 cell lines which exhibit variable levels of gpt mutagenesis. *Environ. Molec. Mutagen.* In press
47. Christie, N. T., Tummolo, D. M., Klein, C. B., Rossman, T. G. 1990. The role of Ni(II) in mutation. In *Nickel and Human Health: Current Perspectives*, ed. E. Nieboer. New York: J. Wiley. In press
48. Conway, K., Costa, M. Nonrandom chromosomal alteration in nickel-transformed Chinese hamster embryo cells. *Cancer Res.* 49:6032–38
49. Brown, C. J., Willard, H. F. 1990. Localization of a gene that escapes inactivation to the X chromosome proximal short arm: Implications for X inactivation. *Am. J. Hum. Genet.* 46:273–79
50. Conway, K., Annab, L., Wang, X. W., Lin, X. H., Klein, C. D., et al. 1990. Chinese hamster X chromosome induces senescence of a nickel-transformed cell line with Xq deletion. UCLA Symposia on Molecular and Cellular Biology (Negative Controls of Cell Growth). *J. Cell. Biochem.* Suppl. 14C:280 (Abstr.)
51. Wang, X. W., Klein, C. B., Lin, X., Bhamra, R. K., Cohen, M. D., et al. 1990. X chromosome transfer into a nickel-transformed male Chinese hamster (CH) cell line with an Xq deletion induces senescence and suppression of agar growth. *Proc. Am. Assoc. Cancer Res.* 31:135 (Abstr.)